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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, workshop, roundtable, platform and poster sessions of the 43rd Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 21-25, 2004.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 443.

The document also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 473.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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1 HERBALS AND DIETARY SUPPLEMENTS IN ATHLETIC PERFORMANCE ENHANCEMENT: FACT VS. FICTION.

T. S. Tracy. *University of Minnesota, Minneapolis, MN.* Sponsor: A. Fuciarelli.

Herbal products and dietary supplements have been used for years in an attempt to enhance athletic performances. However, this usage has not always been based on scientific data. Recent tragic cases, such as those involving ephedra supplements, have highlighted the need for an unbiased assessment of available data and additional research into their actions and effects. This presentation will discuss the various products used for athletic performance enhancement such as ephedra, androstenedione and androgens, creatine, gamma hydroxybutyrate, dimethylglycine, and others. Their promoted uses, purported mechanism of action, adverse effects/toxicities, and available clinical data will be presented to provide a perspective of what is known and areas in need of additional research. Additionally, the current regulatory status will be discussed and what factors may impact upon changes in this status.

2 BASIC NEUROTOXICOLOGY.

W. Slikker, Jr[1], S. L. Schantz[2], M. G. Paule[1], E. C. Tiffany-Castiglioni[3] and J. P. O'Callaghan[4]. *[1]National Center for Toxicological Research, Jefferson, AR, [2]University of Illinois Urbana, Urbana, IL, [3]Texas A&M University, College Station, TX and [4]CDC-NIOSH, Morgantown, WV.*

Neurotoxicity may be defined as any adverse effect on the structure or function of the central and/or peripheral nervous system by a biological, chemical, or physical agent. Adverse effects can include both unwanted effects and any alteration from baseline that diminishes the ability of an organism to survive, reproduce or adapt to its environment. Neurotoxic effects may be permanent or reversible, and may result from direct or indirect actions on the nervous system. A multidisciplinary approach is necessary to assess neurotoxicity due to the complex and diverse functions of the nervous system. Many of the relevant effects can be measured by neurobiological, neurophysiological, neuropathological or behavioral techniques, as well as epidemiological approaches. After a general overview of neurotoxicity assessment from genes to human response, this basic course will present in greater depth the methods used to study populations, individual animals, cells, and genomes. Each speaker will review the basic concepts underlying the methodological approach presented. Selected neurotoxicants, including heavy metals, polyaromatic hydrocarbons, and drugs of abuse, will be used to illustrate principles. The first two lectures will address neurotoxic effects as studied by epidemiology in human populations and behavioral assessment in animal models, respectively. The next lecture will address the cellular responses of neurons, astrocytes, and oligodendrocytes to neurotoxicants. The course will be concluded with a description of a molecular approach to neurotoxicology including genomics. This course will be of interest to a broad range of scientists including drug developers, pharmacologists, neuroscientists, psychologists, regulators, and toxicologists.

3 TOOLS FOR FUNCTIONAL GENOMICS (PM10 REPEATED).

H. I. Swanson[1], E. D. Thompson[1], Y. Tian[2] and K. Kim[1]. *[1]University of Kentucky, Lexington, KY and [2]Texas A&M University, College Station, TX.*

The goal of this course is to discuss cutting-edge tools and techniques that may be used in ascribing hierarchical, functional analyses of gene products following DNA microarray experiments. First, we will discuss the advantages and disadvantages of a variety of pharmacological and molecular tools (i.e., antagonists, dominant negative approaches, siRNA). We will also discuss the means by which the molecular tools may be introduced into the cell or animal model, including the use of retro- and adenoviruses. Our second presentation will use data obtained in the laboratory to demonstrate the approaches that are typically used for determining whether the observed changes in mRNA of the gene product of interest occurs at the transcriptional or post-transcriptional levels. The third presentation will focus on use of the chromatin immunoprecipitation (CHIP) assay to demonstrate whether candidate transcription factors are involved in the regulation of the gene product of interest. Finally, our last presentation will introduce a novel approach, chemical genetics, that may be used to either activate or inactivate target gene products in able to discern their functional role(s) either the toxic or disease-related events.

4 OF MICE AND MAGNETS: METABONOMICS TECHNOLOGY IN SAFETY ASSESSMENT.

D. G. Robertson[1], L. D. Lehman-McKeeman[2], J. D. Baker[3] and D. A. Casciano[4]. *[1]Pfizer Global Research & Development, Ann Arbor, MI, [2]Bristol Myers Squibb Company, Princeton, NJ, [3]Pfizer, Inc., Ann Arbor, MI and [4]National Center for Toxicological Research, Jefferson, AR.*

Although metabonomics as a technology has been in the literature for over a decade, it is only in the past 3 to 4 years that the technology has gained widespread attention within the industrial sector. Metabonomics as a topic was introduced to the Society in a well-received sunrise mini-course in 2000. This was followed by a highly attended IAT symposium and poster session on metabonomics at the 2002 meeting. The technology has reached the level of maturity such that a full CE course is called for. The objectives of this basic level course will be to introduce the technology to SOT members unfamiliar with it, emphasizing the strengths and weaknesses of the technology in a practical way. The presentations will be from a toxicologist's perspective - communicating essential principles, but will avoid NMR and statistical jargon. The course will be primarily from a pharmaceutical development point of view, but will be broad enough to provide useful information for anyone interested in the technology.

5 FUNCTIONAL FLOW CYTOMETRY: APPLICATIONS IN TOXICOLOGY (PM12 REPEATED).

C. D. Bortner[1], D. Laskin[2], N. I. Kerkvliet[3] and S. W. Burchiel[4]. *[1]NIEHS, Research Triangle Park, NC, [2]Rutgers University, Piscataway, NJ, [3]Oregon State University, Corvallis, OR and [4]University of New Mexico, Albuquerque, NM.*

Flow cytometry provides a powerful tool for analyzing multiple characteristics of individual cells in a complex mixture of cell types without having to physically separate the cells. Yet, even though each cell is examined individually, the flow cytometer can process thousands of cells within a few seconds, allowing superior sampling of the population as compared to microscopic counting. The myriad of phenotypic and functional characteristics of cells that can be measured by flow cytometry continues to expand with the development of novel fluorescent probes to a variety of cellular components. The field of immunotoxicology has been greatly influenced by the use of flow cytometry with applications ranging from screening for toxic effects on immune cells to elucidating the mechanisms of toxic action on specific subpopulations of cells. However, other areas of toxicology are beginning to recognize the value of flow cytometry for mechanistic investigations as well. To address this growing interest, the intent of this course is to introduce the audience to novel applications of flow cytometry that have been used to assess tissue injury and mechanisms of toxicity at the whole animal, cellular, and biochemical levels. Although the context of many of the examples will emanate from immunotoxicology studies, each speaker will focus less on the immunology and more on the methods used in their studies that are broadly applicable to other areas of toxicology. Examples of methods to be covered include: apoptosis, oxidative stress, membrane integrity and fluidity, cell cycling using carboxyfluorescein (CFSE), and cell signaling.

6 UNDERSTANDING LIFESPAN CHANGES IN FORM AND FUNCTION OF THE FEMALE REPRODUCTIVE SYSTEM TO ASSESS AND INTERPRET TOXICITY.

P. M. Iannaccone[1], P. E. Blackshear[2], J. M. Cline[3] and P. J. Wier[4]. *[1]Northwestern University, Chicago, IL, [2]Integrated Laboratory Systems, Inc., Research Triangle Park, NC, [3]Wake Forest University School of Medicine, Winston-Salem, NC and [4]GlaxoSmithKline, King of Prussia, PA.*

This course reviews the basic morphology and endocrinology of the female reproductive system in rodents and primates as a basis for interpreting toxicity. Each of the 4 lectures will emphasize fundamental changes and vulnerabilities of the reproductive tract over the lifespan of the female. Both rodent and non-human primates will be discussed with respect to relevance to humans. The first lecture covers embryological development of the female reproductive system and will include key developmental and molecular events with an emphasis on timing of events in rodents and primates and potential periods of susceptibility to toxicity. The second lecture details the morphology and endocrinology of the female reproductive tract in rodents and will relate hormones and histology of the adult rodent reproductive tract from the onset of puberty to reproductive senescence and important sites of toxicity. The third lecture details the morphology and endocrinology of the female reproductive tract in nonhuman primates with emphasis on similarities and differences to rodents. The final lecture will combine the information of the first lectures and analyze issues of study design, endpoints to examine and interpretation of results in assessing female reproductive toxicity data.

7 THE SAFETY ASSESSMENT OF PROTEINS: APPLICATIONS TO AGRICULTURAL BIOTECHNOLOGY.

B. Petersen[1], J. Kough[2], M. Pariza[3] and J. D. Astwood[4]. [1]*Exponent, Inc., Washington, DC,* [2]*USEPA, Washington, DC,* [3]*University of Wisconsin-Madison, Madison, WI and* [4]*Monsanto Company, St. Louis, MN.* Sponsor: B. Hammond.

Biotechnology has made it possible to introduce proteins into food crops to achieve desired biological effects. Introduced proteins can impart important agronomic properties such as tolerance to topically applied herbicides to control weeds or protection of food crops against insect pest damage. Enzymes can be introduced into food crops that enhance the existing production of essential nutrients, or introduce nutrients into food crops that have potential health benefits. Proteins are also produced by microorganisms *via* fermentation such as enzymes used in food processing or pharmaceuticals (i.e. somatotropins) used to enhance the efficiency of milk production in dairy cows. A group of experts in the field of protein safety assessment will share their experience and learnings. The subject of protein allergy assessment will not be covered in this course as it has been thoroughly addressed in other courses and workshops held at SOT meetings. Toxicologists who attend this course will have a better understanding of the safety assessment strategies that have been developed for proteins in relationship to food safety. These strategies will differ in some respect from traditional safety testing approaches used for chemical xenobiotics that come in contact with food.

8 SAFETY PHARMACOLOGY AFTER ICH S7A & S7B.

A. Bass[1], P. Siegl[2], D. J. Murphy[3], S. Lindgren[4] and L. B. Kinter[5]. [1]*Schering Plough Research Institute, Kenilworth, NJ,* [2]*Merck Research Labs, West Point, PA,* [3]*GlaxoSmithKline, King of Prussia, PA,* [4]*AstraZeneca Pharmaceuticals, Sodertalje, Sweden and* [5]*AstraZeneca Pharmaceuticals, Wilmington, DE.*

Safety Pharmacology evaluations for human pharmaceuticals are dramatically redefined following implementation of International Conference on Harmonization Guidances S7A (2000), and finalization of S7B (anticipate in 2003). Those guidelines mandate evaluations for new drugs for unintended effects on cardiovascular, respiratory, and central nervous system functions (S7A core battery), renal and electrophysiological aspects of the cardiac repolarization (S7B) in support of phase I (first in man) programs. This introductory course will familiarize participants with rationale and tactics for modern safety pharmacology evaluations for expeditious development of human pharmaceuticals. An international faculty will present strategies for successful implementation of the core battery evaluations, including critical experimental endpoint, criteria for species selection, study design alternatives, dose selection, data analysis and interpretation, Animal Welfare and Good Laboratory Practice (GLP) issues. The Course will be of broad interest to both academic and industrial SOT Members engaged in pharmaceutical safety assessment and risk management.

9 SKIN SENSITIZATION AND ALLERGIC CONTACT DERMATITIS.

I. Kimber[1], D. A. Basketter[2], G. F. Gerberick[3] and D. M. Sailstad[4]. [1]*Syngenta, Macclesfield Cheshire, United Kingdom,* [2]*Unilever Research US Inc., Bedfordshire, United Kingdom,* [3]*Procter & Gamble Company, Cincinnati, OH and* [4]*USEPA, Research Triangle Park, NC.*

Skin sensitization resulting in allergic contact dermatitis is a very common occupational and environmental health problem and is without doubt the most common manifestation of an immunotoxic response. As a consequence there is a need to identify and characterize skin sensitization hazards and for accurate risk assessment paradigms. The last decade has witnessed very significant advances in our understanding of the cellular and molecular mechanisms that are associated with, and required for, the induction of skin sensitization and the elicitation of allergic contact dermatitis. In parallel there has been a growing appreciation of the characteristics that confer on chemicals the ability to cause allergic sensitization and the nature of apparent inter-individual differences in susceptibility. Such advances have translated into new opportunities for hazard identification, for assessment of relative skin sensitizing potency and for the development of new approaches to risk assessment. This basic continuing education course will describe for a general audience the immunobiology and chemistry of skin sensitization and clinical aspects of allergic contact dermatitis. This will be followed by a description of the methods available for hazard identification and for the determination of potency, approaches to risk assessment and the current global regulatory environment. This course will be of interest to immunotoxicologists, dermatotoxicologists, those involved in the safety assessment of chemicals and regulatory toxicologists. The course is sponsored jointly by the Dermal Toxicity and Immunotoxicology.

10 TOOLS FOR FUNCTIONAL GENOMICS (AM03 REPEATED).

H. I. Swanson[1], E. D. Thompson[1], Y. Tian[2] and K. Kim[1]. [1]*University of Kentucky, Lexington, KY and* [2]*Texas A&M University, College Station, TX.*

The goal of this course is to discuss cutting-edge tools and techniques that may be used in ascribing hierarchical, functional analyses of gene products following DNA microarray experiments. First, we will discuss the advantages and disadvantages of a variety of pharmacological and molecular tools (i.e., antagonists, dominant negative approaches, siRNA). We will also discuss the means by which the molecular tools may be introduced into the cell or animal model, including the use of retro- and adenoviruses. Our second presentation will use data obtained in the laboratory to demonstrate the approaches that are typically used for determining whether the observed changes in mRNA of the gene product of interest occurs at the transcriptional or post-transcriptional levels. The third presentation will focus on use of the chromatin immunoprecipitation (CHIP) assay to demonstrate whether candidate transcription factors are involved in the regulation of the gene product of interest. Finally, our last presentation will introduce a novel approach, chemical genetics, that may be used to either activate or inactivate target gene products in able to discern their functional role(s) either the toxic or disease-related events.

11 COMPUTATIONAL BIOLOGY, DOSE & RESPONSE.

J. W. Fisher[1], M. Craven[2], D. McMillen[3] and M. E. Andersen[4]. [1]*University of Georgia, Athens, GA,* [2]*University of Wisconsin, Madison, WI,* [3]*University of Toronto at Mississauga, Mississauga, ON, Canada and* [4]*CIIT Centers for Health Research, Research Triangle Park, NC.*

The past 40 years witnessed increasing emphasis on development of computational simulation models, including physiologically based pharmacokinetic (PBPK) and, on a more limited scale, physiologically based pharmacodynamic (PBPD) models for biological responses. The fidelity of model parameters with actual biological processes has steadily increased in concert with the explosion of basic biological information. Today computational biology and computational toxicology are undergoing rapid evolution to keep pace with the enormous expansion of our biological knowledge base. A variety of new computational tools and new software are available for computation and the breadth of problems accessible to computational analysis in biology has also increased. The insights derived from computational approaches in biology will influence research strategies to develop biologically based dose-response (BBDR) models in toxicology/pharmacology and undoubtedly form the basis of the next generation of mechanistic approaches for risk and safety assessments. This session consists of 4 talks covering (1) recent progress in PBPK modeling of xenobiotic and endogenous compounds, (2) development of new computational tools to examine cellular signaling networks, (3) modeling approaches for examining relationships between cellular circuitry and cellular function and (4) the possibility that cellular circuits may be regarded as targets for toxic responses. The session is designed to capture the status, current directions and future opportunities of computational biology that are likely to influence toxicological research strategies and risk and safety assessment.

12 FUNCTIONAL FLOW CYTOMETRY: APPLICATIONS IN TOXICOLOGY (AM05 REPEATED).

C. D. Bortner[1], D. Laskin[2], N. I. Kerkvliet[3] and S. W. Burchiel[4]. [1]*NIEHS, Research Triangle Park, NC,* [2]*Rutgers University, Piscataway, NJ,* [3]*Oregon State University, Corvallis, OR and* [4]*University of New Mexico, Albuquerque, NM.*

Flow cytometry provides a powerful tool for analyzing multiple characteristics of individual cells in a complex mixture of cell types without having to physically separate the cells. Yet, even though each cell is examined individually, the flow cytometer can process thousands of cells within a few seconds, allowing superior sampling of the population as compared to microscopic counting. The myriad of phenotypic and functional characteristics of cells that can be measured by flow cytometry continues to expand with the development of novel fluorescent probes to a variety of cellular components. The field of immunotoxicology has been greatly influenced by the use of flow cytometry with applications ranging from screening for toxic effects on immune cells to elucidating the mechanisms of toxic action on specific subpopulations of cells. However, other areas of toxicology are beginning to recognize the value of flow cytometry for mechanistic investigations as well. To address this growing interest, the intent of this course is to introduce the audience to novel applications of flow cytometry that have been used to assess tissue injury and mechanisms of toxicity at the whole animal, cellular, and biochemical levels. Although the context of many of the examples will emanate from immunotoxicology studies, each speaker will focus less on the immunology and more on the methods used in their

studies that are broadly applicable to other areas of toxicology. Examples of methods to be covered include: apoptosis, oxidative stress, membrane integrity and fluidity, cell cycling using carboxyfluorescein (CFSE), and cell signaling.

13 ADRENAL GLAND: MECHANISMS OF TOXICITY AND CARCINOGENESIS.

G. L. Foley[1], C. C. Capen[2], A. S. Tischler[3] and J. D. Obourn[4]. *[1]Pfizer Global Research & Development, Ann Arbor, MI, [2]Ohio State University, Columbus, OH, [3]Tufts New England Medical Center, Boston, MA and [4]Pfizer Global Research & Development, Groton, CT.*

The adrenal gland is a common target organ in safety assessment studies. Many times adrenal changes are attributed to "stress, " because this organ produces glucocorticoid hormones and catecholamines. However, that simplistic interpretation ignores the complexity of this organ, of which a fuller understanding will facilitate the ability of toxicologists to investigate potential alternative mechanisms of action. For instance, the adrenal gland has a cortex with three defined zones (zona glomerulosa which produces mineralcorticoids; zona fasciculate which produces glucocorticoids; and, zona reticularis which produces sex steroids) and a medulla which contains chromaffin cells which synthesize catecholamines (predominantly epinephrine and norepinephrine). The goal of this continuing education course is to illustrate the various physiological roles of the adrenal gland, to provide several examples of toxicity including carcinogenicity, and to illustrate the tools necessary to investigate mechanisms of adrenal toxicity. The first speaker will review the physiology of the adrenal gland, focusing on the hypothalamic-pituitary-adrenal axis that regulates adrenal cortical function and the sympathetic control of adrenal medullary function. In addition, the comparative anatomy of the adrenal gland will be discussed, focusing on the common species used in toxicology studies (mouse, rat, dog, primate). The second and third speakers will build upon the physiology of the adrenal by describing mechanisms for adrenal cortical and medullary toxicity and carcinogenesis. These speakers will highlight mechanisms of toxicity, illustrate methods to assess adrenal toxicity, and discuss human relevance. The last speaker will provide a case study where the mechanism of adrenal cortical tumors induced by a selective estrogen receptor modulator (SERM) was elucidated and how this information was applied in assessing risk to patients.

14 LIFE AS A TOXICOLOGIST - A GRADUATE STUDENT AND POSTDOC PRIMER TO CAREERS IN TOXICOLOGY.

D. Robinson[2] and R. J. Gerson[1]. *[1]Endo Pharmaceuticals, Chadds Ford, PA and [2]Worldwide Safety Sciences, Pfizer Global R&D, New London, CT.*

The proposed course will familiarize graduate students and post-docs with the day-to-day responsibilities, scientific challenges and activities of practicing Toxicologists in various professional fields of employment. The symposium will include presentations by Toxicologists from the Chemical/Agro Chemical, Pharmaceutical, Contract & Consulting arenas as well as Toxicologists from the EPA and FDA. The purpose of these presentations will be to familiarize aspiring Toxicologists with the specific activities and scientific challenges associated with these careers in Toxicology and provide perspective on career choices in Toxicology. Each presentation will include specific case studies of how Toxicology data are used and integrated within the specific career discipline. This course offering is designed to provide insight to Toxicology graduate students and post-docs as they begin to ponder their careers following their graduate/post-graduate education.

15 THE ROLES OF A TOXICOLOGIST IN A PHARMACEUTICAL COMPANY.

M. V. Kindt. *Safety Assessment, Merck & Co., West Point, PA.*

Toxicologists serve in a variety of roles in the preclinical development of pharmaceutical products. They conduct laboratory research in specialty areas such as acute, genetic, developmental and reproductive toxicology, as well as, general/chronic toxicity, safety pharmacology, and toxicokinetics. This research can range in focus from methodology development to drug candidate safety evaluation to mechanism-based research. For example, the toxicologist on general/chronic toxicity studies would likely be involved in study design, data interpretation (eg. body weight, food consumption, physical sign, clinical pathology, and electrocardiogram data), and report preparation. When adverse effects are identified with the drug development candidate, efforts may shift to mechanistic studies designed to determine the effects's importance for clinical development of the compound. Toxicologists may also serve as study directors for nonclinical safety studies, i.e., as the central point of overall control and responsibility for the study. The study director integrates the antemortem, postmortem, and toxicokinetic data from the study. Finally, toxicologists also often represent their non-clinical safety department on interdepartmental teams that oversee the development of drug development candidates from basic chemistry through clinical trials. As the compound or product manager, the toxicologist works closely with the synthetic chemists to address impurity issues, with formulation chemists to approve vehicles for nonclinical and clinical use, and with clinicians to design a nonclinical program that will support the clinical development of the drug candidate. Often the toxicologist as compound/product manager is responsible for preparing regulatory documents and for representing in the company in discussions with regulatory agencies. General examples will be provided for illustration.

16 THE ROLE OF TOXICOLOGY IN THE DEVELOPMENT OF HUMAN THERAPEUTICS - AN FDA PERSPECTIVE.

A. Weir. *FDA/Center for Drug Evaluation and Research/ODE VI, Rockville, MD.*

The Food and Drug Administration (FDA) has five centers regulating food and medical products intended for use in humans and animals. Each center has career opportunities for toxicologists, with the Center for Drug Evaluation and Research (CDER) providing opportunities to those interested in regulating human therapeutics such as small molecular-sized drugs and biotechnology-derived products (e.g. monoclonal antibodies and therapeutic proteins) intended for the treatment of various medical conditions, and more recently as defense against bioterrorism. Before starting clinical trials in humans, pharmaceutical companies must submit an investigational new drug application (IND) to CDER for a scientific team comprised of a toxicologist, a physician, and a chemist to review. A typical IND has data from a variety of toxicology/related studies, a protocol for the clinical trial, and data defining the product's chemistry and manufacturing. The toxicologists evaluate the design of the toxicology/related studies, identify toxicities, recommend safe doses for the clinical trial, and communicate issues to their review team and to their counterparts in pharmaceutical companies. After clinical trials are initiated, the toxicologists review additional studies needed to support the continued safety of clinical trials and, ultimately, the approval of the product after a biological licensing application (BLA) or new drug application (NDA) is filed with CDER. The wide variety of studies submitted with an IND and BLA/NDA, which will be illustrated by a sample data set, gives toxicologists the opportunity to develop specialized skills in areas such as toxicokinetics, reproductive toxicology, and immunotoxicity. CDER gives toxicologists the opportunity to actively participate in the development of therapeutic products, to keep pace with the cutting edge technology behind new therapies and their safety evaluation, and to actively engage in regulatory/scientific decision-making at the national and international level.

17 ENVIRONMENTAL PROTECTION AGENCY: SCIENTIFIC CHALLENGES.

V. Dellarco. *Office of Pesticide Programs, USEPA, Washington DC, DC.*

The USEPA (EPA) is responsible for remediating the damage done to the natural environment, establishing standards to ensure a cleaner environment, and the protection of ecosystems and human health. EPA employs approximately 18, 000 people in the program offices, regional offices, and laboratories. More than half are scientists, engineers or environmental protection specialists from a wide range of disciplines such as biology, chemistry, ecology, epidemiology, hydrology, oceanography, statistics/computer science, and toxicology. Implementation of environmental laws requires many different kinds of skills. Some programs have staff scientists who conduct health risk assessments on pollutants (e.g., Office of Air, ozone; Office of Water, arsenic; Office of Pesticide Programs, carbaryl). Other program office scientists may be involved in the development of standard test protocols or policy and guidance documents, or develop databases and risk tools that are used to identify and analyze human health or environmental effects. Some EPA scientists are responsible for managing major environmental programs such as drinking water disinfection by-products. Some have chosen to go into management and supervision of other scientists. Additionally, there are EPA scientists who conduct fundamental research. For example, scientists in the National Health and Environmental Effects Laboratory (located in Research Triangle Park, NC) are investigating the potential human health effects of exposure to environmental agents. Some of the things that attract scientists to EPA are the challenge of solving complex problems, contributing to environmental decision-making and working to protect the environmental and human health (frequently interacting with scientists from different disciplines inside and outside of EPA), and the constant learning of new science. A case study will be provided to illustrate how EPA uses toxicity and mode of action information to determine the potential human risks associated with exposure to environmental chemicals.

18 LIFE AS A TOXICOLOGIST IN THE CHEMICAL AND AGROCHEMICAL INDUSTRY.

M. S. Bogdanffy. *DuPont Haskell Laboratory, Newark, DE.*

Toxicology is a broad interdisciplinary field made even more diverse by its intent to understand chemical-induced harm in all sectors of society. In the chemical and agrochemical industries, the products are also quite diverse but there are two issues that are constant. First, many of the products and process intermediates are hazardous dictating a high level of concern for product safety. Second is the high degree of regulatory oversight expected by society and imposed on the industry by various governments. Consequently, life as a toxicologist in the chemical and agrochemical industry is very much driven by issues of workplace safety and environmental regulatory approvals necessary for freedom to operate. With the globalization of economies these hurdles become a maze of international regulatory complexities that the toxicologist must navigate. Toxicology testing, research and consulting are all part of the job although for all but the largest of chemical and agrochemical companies, much of the testing and research are farmed out to contract research organizations or universities. Therefore, in addition to good problem solving skills that make use of broad toxicology expertise, project management is an important component of the corporate toxicologist's life. Hands-on study director responsibilities and laboratory research opportunities exist for toxicologists of the larger chemical and agrochemical companies. Toxicologists of agrochemical companies frequently migrate to international product registration positions. The diversity of products and issues that a toxicologist in the chemical and agrochemical industry must attend to leave ample opportunity for very interesting and often long-term involvement in programs of product stewardship. This presentation will illustrate many of the day-to-day and year-to-year challenges facing a toxicologist in this industry by following the story of a high production volume carcinogenic chemical, the development of research to understand its mode of action and how this information impacts regulatory decision-making.

19 ON THE SERVICES SIDE: LIFE AS CRO SCIENTIST AND CONSULTANT.

D. J. Kornbrust. *Consultant, Reno, NV.*

The contract research organization (CRO) industry presents a wide range of career opportunities. Many of these institutions cater to the needs of the pharmaceutical industry, in terms of providing support for drug development programs, although some are more oriented towards assisting chemical companies. Services provided can range across the entire spectrum of nonclnical activities that comprise a therapeutic development program, from manufacturing operations to formulations to the performance of pharmacology, toxicology and pharmacokinetic studies that enable clinical testing of prospective pharmaceuticals. In general, the scientists within CROs are commonly referred to as "study directors" (SD). The role of the SD is to develop protocols and oversee the implementation, conduct and reporting of nonclinical studies that are "placed" with the CRO by the "Sponsor". This experience can differ substantially from that of a similar scientist role within a pharma or chemical company mainly with respect to the diversity of projects. The tradeoff is that opportunities to become deeply immersed in a project and obtain continuity of experience with individual programs are limited. Another distinguishing aspect of this line of work is that, as the SD develops skills and experience, he/she may become increasingly drawn into business development activities, possibly to an extent that dictates diminishing involvement with the basic science. Consequently, good business acumen and communication skills are highly valued in this industry. A background in the pharmaceutical industry combined with CRO experience can be an excellent springboard to move to independent consulting. Of course, one must develop a wide array of skills and be sufficiently "seasoned" to be successful in this endeavor, and there is considerable competition. To illustrate how projects can evolve from a basic service contract to a consulting role, an example of a program involving preclinical development of a biologic agent (human clotting factor) will be discussed.

20 TAKING COMMAND OF YOUR CAREER.

L. M. Kamendulis[1] and W. A. Toscano[2]. *[1]Division of Toxicology, Indiana University School of Medicine, Indianapolis, IN and [2]Division of Environmental and Occupational Health, University of Minnesota School of Public Health, Minneapolis, MN.*

An important mission of the Placement committee is to expand the traditional service role to that of a resource for career development issues for all career stages. This career development workshop, targeted at entry- to mid-career SOT members, addresses issues related to setting and achieving career goals in a dynamic and technical field. Many mechanisms exist for career advancement, however, the tools and skills needed to advance are not always realized. The technical tools and prowess to keep on the cutting edge may often times get lost as increased managerial demands and other responsibilities are placed on individuals. The presentations in this workshop will focus on: How to take charge of your career to assure success, how to challenge oneself intellectually and scientifically in a subject matter for which one has both expertise and interest, how to garner and maintain the tools and skills necessary skills to make career advancements, and anticipating future trends in Toxicology. An interactive panel discussion, directed by questions from the audience will follow the presentations.

21 KEEPING SKILLS UP TO DATE.

J. E. Manautou. *School of Pharmacy, University of Connecticut, Storrs, CT.*

Staying competitive early in your professional career is essential in order to maximize your career growth opportunities. Since many avenues exist to maintain this competitive edge, it is key to develop a strategy to take advantage of all available resources while maintaining your current work responsibilities. Several widely available resources exist to develop your strategy and include attending continuing education classes, developing a scientific network with external colleagues, gathering information from recruiters seeking to place individuals with your expertise, and establishing career mentors. While solidifying your early and mid-career goals, it is important to expand your scientific and technological expertise as well. Staying current with the scientific literature is fundamental to keeping abreast of scientific knowledge and emerging technologies, however, other approaches such as engaging in scientific collaborations and sabbaticals provide excellent avenues to expand your scientific skills.

22 SEEKING MID AND LONG TERM CAREER GOALS: PERSPECTIVES OF AN INDUSTRY TOXICOLOGIST.

J. Bus. *TERC, Dow Chemical Co., Midland, MI.*

One of the most exciting, but also most challenging aspects of a career in toxicology is that it potentially can touch all disciplines of biology. In an industry environment, toxicologists often are directed into two broad career options. These are: 1) being an expertise leader in one of the primary subdisciplines of toxicology, e.g., neurotoxicity, developmental toxicity, etc.; and 2) providing generalized toxicology expertise in support of business units, i.e., using your expert knowledge to guide business decisions on safety of products or processes. Several considerations can greatly facilitate choices that ultimately lead to satisfying and rewarding careers. Successful careers are always accompanied by technical excellence; lifelong learning and curiosity is essential and expected. Attention to development of communication and interpersonal skills, however, are equally important to expanding the breadth of available career options. Since the science of toxicology is very multidisciplinary, taking full advantage of professional networks is also essential to defining future career options. Seeking early opportunities to build relationships with trusted mentor(s) are critical. Mentors may be found through activities pursued within professional societies, within trade associations, or colleagues within your institution. Finally, toxicologists seeking careers within industry must develop a passion for applying the highest quality science to protecting the health of workers, customers and the public.

23 LEADERSHIP - STEERING YOUR CAREER TO ACHIEVE SOCIETAL AND PERSONAL GOALS AND THE WONDERFUL THINGS YOU LEARN ABOUT YOURSELF ALONG THE WAY.

V. P. Wilson. *Brown University, Providence, RI.* Sponsor: W. Toscano.

Inspiring others to follow your vision to a better future is leadership. Some people are born with the qualities of leadership, but many others must learn to cultivate their abilities to become leaders. The rapidly changing scientific environment of toxicology provides novel challenges to emerging leaders. An effective leader must have a vision of the scientific and societal implications of modern toxicological sciences. This presentation will focus on how to develop and demonstrate effective leadership skills. Topics will include qualities of leadership in academic, government and industrial positions; how to motivate colleagues; and the use of leadership skills in career advancement.

24 MAINTAINING TECHNICAL SKILLS WHILE RISING THROUGH MANAGEMENT.

L. D. Lehman-McKeeman. *Discovery Toxicology, Bristol-Myers Squibb, Princeton, NJ.*

As practicing toxicologists gain knowledge and experience, evolving career opportunities increasingly make demands on time or take scientists completely away from direct involvement in research or technical issues. As such, it is difficult to sustain a technical focus while assuming greater management or other leadership responsibilities within an organization. In order to find a balance between these often-competing priorities, it is first essential to recognize that no one person can do everything, and decisions on what is personally and professionally fulfilling and rewarding must be a deliberate choice. This is also a decision that all scientists need to re-examine and re-evaluate periodically. When a scientist decides that maintaining a technical focus is important, it is imperative to focus on sustaining a learning environment. In particular, this means that it is necessary to challenge oneself to continue to learn and to expand expertise into new technical areas. Whereas this is personally beneficial, it also helps to foster a learning environment within the larger organization. It is also essential to hire good researchers to work with you and to help and courage them to learn, grow and assume more responsibility as well. Coaching and mentoring good scientists provides them with the opportunity to expand their skills and is usually a long-term investment in the overall capacity of the laboratory or organization. Finally, it is important to learn from others, by asking questions, engaging in dialogue and discussion and being challenged to think about issues from different points of view. In the end, personal and organizational development is enhanced and job satisfaction is high.

25 TRANSDISCIPLINARY RESEARCH: RIDE THE WAVE.

J. Barrett, J. S. Wiest and L. Bennett. *Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD.*

The exponential rate at which discoveries are being made has resulted in science becoming more and more complex. In order to meet the challenges presented by this complexity, the new generation of independent investigators needs to work collaboratively; the time of the independent investigator working in isolation is vanishing. Today scientists will benefit from developing a solid understanding of the translational research process; moving discoveries made in the laboratory through a developmental process and into the clinical setting. No individual can be an expert in all areas of science, but by employing team science and exploiting scientific advances an individual can contribute to major scientific discoveries. Current training strategies should include plans for participating in transdisciplinary science. Having this expertise will provide the insights necessary to assemble a multidisciplinary team to tackle a problem and will empower the individual to establish himself or herself as a leader on a project and to assume the responsibility to make sure progress is made. Similarly, the necessity of contributing to a project for which one may not have a lead role will be appreciated. These strategies will be critical for recognizing contributions toward project success and for receiving the rewards that enable the science to move forward.

26 STEROID INACTIVATION: ALTERNATIVE MECHANISMS OF ENDOCRINE TOXICITY.

L. You. *CIIT Centers for Health Research, Research Triangle Park, NC.*

Normal reproductive development depends on the action of steroid hormones at specific tissue sites. Agents interfering with this process can elicit malformation or malfunction in the reproductive tract or other organs that rely on steroids to maintain normal physiology. While effects mediated by the steroid receptors have thus far been most extensively studied as targets for xenobiotic endocrine modulation, the consequences of increased or decreased inactivation of hormone ligands in relation to overall endocrine functions are less known. Many of the enzymes that control steroid biotransformation are responsive to xenobiotic induction. These enzymes include steroid hydroxylases that are members of the cytochrome P450 (CYP) family and the conjugation enzymes of sulfotransferases (ST) and uridine diphosphate-glucuronosyltransferases (UGT). Cloning and characterization of the nuclear receptor CAR and PXR in recent years have greatly improved the understanding of how some key members in the CYP enzyme family are transcriptionally regulated. Exposure to CAR and PXR receptor activators leads to up-regulation of CYP2B and 3A, which utilize steroid hormones as substrates. Similarly, xenobiotics can cause changes in the transcriptional control of ST, leading to alteration of its regulation on the bioavailability of free steroids. While these enzymatic changes may account for enhanced steroid metabolism, the physiological and toxicological consequences of such enzyme effects will require more assessment.

27 XENOBIOTIC INTERFERENCE WITH HORMONE TRANSPORT PROCESSES.

G. A. LeBlanc. *Environmental & Molecular Toxicology, North Carolina State University, Raleigh, NC.*

The clearance of steroid and other hormones from critical target organs is regulated by enzymes that modify the mobility of the hormone. These enzymatic biotransformations also can target the hormone to active transporters that facilitate elimination of the hormone. Many of these enzymes and transporters also are involved in xenobiotic processing and are induced by chemical exposure through the regulatory action of xenobiotic sensors such as CAR and PXR. Such xenobiotic-mediated increases in these activities can result in enhanced elimination of some homones. For example, thyroid hormone (T4) is cleared from the body through its conjugation to glucuronic acid. This process is mediated by the glucuronosyl transferase enzyme UGT1 and is induced in response to CAR and PXR activators. Exposure of rats to some PCBs causes a significant increase in T4 glucuronidation and a commensurate decrease in serum T4 levels. Such effects of PCBs have been associated with cognitive deficits in exposed human populations. Xenobiotics also may suppress steroid modulating proteins resulting in increased levels of hormone. For example, acyl CoA-testosterone acyltransferase is responsible for conjugating fatty acids to testosterone. These conjugates have a prolonged half-life and are sequestered within lipid stores in the body for subsequent use. This enzyme is sensitive to inhibition by the biocide tributyltin. In the mud snail, Ilyanassa obsoleta, the inhibition of testosterone-fatty conjugation by tributyltin results in elevated testosterone levels. The increased testosterone is causally associated with an intersex condition whereby females express male sex characteristics. This endocrine toxicity has been demonstrated in over 150 snail species worldwide and is causally associated with environmental levels of tributyltin. Understanding the coordinated regulation of steroid biotransformation and transport proteins and their susceptibility to modulation by xenobotics may facilitate identification of modes of endocrine toxicity of many xenobiotics.

28 THE NUCLEAR RECEPTOR CAR IN REGULATION OF ESTROGEN METABOLISM.

M. Negishi. *LRDT, NIEHS, Research Triangle Park, NC.* Sponsor: L. You.

CAR and other xenosensors, such as PXR, may form a regulatory network that governs endocrine homeostasis by altering the levels of steroid-metabolizing enzymes such as CYP2B, CYP3A, SULT and UGT1A1. In response to steroid hormones, in addition to xenobiotics, CAR activates genes that encode those enzymes. Estradiol and estrone at pharmacological doses activated both mouse and rat CAR but not human CAR in cell-based transfection assays, while non-estrogenic metabolites estriol, estetrol and the synthetic diethylstilbestrol could not activate CAR. On the other hand, testosterone repressed CAR activity. Estrogen also elicits nuclear accumulation of CAR in mouse liver and primary hepatocytes. As estrogens and androgens have opposing actions on CAR, their endogenous role in CAR regulation was examined by ovariectomization and castration of female and male mice, respectively. Ovariectomy did not alter the level of CAR in the nucleus indicating that endogenous estrogen does not regulate CAR, whereas castration increased both basal and E2-induced CAR levels suggesting that endogenous androgen may repress CAR in male mice. These results suggested that CAR may play a role in regulating the levels of active estrogens.

29 MOLECULAR REGULATION OF HEPATIC SULFOTRANSFERASES.

M. Runge-Morris. *Inst. Environment Health Sciences., Wayne State University, Detroit, MI.*

Sulfonated metabolites of thyroid hormone and estradiol are receptor inactive. Therefore, hormone metabolism by sulfotransferase enzymes represents a critical check-point for the control of bio-active intra-tissue hormone levels. Several of the cytosolic sulfotransferases function as key components of intra-hepatic steroid metabolism. For example, hepatic aryl sulfotransferase (SULT1A1) is responsible for sulfonating xenobiotic phenols, and thyroid hormone, as well as a variety of therapeutic agents such as acetaminophen, minoxidil, and estradiol at pharmaceutical concentrations. Hydroxysteroid sulfotransferase (SULT2A) catalyzes the sulfonation of xenobiotics, bile acids and the most abundant adrenal androgen in humans, dehdyroepiandrosterone (DHEA). The consequences of DHEA sulfonation are multifaceted. In mice, DHEA-3-beta-sulfate was reported to induce peroxisome proliferator gene expression, an effect that was ablated in PPAR-alpha knock-out mice. More recently, DHEA and DHEA-sulfate were shown to activate the expression of target genes through the constitutive androstane receptor transcription factor. Because of their important roles in xenobiotic detoxication and in the regulation of intra-tissue hormone homeostasis, it is critical to achieve a clearer

understanding of the transcription factor machinery that regulates sulfotransferase gene expression. Our laboratory has demonstrated that glucocorticoid-inducible rat hepatic SULT2A gene transcription occurs as a result of glucocorticoid receptor, pregnane-X-receptor, and liver-enriched CCAAT binding enhancer protein-mediated mechanisms. We have also implicated a role for steroid-sensitive nuclear receptors in the transcriptional control of SULT2A gene expression in primary cultured human hepatocytes. In parallel studies, we demonstrated that glucocorticoid-inducible hepatic SULT1A1 gene transcription occurs as a result of a classical glucocorticoid receptor-mediated mechanism. These investigations provide a foundation for future work on the integrated role of nuclear receptors and liver-enriched transcription factors in the molecular regulation of hepatic sulfotransferases.

30 NUCLEAR RECEPTOR PXR- AND CAR-MEDIATED INDUCTION OF STEROID BIOTRANSFORMATION ENZYMES AND RELATIONSHIP WITH REPRODUCTIVE DEVELOPMENT.

L. You, H. B. Hoffman, A. R. Laughter, E. J. Bartolucci-Page, S. Kirwan and M. E. Wyde. *CIIT Centers for Health Research, Research Triangle Park, NC.*

The actions of steroid hormones are maintained in part through a dynamic regulation of ligand bioavailability. Steroid hormone inactivation occurs at the liver as well as at target tissues. In the liver, steroid hydroxylation is mainly mediated by cytochrome P450 (CYP) family enzymes and conjugation by the sulfotransferases (ST) and uridine diphosphate-glucuronosyltransferases (UGT). These enzymes have wide spectra of endogenous and exogenous substrates including steroid hormones. Hepatic enzyme inducers can enhance the inactivation of pharmacological steroids, leading to diminished bioavailability and therapeutic efficacy. Whether such enzyme inducers can affect steroid-dependent physiology though altering the availability of endogenous steroid hormones is less clear. We conducted studies to evaluate the potential of xenobiotics to impact reproductive development through induction of liver enzymes. Several compounds, including DDE, phenobarbital (PB), and pregnenolone 16a-carbonitrile (PCN) were demonstrated to induce multiple hepatic biotransformation enzymes, partly due to their ability to activate the nuclear receptor constitutive androstane receptor (CAR) and pregane X receptor (PXR). These nuclear receptors are transcriptional regulators for several hepatic enzymes catalyzing steroid hydroxylation and conjugation. We observed altered biotransformation of injected testosterone in castrated rats treated with these enzyme-inducing compounds. However, administration of PB, PCN, and other liver enzyme inducers during the pubertal stage did not result in discernible interference with pubertal sexual development in male rats. Our data suggest that, while induction of hepatic enzymes would result in altered biotransformation for steroid hormones, regulatory mechanisms for steroid homeostasis in intact animals may be able to adapt to such enzyme induction. (Supported by the Long-Range Research Initiative of the American Chemistry Council.)

31 ASSURANCE OF ANIMAL WELFARE IN RESEARCH: COEXISTENCE OF TOXICOLOGY STUDIES WITH HUMANE ENDPOINTS.

S. M. Lasley[1] and J. I. Everitt[2]. *[1]Department of Biomedical & Therapeutic Sciences, University of Illinois College of Medicine, Peoria, IL and [2]Comparative Medicine & Investigator Support, GlaxoSmithKline, Research Triangle Park, NC.*

In the performance of toxicology studies, whether for purposes of product safety testing or identifying mechanisms of toxicant action, it is necessary to incorporate multiple regulatory, scientific, humane, and ethical factors into the use and care of laboratory animals. This Workshop will provide a forum for discussion of these various factors from different vantage points to better inform the audience, particularly with respect to utilization of humane endpoints. These issues are of timely importance because of continually increasing regulatory oversight of animal care and use, and thus this forum will be of broad interest to toxicologists. Consideration of these factors will be addressed from the standpoint of regulatory requirements and the types of data that must be submitted (Schechtman). A veterinary medicine perspective will be presented, highlighting the development of humane endpoints and their use to determine when study interventions are necessary (Stokes). The role of the IACUC will be defined, particularly in the refinement of the project experimental design and optimization of the proposed numbers of animals (Brown). The conduct of toxicology studies will also be presented from the viewpoint of the investigator, who must balance these factors to produce sound and reliable data (Mattsson). The final presentation will provide a European Union perspective, highlighting the manner in which approaches to these animal care issues are addressed differently in those countries, and indicating trends in regulatory oversight that may soon reach North America (Donovan).

32 ASSURANCE OF ANIMAL WELFARE IN RESEARCH: COEXISTENCE OF TOXICOLOGY STUDIES AND HUMANE ENDPOINTS - ISSUES IN TOXICITY TESTING FOR REGULATORY PURPOSES.

L. M. Schechtman. *NCTR/WO/HFT-10, US Food and Drug Administration, Rockville, MD.*

Historically, toxicology studies for safety/hazard assessment have been primarily animal-based but with little regard for animal welfare. The emergence of alternative toxicological methods that employ fewer animals, refine procedures (cause less pain and distress), and rely on non-animal endpoints, is altering that paradigm. From a regulatory perspective, validation of an alternative method (determination of its reliability and accuracy for a specific purpose) lends itself to acceptance and implementation of methods for regulatory purposes. To address such validation issues, 15 US federal research and regulatory agencies have assembled to form the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), supported by its operational/scientific support center, the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). Different federal agencies and different regulatory units within a given agency often accept/solicit data from different test methods or batteries of tests, often dictated by the products within their purview and the regulatory mandates to which they respond. Data requirements for safety/hazard evaluations differ from one regulatory agency to another and those data may be generated using validated, unvalidated, traditional, and/or non-traditional test methods. Although regulatory agencies do have the option of considering data derived from non-conventional tests, often such data are used in a supplementary manner (e.g. to provide mechanistic information) to support the regulatory decision-making process. Some agencies (e.g. FDA) and its respective regulatory entities, provide direction to sponsors in the form of test guidances that help determine acceptable testing strategies/procedures for evaluating a regulated product. A number of these guidances have either directly or indirectly benefited animal welfare and have incorporated validated, animal-friendly methods that supplement or replace some of the routinely employed classical toxicological tests.

33 ASSURANCE OF ANIMAL WELFARE IN RESEARCH: COEXISTENCE OF TOXICOLOGY STUDIES WITH HUMANE ENDPOINTS - VETERINARY MEDICINE AND ANIMAL WELFARE ISSUES.

W. S. Stokes. *DHHS/NIH/NIEHS, National Toxicology Program, Research Triangle Park, NC.*

Toxicology studies using laboratory animals are conducted to determine the safety or potential adverse health effects of new chemicals and products. Local or systemic toxicity to animals in these studies may result in injury or disease involving significant pain and distress. Standard veterinary care would normally involve treatment with drugs to minimize or alle*via*te pain and distress, or removal of the involved animal(s) from the study. However, such veterinary care interventions can become problematic if they interfere with attainment of the study objectives. Decisions on appropriate interventions can be assisted by anticipating possible toxic effects and establishing, prior to the initiation of a study, appropriate criteria that can serve as the basis for ending a procedure when pain and distress become apparent. Such criteria are considered humane endpoints if they reduce the severity and/or duration of pain and distress experienced by an animal. Humane endpoints can also be established by systematic identification of early clinical signs or other physiological parameters that are predictive of specific toxicity outcomes of interest. Mechanism-based biomarkers of toxicity that serve as earlier more sensitive indicators of toxicity that can also be used to end experiments before the onset of significant morbidity or mortality. These biomarkers and endpoints should undergo appropriate validation to determine that they provide equivalent or better prediction than previously used endpoints. International guidance for the use of humane endpoints in toxicology studies has been established and should be considered when designing studies. The incorporation of humane endpoints in toxicology studies can be expected to facilitate decisions on appropriate interventions by veterinary care staff and study directors and provide for improved animal welfare.

34 THE IACUC AS A VALUE-ADDED COMPONENT OF TOXICOLOGY RESEARCH.

M. J. Brown. *Animal Welfare and Training, Charles River Laboratories, Wilmington, MA.* Sponsor: J. Everitt.

Toxicologists involved in animal research and testing must answer to many regulatory masters: FDA, USEPA, OECD, ICH, CCAC, USDA and AAALAC. The beneficiary of this research and testing, the public, has ever increasing demands for quality health care and a safe environment but is also highly sensitive about the use

of research animals. Added to these demands of the public and the regulatory community are the interests of study sponsors and those of contract research organizations. The Institutional Animal Care and Use Committee or Ethics Committee (IACUC/EC) and Study Director (SD) must work within these various demands and constraints to assure humane care of research animals and high quality science. Various regulatory agencies outline the roles and responsibilities of both the IACUC/EC, and the SD. These roles are often contradictory so it is not surprising that conflicts arise. This presentation will briefly discuss the roles and responsibilities of the IACUC/EC and SD and present strategies to minimize conflict and enhance both animal welfare and the quality of the science. Emphasis will be placed on protocol review activities that increase collaboration and communication. Mechanisms will be suggested to streamline the process, making it both more efficient and less time consuming while maintaining the quality of the review. Using examples of potential issues (such as the ordering of animals prior to IACUC approval, determining criteria for veterinary intervention, establishing humane endpoints, etc.), suggested mechanisms will be discussed.

35 ANIMAL TESTING: THE DICHOTOMY BETWEEN NATURAL TOXICANTS IN FOOD AND SYNTHETIC PESTICIDES POINTS TO A PROBLEM.

J. L. Mattsson. *Dow AgroSciences LLC, Indianapolis, IN.*

The most highly regulated area of toxicology is the registration of pesticides, which will be the focus of this presentation. At this time, a theoretical minimum of some 7000 mammals and 3000 birds are required to register one pesticide for food use. The actual numbers of animals used per pesticide are actually much higher and are likely to increase still further unless there is a radical change in regulatory policy. In practice, pesticide residues in food are below the level of detection about 40% of the time, and other residue levels are typically very low (USDA Pesticide Data Program, 2000). On the other hand, natural toxicants such as glycoalkaloids and furocoumarins are present in food at much higher levels, and often, are more potent than synthetic chemicals found in food (Beier and Nigg. Ch. 2, Foodborne Disease Handbook, Vol. 3, Marcel Dekker, 2001; Carcinogens and Anticarcinogens in the Human Diet. National Research Council, 1996). In spite of these stark differences in dietary exposure, there is no systematic approach to safety evaluation of natural toxicants in food. This dramatic dichotomy in the regulation and testing of natural and synthetic toxicants in food cannot be justified scientifically. Under current paradigms, there is either an unreasonable excess in animal-based testing of synthetic chemicals, or a dramatic under testing of natural chemicals in foods. If animal testing of synthetic chemicals is excessive, then there exists a clear ethical problem on the use of animals. If animal testing of natural chemicals in foods is deficient, then there exists a clear ethical problem on the protection of public health. My opinion is in the middle ground, that there is both an excessive animal-based testing of food-use synthetic pesticides, and a deficient testing of natural toxicants of food. In the interests of food safety and ethics of animal experimentation, a more animal-efficient testing strategy is needed to allow proper resource allocation to all chemicals in food.

36 EUROPEAN PERSPECTIVES ON ANIMAL WELFARE AND SCIENTIFIC ENDPOINTS IN ANIMAL STUDIES.

J. C. Donovan. *BioResources, Wyeth Research, Collegeville, PA.* Sponsor: J. Everitt.

The growing internationalization of science has brought greater focus on differing approaches to animal welfare in biomedical research. Cultural attitudes and the evolution of animal welfare standards in various countries have resulted in practices which vary significantly. Not only do animal care, housing, and animal health standards influence research results, but there are other areas that have a more direct scientific impact. For example, decisions on study design, dosing/sampling volumes, environmental enrichment and scientific endpoints have important effects on the outcomes of studies using animals. Clearly, the comparability of results among academic, industrial and governmental institutions is challenged when differing practices governing animal research are applied. Animal welfare regulations and guidelines continue to evolve in Europe and the United States. Understanding the trends in regions having the most influence on scientific development can help steer the community towards common practices which are both scientifically sound and sensitive to animal welfare. European perspectives are already having a significant impact in the US in areas such as genetically-modified organisms, agricultural practices and animal welfare. A general lack of acceptance of genetically-modified food and the use of hormone growth promoters in the animal industry is effecting trade and politics with the US A growing number of Americans now feel concern is warranted. In the realm of research animal welfare it is generally accepted that, due largely to public demands for more protections for animals, European approaches have been more "progressive". Many of these attitudes are migrating to the US in the form of animal rights activism, changing perspectives in school-aged children towards animals and escalating animal welfare regulations. This presentation will look at animal welfare trends, guidelines and regulations in Europe, including scientific endpoints in animal studies and dosing/sampling guidelines, and how they may influence research practices in the US

37 THE IMPORTANCE OF ELECTROPHYSIOLOGY IN NEUROTOXICOLOGY EVALUATION.

C. G. Markgraf and A. Bass. *Safety Pharmacology, Schering-Plough, Lafayette, NJ.*

Neurons differ in their vulnerabilities to toxic agents, thus techniques that can selectively evaluate functional neuronal systems fill an important role in neurotoxicology. Electrophysiologic recording has long played such a role. Neuroelectrophysiology techniques add important information to studies that have pathology and/or behavior as outcome measures. Electrophysiologic recordings are able to detect toxic effects on the nervous system that may occur without morphologic correlates. In addition, techniques can be applied specifically to a functional neuronal system, allowing study of just that system. Data are quantifiable, reproducible and can be obtained repeatedly from an individual animal. The same techniques can be used in clinical studies, forming a stable bridge in determination of human risk. Furthermore, the extensive history of electrophysiology creates a strong database which can be used to provide perspective for data on new drugs. While these similarities are shared among the various techniques, there are important differences in evaluation of neuronal systems that can influence the interpretation of data, and there are different sensitivities to detection of abnormal results. The speakers in this workshop will discuss the strengths of electrophysiologic recording and factors critical to data interpretation in various functional systems, including the peripheral nervous system, sensory systems, hippocampal function and cortical dysfunction. This integrated examination of neuronal system functions using electrophysiology will provide insight into these data in the evaluation of new chemical entities, and their place in human risk assessment.

38 NEUROPHYSIOLOGICAL EVALUATION OF SENSORY SYSTEM FUNCTION.

D. W. Herr. *Neurotoxicology, USEPA, ORD/NHEERL, Research Triangle Park, NC.*

Exposure to many neurotoxic compounds has been shown to produce a sensory system dysfunction. Neurophysiological assessment of sensory function in humans and animal models often uses techniques known as sensory evoked potentials. Because both humans and animals show analogous responses to sensory stimuli, extrapolation of effects from the laboratory to a clinical situation is simplified. Additionally, the methods are amendable to repeated testing, allowing the time-course of changes in sensory system function to be monitored. Furthermore, examination of multiple sensory modalities allows the determination of the specificity of a sensory dysfunction. A change in a sensory evoked potential can often be associated with pathological damage. Auditory system function at the level of the periphery and the brainstem can be assessed using Brainstem Auditory Evoked Responses (BAERs). Decreased amplitudes of the early BAER peaks have been associated with a frequency-dependent loss of cochlear hair cells. In contrast, decreased amplitudes of later BAER peaks have been associated with brainstem lesions. Evoked potentials can also be used to investigate changes in the visual system from the level of the retina to the cortex. Alterations in the early portions of cortical visual evoked potentials have been associated with pathological changes in the retina, thalamus, or cells involved in the early cortical processing of the stimulus. Besides quantifying changes in sensory function produced by neuroanatomical lesions, knowledge of the neuropharmacology of evoked potentials can be used to interpret changes after exposure to substances known to alter specific neurotransmitter systems. This allows the use of evoked potential technology to assess not only undesirable effects, but also to monitor for intended pharmacological actions. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

39 NEUROELECTROPHYSIOLOGICAL ENDPOINTS IN SAFETY STUDIES AND SAFETY PROGRAMS.

J. F. Ross. *Ross Toxicology Services, LLC, Cincinnati, OH.*

A subset of neuroelectrophysiological endpoints includes relatively noninvasive tests (e.g., peripheral nerve conduction velocity, auditory evoked potentials) that enjoy widespread use in both human and veterinary neurology practice. These tests are becoming popular additions to neurotoxicology safety programs, partly because they can provide detailed information about neurological disease without requiring the sacrifice of the subjects. Since neuroelectrophysiological tests are most often used in 2nd- or 3rd-tier safety studies, a primary set of data is generally available to help choose among the wide variety of available tests. The most important parts of

this data set include the neurological examination (e.g., observational battery) and neuropathology. The ultimate utility of the neuroelectrophysiological endpoints depends on a good fit with the neurological and neurohistopathological results.

40 USE OF ELECTROENCEPHALOGRAPHY (EEG) IN DETECTING NEUROTOXIC EFFECTS.

J. C. Arezzo and M. S. Litwak. *Neuroscience, Albert Einstein College of Medicine, Bronx, NY.* Sponsor: A. Bass.

EEG is arguably the oldest direct physiologic measure of CNS function. When recorded from the intact scalp, it provides a non-invasive index of the overlapping activity pattern of large ensembles of neurons. Thus it can provide a highly sensitive, but non-specific measure of neurotoxic insult. Standard measures of EEG activity, such as power in specific frequency bands (eg., alpha, theta), voltage suppression or the presence of paroxysmal activity, are applicable to both human and experimental animal studies. EEG is sensitive to a wide range of neurotoxic effects including: hyper- or hypo-excitability, inflammation, anoxia, cortical neural degeneration, alterations of sleep-wake patterns, metabolic disorders and toxic encephalopathies. The identification of compound-induced epileptiform activity has always been a principal strength of EEG procedures. Studies have documented dose thresholds for seizure activity, the time course and duration of seizures, as well as frequency changes and subtle paroxysmal patterns that antecede and may predict seizure onset. Recent advances in computer modeling and in the analysis of nonlinear dynamic systems have greatly expanded the sensitivity of EEG to other deficits; while computer-aided spatiotemporal analysis allows exploration of neurotoxic effects distributed over both cortical and sub-cortical regions. An emerging strength of EEG is its ability to explore the effects of putative neurotoxins on inherent rhythms of the brain, such as the cortical beta/gamma waves (33-45 Hz). Finally, intracranial studies using two-dimensional spatial grids (eg., 12x12) or linear multicontact arrays, allow exploration of neurotoxic effects isolated within specific cortical laminae, or effects on the synchrony of EEG activity measured simultaneously across distinct cortical areas.

41 ASSESSING HIPPOCAMPAL CHANGES INDICATIVE OF NEUROTOXIC EFFECTS.

M. E. Gilbert. *Neurotoxicology, USEPA, Research Triangle Park, NC.*

Subtle changes in cognitive function are often the earliest indication of neurotoxic effects in humans. The hippocampus is a large forebrain structure subserving specific kinds of information encoding and consolidation in humans and other animals. Because of its laminar structure, it is ideally suited for neurophysiological assessment and it is readily accessible for both *in vitro* and *in vivo* examination. The homogeneity of the neural populations within each cell body layer results in large field potential recordings that faithfully reflect intracellular current flow. As such, these field potentials can be directly translated to activity at the cellular level. Hippocampal field potentials also display a high degree of synaptic plasticity and a well accepted synaptic model of memory, long-term potentiation, has been characterized in this structure. However, toxicological assessments of hippocampal function using this approach are relatively sparse, and *in vitro* assessments using slices or cultures are more prevalent than *in vivo* investigations. The bulk of neurotoxicological investigations *in vivo* have examined the long-term consequences of developmental exposure, although acute effects of chemical exposures have also been reported for pyrethroid, formamadine, and organochlorine pesticides. Developmental insults include pre and/or postnatal exposure to ethanol, carbon monoxide, lead, antimitotic agents, and thyroid disrupting compounds. The majority of these studies have demonstrated altered hippocampal synaptic function that in all likelihood represents permanent alterations in cell communication following early developmental insult. These perturbations are often present in the absence of overt pathological changes in structure and in the absence of behavioral impairments based on standard learning and memory assessments. Abstract does not necessarily reflect USEPA policy.

42 LOW-DOSE EXTRAPOLATION: TIME FOR A FRESH LOOK AT AN OLD PROBLEM, OVERVIEW.

J. Bus[1] and R. Henderson[2]. *[1]TERC, Dow Chemical Co., Midland, MI and [2]Inhalation Toxicology Research Institute, Albuquerque, NM.*

The basic tenet of toxicology is the dose makes the poison. Thus, characterization of the dose-response remains a foundational element in the translation of animal toxicity information to estimation of potential human health hazard and risk. However, due the significant impact of regulatory and product stewardship interventions over the last 30 years, animal toxicity observed over the range of conventionally-determined dose-response evaluations is becoming increasingly disparate from real-world exposures to many environmental chemicals. Given these growing disparities, toxicologists are increasingly challenged to provide rational methods and mechanisms to understand true adverse human health outcomes associated with these low-level chemical exposures. In order for these approaches to be scientifically credible, toxicologists must direct attention to how such mechanisms can differentiate health effects associated with low-dose environmental exposures to synthetic chemicals from those that might be due to the many thousands of toxicologically similar, but likely health beneficial natural compounds present in everyday diets. Future low-dose extrapolation paradigms failing to address this important issue will result in scientifically indefensible decisions regarding strategies designed to protect public health from adverse consequences of low-dose chemical exposures, be they synthetic or natural.

43 HISTORICAL DEVIATIONS FORM THE LNT MODEL: HARMONIZATION OF CANCER AND NONCANCER ENDPOINTS.

M. L. Dourson. *Toxicology Excellence for Risk Assessment (TERA), Cincinnati, OH.*

Cancer and noncancer dose response assessment has historically been done differently due in part to differences in underlying assumptions, for example, whether the low dose response is due to a stochastic process or not. Recent understanding of underlying modes of action of cancer and noncancer endpoints, however, has created the basis for replacing such assumptions with data. Efforts to harmonize cancer and noncancer dose response assessment by SOT, USEPA and others have lead to new approaches to low dose understanding and response. Examples are given for perchlorate and chloroform, where the integration of cancer and noncancer toxicity has resulted in harmonized dose response assessment in the low dose range.

44 USE OF MECHANISTIC DATA TO HELP DEFINE DOSE-RESPONSE CURVES.

J. Preston. *Environmental Carcinogenesis Division, University.S.Environmental Protection Agency, Research Triangle Park, NC.* Sponsor: R. Henderson.

The cancer risk assessment process described by the USEPA necessitates a description of the dose-response curve for tumors in humans at low (environmental) exposures. This description can either be a default linear one when appropriate data are lacking, or a linear or nonlinear one when specific informative data are available. The challenge is to define the nature and extent of these specific informative data. Ideally, to use nontumor data for predicting tumor outcomes, the most informative endpoint is one that most accurately predicts the tumor outcome, namely an outcome that is part of the tumor response itself or is proximate to this response and along the tumor pathway. The current rapid development of whole genome response methods, such as RNA or protein array approaches, promises to aid substantially in the selection of informative biomarkers of response. Until progress has been made, it remains a common practice for genotoxic carcinogens to use overall genetic alterations (e.g. mutations and chromosome aberrations) as a tumor surrogate marker. The problem with this selection is that rarely are the appropriate data collected for defining shape of dose-response curve. In addition, since cancer is a multistep, multimechanism process, it is necessary to develop dose-response curves based on interactive processes for predicting tumor outcomes. For example, dose-response curves for mutations need to consider the impact of cell proliferation on the mutation frequency. Other genotypic and phenotypic factors can equally influence the shape of a dose-response curve for mutations. The shape of a dose-response is definable for different scenarios when the mechanistic data to be used are developed on the basis of the need for which they are to be used, thereby helping to avoid their inappropriate use. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

45 NEW DATA SUPPORT REVISED LOW DOSE EXTRAPOLATION MODELS.

B. R. Scott. *Lovelace Respiratory Research Institute, Albuquerque, NM.* Sponsor: R. Henderson.

Low-dose extrapolation of carcinogenic risk for radiation and genotoxic chemicals is based on a relative risk (RR) paradigm for which RR is always greater than or equal to 1. Stated differently, no consideration is given to the possibility that RR for cancer induction can be less than 1 after low-dose exposure of humans. However, new in-vitro (mutation, neoplastic transformation), in-vivo (animal carcinogenesis), and epidemiological (cancer-induction) data are emerging that support the view that low-dose photon radiation (x and gamma rays) and certain chemicals (e.g. ethylene, propylene) can turn on protective processes (possibly mediated *via* apop-

tosis) that lead to a reduction in the frequency of stochastic effects below the spontaneous rate. These data strongly support a low-dose cancer risk assessment paradigm that includes RR < 1. Implications of the data for low-dose risk assessment for single-agents (radiation, genotoxic chemical) and combined exposures (different radiations, different chemicals, radiation and chemicals) will be briefly discussed including the need for revised low-dose, risk-extrapolation models. (Research supported by the US Department of Energy Offices of Science and Environmental Management).

46 BIOLOGICAL BASIS FOR AND CONCEPTUAL APPROACHES TO LOW-DOSE NONLINEARITY.

R. Conolly. *CIIT Centers for Health Research, Research Triangle Park, NC.*

The initial interaction of a xenobiotic ligand with its biological receptor is low-dose linear. The initial interaction is usually the first step in a mechanism leading to a frankly adverse effect. For many, if not all environmental toxicants, adaptive and homeostatic mechanisms compensate for toxicant-induced changes to maintain a relatively constant internal environment. While the initial interaction may be low-dose linear, the ability of biological systems to compensate means that shapes of dose-response curves can change as we move downstream from the initial interaction. The shapes of dose-responses for frankly adverse effects are thus expected to differ from the dose-response shape of the initial interaction. Thresholds and nonmonotonic curves are possible. Numerous experimental studies are consistent with nonlinear and nonmonotonic dose-response behaviors. Theoretical modeling studies of mechanisms involving adaptive processes readily demonstrate threshold and nonmonotonic dose-responses. The assumption of low-dose linearity makes sense as a health protective measure in the absence of mechanistic data. Toxicologists and risk assessors need to think broadly, however, about the determinants of the shape of the dose-adverse effect curve. We should continue to characterize initial interactions and pharmacokinetic nonlinearities but we also need to evaluate how the organism responds to the toxicant. Characterization of compensatory processes is particularly important at low, environmentally relevant doses, where the risk of toxic effects is typically only inferred from effects observed at much higher doses. Biological compensation is most likely to be operative in this low-dose range, which is not studied in experiments that focus on demonstration and analysis of frankly adverse effects. A redefinition of the concept of toxicological mechanism to include elicited homeostatic and adaptive responses is needed. This redefinition is no more than a natural extension of the progression away from default-based towards mechanism-based human health risk assessment.

47 HORMESIS: ITS IMPLICATIONS FOR HAZARD AND RISK ASSESSMENT.

E. J. Calabrese. *Environmental Health Sciences, University of Massachusetts, Amherst, MA.*

An ongoing comprehensive evaluation of the toxicology and pharmacology literature suggests that the biphasic dose response that characterizes hormesis is much more wide spread than is commonly recognized and may come to rival currently favored concepts/models (e.g., linear and threshold) describing toxicological dose responses used in risk assessment. The data indicate that hormetic responses occur in a high percentage of appropriately designed toxicological studies, are reproducible and that these responses are highly generalizable being independent of biological model, endpoint measured and chemical class. Numerous mechanistic studies have also been published which account for the hormetic biphasic dose response relationships in multiple biological dose response systems. The implications of these findings are significant since they have the capacity to affect not only our concept of the dose response in toxicology but also the selection of animal model, study design features, endpoints measured, and risk assessment applications.

48 LIPOMICS, AN IMPORTANT COMPONENT OF METABOLOMICS, AND POSSIBLE USE IN TOXICOLOGY STUDIES.

M. C. Madden. *NHEERL\Human Studies Division, USEPA, Chapel Hill, NC.*

Metabolites of endogenous biochemical substances can be considered to represent the ultimate organ and cellular responses to toxicants or other changes in an organism's environment. An important fraction of these endogenously produced metabolites are lipids; the comprehensive study of the production of these lipids is termed lipomics or liponomics. Lipids of various chemical classes have been implicated in mediating human diseases in the lung, cardiovascular, brain, and other organ systems. The emphasis of this session will be to provide an overview of strategies for quantifying lipids and key lipid metabolic steps, and subsequently organizing the resulting data into more usable and understandable formats. A brief overview of the biological relevance of lipids will initiate the session. A presentation on lipid chemistry and analytical chemistry strategies (along with the associated strengths and shortcomings) will follow in order to provide the audience with insights on some of the technologies needed to perform the first step involved in lipomics. Additional presentations will show: comprehensive lipid analyses (>400 lipids) of mice treated with the anti-hyperlipidemic agent rosiglitazone and subsequent data manipulation into a informative database; alterations of lung lipids collected in breath condensate from humans and animals models (mice, pigs) of lung disease; and using lipomics to monitor microbial biomass and composition for use in environmental remediation strategies, microbial ecology studies, and minimizing microbial populations in occupational settings. Use of lipomics, in combination with proteomics and genomics, can provide a more complete view of cellular responses. Monitoring of these responses can be used to assist in optimizing drug therapies, examining effects from toxicant exposures, determining the influence of nutrition on responses, and screening of the environment for microbial populations. [This abstract may not represent official EPA policy.]

49 LIPIDS: A PRIMER ON MEASUREMENT, CLASSIFICATION AND FUNCTION.

J. Jackman[1] and D. C. White[2]. *[1]Applied Physics Laboratory, Johns Hopkins University, Laurel, MD and [2]Center for Biomarker Analysis, University of Knoxville, Knoxville, TN.*

Lipids are a structurally diverse class and functionally critical components of all biological organisms. Due the central role of lipid and lipid containing molecules in cell responses, there has been an enhanced interest in lipomics or the total lipid profile of cells. The revolutions in chromatographic resolution, high-resolution mass spectral detection, improvements in extraction, ionization methods and computing have increased the scope of lipomics. While most lipids can be extracted with organic solvents by classical extraction methodology, lipids covalently bound to carbohydrates (lipopolysaccharide, lipoproteins) are generally non-extractable due to poor solubility. Hence new techniques for extraction such as solid phase extraction methods and analysis such as mass spectrometry (MS) are being developed to overcome some of these issues. A convenient functional classification of lipids is based on the normal-phase bulk elution of components using gradients of polar phases. Neutral lipids (e.g., waxes, sterols, tri- and diglycerides) elute first; glycolipids elute next; followed by polar lipids (e.g., phospholipids). Each lipid class can be fractionated by normal or reverse phase chromatography. Lipid classes, fragments or total extracts can be hydrolyzed and derivatized to make them volatile for high-resolution capillary GC/quadrupole MS. Additional insight into the structural details can be greatly increased by utilizing ion traps capable of multiple fragmentations or tandem quadrupole MS for precursor and product ion scans. Replacement standard GC/MS systems with atmospheric pressure ionization (API) have expanded the mass range of analysis to include intact lipids. Increasing the mass resolution with coupled time-of flight, advanced quadrupole, or Fourier transform ion-cyclotron resonance MS with generation of multiply charged analytes allows the technologies developed for proteomics to be applied to complex glycolipids like gangliosides. Together these technologies have revolutionized the detection and functional analysis of lipids.

50 LIPOMIC PROFILING APPLICATIONS IN TOXICOLOGY.

S. M. Watkins. *Lipomics Technologies, Inc., West Sacramento, CA.* Sponsor: M. Madden.

Diet, environmental exposure, and genetic background exert profound influences on human metabolism that results in changes in lipid metabolites. Lipomics employs a quantitative and comprehensive assay that measures lipid metabolites from biological tissues and fluids. This technology is used to improve the understanding of the role of lipid metabolism in disease and to develop diagnostic profiles of drug safety and efficacy. A critical benefit of producing quantitative data on known metabolites is that the results of each analysis can be mapped against the known biochemical pathways. This presentation will demonstrate the application of quantitative metabolite profiling in determining the metabolic response to several chemical compounds including insulin-sensitizing agents and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. In the first example, a PPARs-γ agonist and a β3-adrenergic receptor agonist are shown to elicit dramatically different metabolic

responses in obese, diabetic mice, despite producing similar clinical chemistry profiles. In the second example, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin is shown to selectively dysregulate cholesterol and phospholipid metabolism in pregnant rhesus monkeys. Lipomic profiling is uniquely suited for illuminating the metabolic basis of phenotype and advancing our understanding of adverse events. This technology should vastly improve therapeutic and diagnostic capabilities in the near future.

51 LIPIDS FROM BREATH CONDENSATE AS NON-INVASIVE BIOMARKERS FOR RESPIRATORY PATHOPHYSIOLOGY.

D. C. White[1], R. Geyer[1], J. Cantu[1], S. Mani[2], M. Jett[2], J. Jackman[3] and M. Karlstad[4]. *[1]Center for Biomarker Analysis, University of Tennessee, Knoxville, TN, [2]Walter Reed Army Institute of Research, Silver Spring, MD, [3]Applied Physics Laboratory, Johns Hopkins University, Laurel, MD and [4]Medical Center, University of Tennessee, Knoxville, TN.*

Regulatory lipids readily form aerosols that can be recovered non-invasively by cooling expired breath to form breath condensate (BC). BC includes eicosanoids as well as the phosphatidylcholine (PC) based lipid surfactants, lysoPC and platelet activation factors (PAF) that modulate inflammatory and immune processes, and are reflective of oxidative stress and toxicity. Detection by mass spectrometry (MS) provides insights into presumptive harbingers of respiratory pathobiology. BC lipids recovered by solid phase extraction contain PC lipids (detected by HPLC/MS) and eicosanoids (derivatized prior to GC/MS). Responses of BC lipids and eicosanoids from piglets and rats exposed to aerosolized lipopolysaccharide (LPS) or Staphylococcal enterotoxin (SEB) showed similar responses as observed in exposed cultured cell supernatants and rat lung lavage. Molecular species of PC derived lipids and eicosanoids showed differential responses to the toxins and bacterial infections. Initial targets include prostaglandins, (PGE2, PGF2α), thromboxane (TXB2), and prostacyclin (as 6-Keto PGF1α) that show differential responses to inflammation; leukotriene B4 and PGD2 for allergic responses; and isoprostanes for free radical oxidative stress. PAFs and lysoPAFs have been shown to increase with inflammation. Pulmonary responses of piglets to intratracheal exposure of toxicants (LPS & SEB) show differential patterns of lipids and eicosanoids. Human BC is readily collected within 10-20 min with a disposable, commercially available apparatus, and its clinical human use has been approved in Germany. Preliminary data indicate numerous other BC eicosanoids are available for assay, and different infectious agents induce different patterns of eicosanoid responses suggesting an important application of lipomics in the non-invasive early assessment of lung pathobiology.

52 LIPID ANALYSES OF MICROBIAL COMMUNITIES-APPLICATIONS FOR ENVIRONMENTAL REMEDIATION, MICROBIAL ECOLOGY, AND MEDICINE.

E. Sobek and A. D. Peacock. *Microbial Insights, Rockford, TN.* Sponsor: M. Madden.

Lipid analysis of microbial communities provides quantitative details of microbial biomass and community composition. The nutritional and physiological status of the microbiota can be determined through lipid analysis. The vast majority of microbes in the environment are unculturable; lipomics circumvents this problem by direct examination of microbes in their natural environments. Lipids are readily concentrated and purified from complex environmental matrices and are amenable to structural analysis by GC/MS. Viable bacteria have intact polar phospholipid membranes and are assessed quantitatively with analysis of phospholipids ester-linked fatty acids (PLFA). PLFA generally do not exist as molecular fossils and may be considered as quantitative measures of *via*ble microbial biomass. The community composition of microbial communities can be quantitatively discerned from the lipid composition which correlates with analyses from isolated rDNA and functional gene sequences. Specific patterns of lipids can indicate physiological and toxin induced stress. Lipid composition reflects both the genotypes and phenotypic responses at the specific microniches of the bacteria as well as providing specific biomass quantitation. Lipomics provides the unique capability of defining the in situ redox conditions which can be critical in subsurface bioremediation. Exposure to toxic environments converts monoenoic to cyclopropane PLFA, and a relative reversible increase in specific trans/cis monoenoic PLFA ratio. Prolonged exposure to low moisture conditions induce cyclopropane PLFA indicating stationary growth phase and increasing proportions of trans PLFA. The formation of poly β-hydroxyalkanoic acid (PHA) provides measures of unbalanced growth and accumulation of intercellular reductants. Specific lack of bioavailable phosphate induces ornithine-lipids in some environmental communities. The usefulness of microbial lipomics is evident in environmental remediation, community microbial ecology of biofouling, and in the persistence and nurture of pathogens in biofilms in the workplace and hospital settings.

53 DEVELOPMENTAL IMMUNOTOXIC EFFECTS OF PRENATAL ATRAZINE EXPOSURE.

A. M. Rowe[1], K. M. Brundage[1, 2], R. Schafer[1] and J. B. Barnett[1, 2]. *[1]Microbiology, Immunology and Cell Biology, West Virginia University, Morgantown, WV and [2]Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, WV.*

Atrazine, 2-chloro-4-ethylamino-6-isopropylamino-s-triazine, (ATR) is the most heavily used herbicide in the United States with over 70 million pounds of atrazine applied annually and it is the most common herbicidal contaminant of ground water in agricultural areas. It is our goal to characterize the effects of prenatal atrazine exposure on the developing mammalian immune system. We hypothesie that in *utero* exposure to ATR will result in a persistent and potentially debilitating effect on the organism's immune system. Using the Balb/c mouse as a model we exposed pregnant female mice to ATR for 21 days starting at day 10 of gestation. ATR or placebo was administered *via* a time-release pellet implanted subcutaneously. The matrix of the pellet allowed for the daily release of 0.7mg of atrazine for 21 days. The pellets were inserted into the mothers at day 10 post coitus. The resulting offspring were immunized with heat killed S. pneumoniae (HKSP) between 9 and 10 weeks of age. Two weeks following immunization the spleens were harvested, and the splenocytes were phenotypically characterized by flow cytometry. The number of B-cells secreting HKSP-specific antibodies was enumerated *via* ELIspot analysis. Male offspring had a statistically significant increase in the number anti-HKSP secreting B-cells compared to the controls. There was not a statistically significant change in the number of total splenocytes or the number of $CD4^+$, $CD8^+$ or $B220^+$ splenocytes. However, atrazine treated mice possessed an increased percentage of $CD4^+$ splenocytes and a decrease in $CD8^+$ splenocytes. Female offspring did not possess a statistically significant change in IgM production compared to controls. And unlike the males, the female offspring had a statistically significant increase in the percent of $B220^+$ splenocytes compared to the controls. These results demonstrate a gender-dependant immunotoxic effect of prenatal exposure to ATR on the offspring. Supported by NIOSH grant OH07686 and NIEHS grant ES010953

54 EFFECTS OF PRENATAL EXPOSURE TO CIGARETTE SMOKE ON TUMOR SURVEILLANCE IN THE OFFSPRING.

S. P. Ng, S. P. Doherty and J. T. Zelikoff. *Environ Med., New York University School of Medicine, Tuxedo, NY.*

More than 1 million infants in America are born each year after either exposure *in utero* from active or passive maternal smoke exposure. Since accumulating epidemiologic evidence suggests that prenatal exposure to intact cigarette smoke (CS) increases the incidence of cancer in the offspring, it is hypothesized that inhalation exposure of pregnant mice to mainstream cigarette smoke (MCS) decreases offspring resistance to tumor challenge due to alterations in surveillance mechanisms critical for the recognition and destruction of cancers. Thus, this study seeks to: (1) ascertain by means of an *in vivo* tumor challenge whether exposure of pregnant dams to MCS alters tumor induction/development in the offspring; and, (2) assess the degree to which exposure of dams to MCS during gestation modulates tumor surveillance mechanisms. Pregnant $B_6C_3F_1$ mice were exposed to MCS for 5 d/wk (4 hr/d from gestational d5 to parturition). Exposure levels of particulate matter and carbon monoxide, measured within the exposure chamber, were 16.2 and 21.5 ppm respectively; carboxyhemoglobin and cotinine levels, assessed as exposure dosimeters, were 3.6% and 28 ng/ml. In addition to this relatively low inhaled dose of MCS reducing litter size by 13% and shifting gender ratios (compared to air-exposed controls), prenatally-exposed male and female pups challenged at 5-wk of age with neoplastic cells demonstrated a 2- and 4- fold increase in tumor incidence, respectively, (relative to age-/gender-matched air-exposed offspring); control males developed significantly more tumors than exposure-matched females. In addition, MCS-exposed offspring had a shorter time to tumor formation. Finally, *in utero*-exposed male pups demonstrated significantly lower T-lymphocyte proliferative responses than age-/gender-matched controls. Results demonstrate that inhalation exposure of pregnant mice to a low dose of MCS alters gestational parameters, reduces immune function and decreases offspring resistance against nascent tumors 5-wk after exposures have ceased. Supported by Philip Morris Foundation

55 THE EFFECTS OF CANNABINOID EXPOSURE ON TUMOR GROWTH AND THE ANTI-TUMOR IMMUNE RESPONSE.

R. McKallip[1], M. Nagarkatti[1] and P. S. Nagarkatti[2]. *[1]Department of Microbiology and Immunology, VCU, Richmond, VA and [2]Department of Pharmacology and Toxicology, VCU, Richmond, VA.*

Previous studies have shown that Delta 9-tetrahydrocannabinol (THC) has both immunosuppressive as well as antitumor activity. However, little is known about the effect of THC on the immune response to and growth of tumors that are resist-

ant to THC-induced apoptosis. In the current study, we tested the central hypothesis that exposure to THC can lead to enhanced growth of tumors that are relatively resistant to THC-induced apoptosis by specifically suppressing the anti-tumor immune response. Exposure of 4T1 breast cancer cells to 20 μM THC led to induction of apoptosis, whereas lower concentrations were relatively ineffective, suggesting that 4T1 tumor cells are relatively resistant to THC-induced apoptosis when compared to the level of sensitivity of normal lymphocytes. Next, we examined the effect of THC exposure on the growth of 4T1 tumor cells *in vivo*. The results showed that exposure to 25 mg/kg body weight of THC led to significantly elevated tumor growth when compared to vehicle-treated mice. Examination of the effect of THC exposure on 4T1 metastasis revealed that exposure to 25-50 mg/kg THC led to a significant increase in the number and size of tumors metastasizing to the lungs. Evaluation of the effect of THC exposure on the immune response showed that exposure to THC significantly inhibited the proliferative response of splenocytes to various mitogens due to induction of apoptosis. Finally, we noted that exposure to THC significantly inhibited the specific anti-tumor immune response to 4T1 tumor cells *in vivo*. Together, the results from this study suggest that exposure to THC can induce apoptosis in immune cells *in vivo* and thereby downregulate the anti-tumor immune functions. Such findings suggest that marijuana abuse may increase the susceptibility to breast cancer as well as other cancers that are resistant to THC-induced apoptosis (Supported in part by NIH grants R01DA016545, R21DA014885, R01ES09098, R01AI053703, R01HL058641, DA14041 and American Cancer Society IRG-100035)

56 MOLECULAR MECHANISM OF ACTION OF THE FUNGICIDE MANCOZEB ON THE INHIBITION OF CYTOKINE PRODUCTION.

E. Corsini[1], S. Birindelli[2], M. Marinovich[1], C. Colosio[2] and C. L. Galli[1]. [1]*Department Pharmacological Sciences, University of Milan, Milan, Italy and* [2]*ICPS, International Centre for Pesticide Safety, Busto Garolfo, Italy.*

We previously observed in agricultural workers exposed to mancozeb a statistically significant decrease in lipopolysaccharide (LPS)-induced tumor necrosis factor-alpha (TNF) production ($p<0.01$). The purpose of this work was to establish an *in vitro* model reflecting *in vivo* data on mancozeb and to characterize its molecular mechanism of action. The human promyelocytic cell line THP-1 was used to develop the *in vitro* model to study the effects of mancozeb and its main metabolite ethylenthiourea (ETU) on LPS-induced TNF release. Cells were treated with increasing concentrations of mancozeb or ETU for different times. TNF release was evaluated following LPS stimulation by specific ELISA. Mancozeb, but not ETU, at non cytotoxic concentrations, induced a dose and time related inhibition of LPS-induced TNF release. The results obtained indicated that THP-1 cells were indeed a suitable model to study the molecular mechanism of action of mancozeb. For the characterisation of the molecular mechanism of action the effect of mancozeb on TNF mRNA expression was first evaluated by semi-quantitavive RT-PCR. We observed that decreased TNF release following mancozeb and LPS treatment was due to a pre-transcriptional event. Successively, in order to better define the molecular target of mancozeb its effect on LPS-induced NF-kB activation and reactive oxygen species (ROS) generation was characterised. Transcription factor activation was evaluated measuring by ELISA the DNA binding by specific transcription factor present in nuclear extract obtained from cells treated in the presence or absence of mancozeb following LPS stimulation. ROS generation was evaluated using a flow cytometric method using the dye dichlorofluorescein diacetate. We demonstrated that mancozeb functioning as an antioxidant causes a direct inhibition of NF-kB activation, which in turn resulted in decreased TNF production in monocytes.

57 IMMUNOTOXICITY OF SILICA: T CELL ACTIVATION AND BAL CELL ANTI-APOPTOTIC PHENOTYPE PRECEDE GRANULOMA FORMATION IN CHRONIC SILICOSIS.

R. J. Langley, N. Mishra and M. Sopori. *Immunology, LRRI, Albuquerque, NM.*

Occupational exposures of low to moderate levels of silica (SL) may lead to silicosis many years after SL exposure. Current experimental animal models indicate that acute silicosis is associated with apoptosis. However, the role of apoptosis during granuloma formation in chronic silicosis is not known. To induce chronic silicosis in an animal model, Lewis rats were exposed *via* inhalation to filtered air or silica (1.75 mm mass median aerodynamic diameter) at an exposure concentration of 6.2 mg/m3, 6 h/d, 5 d/wk for 6 wk; and observed for up to 18 months after the exposure. Immunological changes, silica burden, histopathology and apoptotic phenotypes were monitored at various intervals during this time. In the early phase (0-28 days after exposure) SL is detected in the lung, spleen and brains of the SL-treated animals. SL is mostly cleared from all tissues within 10 wks after the exposure; however polarized microscopy revealed that a few, but significant number of SL particles are retained in the lung and tracheal-bronchus lymph nodes. In the early phase of silicosis, spleen cells from SL-exposed animals show increased antibody-forming cell response and T cell hyper-responsiveness (i.e. increased Con A-induced proliferation and increased intracellular Ca2+ levels in response to TCR-ligation.) These immune changes returned to normal within approximately 10 wks. Around this time, granulomatous changes in the lung were initiated with the accumulation of epitheliod macrophages; SL particles could be found inside the developing granulomas. This was accompanied by the presence of various anti-apoptotic molecules in bronchoalveolar lavage (BAL) cells (i.e. high levels of pro-caspase 3 and Bcl-2, and decreased levels of JNK1 and JNK2). In addition, BAL fluid contained increased levels of MMP2 and MMP9. Thus, silicosis starts with immune activation that is followed by granulomatous changes in the lung around SL particles and a strong anti-apoptotic phenotype of BAL cells.

58 GENE EXPRESSION PROFILES IN HEXACHLOROBENZENE-INDUCED TOXICITY.

J. Ezendam[1, 2], F. Staedtler[3], J. Pennings[2], R. Vandebriel[2], R. Pieters[1], J. Harleman[3] and J. Vos[2]. [1]*Immunotoxicology, IRAS, Utrecht, Netherlands,* [2]*National Institute for Public Health and the Environment, Bilthoven, Netherlands and* [3]*Novartis Pharmacology AG, Basel, Switzerland.*

Hexachlorobenzene (HCB) is a persistent environmental pollutant with toxic effects in man and rat. Reported adverse effects are hepatic porphyria, toxic effects on reproductive and immune system, and neurotoxicity. The Brown Norway (BN) rat is very susceptible to HCB-induced immunopathology and oral exposure causes enlargement of liver, spleen and lymph nodes, increased serum levels of IgM, IgG and IgE and inflammatory skin and lung lesions. The mechanisms of HCB-induced immunopathology are not clear, but both T cells and macrophages seem to be involved. To gain more insight in the mechanisms of HCB-induced toxicity, gene expression levels were studied using microarrays. BN rats were exposed for 4 weeks to a diet supplemented with 0, 150 or 450 mg/kg HCB. Blood, thymus, kidney, liver, spleen and mesenteric lymph nodes (MLN) were collected and analysed using the Affymetrix rat RGU-34A GeneChip microarray. Most significant ($p<0.001$) changes occurred in spleen, followed by liver, kidney, blood and MLN, but only a few genes were affected in thymus. This was to be expected since the thymus is not a target organ of HCB. Known effects of HCB such as immunotoxicity and induction of enzymes involved in drug metabolism, porphyria and reproductive system were confirmed, and in line with histopathology were the increased transcript levels of markers for granulocytes and macrophages. New findings include upregulation of genes encoding mast cell markers, complement, cell adhesion molecules, chemokines, proinflammatory cytokines, acute phase proteins and antioxidants. In general, gene expression data provide evidence that HCB induced a systemic inflammatory response, accompanied by oxidative stress and an acute phase response. In conclusion, this study confirms previously observed toxicological effects of HCB but also reveals several new and mechanistically relevant genes. Thus, genomics can be considered to be an important tool for hazard identification of this compound.

59 DENDRITIC CELLS ARE A SENSITIVE TARGET OF THIMEROSAL AND ETHYLMERCURY.

S. R. Goth, R. A. Chu and I. N. Pessah. *Department of Molecular Biosciences and the Center for Children's Environmental Health and Disease Prevention, UC Davis, Davis, CA.*

Thimerosal is an organic mercurial compound widely used as a preservative in consumer products and vaccines. Dendritic cells (DC) are potent antigen presenting cells capable of initiating primary T cell responses to vaccines and infectious agents. Under normoxic (5% oxygen v/v) and reductant-free conditions, we tested whether thimerosal (THI) exposure modified effector functions of immature and mature DC subsets *in vitro*. After a 20h exposure to THI, DC *via*bility, expression of class II MHC and CD86, and caspase activation were measured by fluorescence cytometry. Thiosalicylic acid (TSA) and ethylmercuric chloride (EtHgCl) were also assayed to determine if products of THI ionization contributed to its effects. The IC50 value for TSA was greater than or equal to 10uM for all parameters measured. The IC50 values for THI and EtHgCl induced cell death in immature DCs were 534nM and 690nM; in mature DCs they were 628nM and 477nM. In propidium iodide negative (*via*ble) cells, the IC50 values for THI and EtHgCl induced downregulation of cell surface class II MHC in immature DCs was 1.2uM and 1.4uM; in mature DCs it was 2.5uM and 2.1uM. IC50 values for THI or EtHgCl induced downregulation of cell surface CD86 in immature DCs was 1.3uM and 1.7uM; in mature DCs it was 329nM and 358nM. Annexin V binding was upregulated in *via*ble immature and mature DC after a 20h exposure to THI. Two hour exposure to 1uM THI and EtHgCl activated caspases in mature but not immature DC. DC pretreated with THI stimulated allogeneic CD4+ or peptide antigen specific CD8+ T cells less strongly than control cells. In summary DC are sensitive targets of THI, exhibiting cytotoxicity in the nanomolar range. Moreover enhanced apoptosis, downregulated expression of class II MHC and CD86, and reduced ability to stimulate allo- and antigen-specific T cells were observed in DC exposed to organic mercury. We conclude that THI and EtHgCl downregulate the *via*bility and function of DCs by a mechanism consistent with apoptosis. Sponsored by NIEHS Grant P01ES11269

60 INORGANIC MERCURY INCREASES SEVERITY AND FREQUENCY OF AUTOIMMUNE MYOCARDITIS IN MICE.

J. F. Nyland[1, 2], D. Fairweather[3], N. R. Rose[1, 2, 3] and E. K. Silbergeld[1]. *[1]EHS, JHU Bloomberg School of Public Health, Baltimore, MD, [2]MMI, JHU BSPH, Baltimore, MD and [3]Pathology, JHU School of Medicine, Baltimore, MD.*

Inorganic mercury (iHg, HgCl2) has a range of immunotoxic effects, including induction of autoimmunity in specific inbred strains of rats and mice. Epidemiological studies have demonstrated an association between Hg exposures and cardiac disease in human populations. We hypothesize that the expression of frank disease may require interactions between Hg and other susceptibility factors, genetic or acquired. To test this hypothesis, we have treated mice with low doses of iHg and then induced autoimmune cardiac disease using Coxsackievirus B3 (CB3) in BALB/c male mice. CB3 induces a biphasic cardiac disease in susceptible mice which is characterized by a primary acute viral phase, peaking at about day 12 after infection, and a chronic autoimmune phase, peaking at about day 35 post-infection. The animals were either pre-treated with iHg (200ug/kg, every other day for 15 days) before infection with CB3 or interim-treated (200ug/kg, every other day for 7 days) between the acute and chronic phases, approximately day 15 post-infection. We found that pre-treatment significantly increased the percent of inflammation in the heart during the chronic, but not the acute, stage. Further, the percentage of mice with dilated cardiomyopathy was significantly increased at the chronic stage with iHg pre-treatment. However, these increased pathological symptoms of myocarditis were not accompanied by changes in cytokine profile in the heart. In contrast, iHg interim-treatment did not significantly change the prevalance of dilated cardiomyopathy, but it did increase the levels of IL-12 and IL-4 in the heart at day 35. Neither iHg treatment reactivated virus in the heart at day 35. These preliminary results suggest that mercury lowers the threshold of activation for autoreactive cells before they have seen their autoantigen (before viral infection damages the cardiac tissue and releases cardiac antigens), and there is evidence for enhanced activity of autoreactive cells with later iHg exposure.

61 EFFECTS OF MERCURY (HG) EXPOSURE ON BIOMARKERS OF AUTOIMMUNITY IN HUMANS POPULATIONS.

E. K. Silbergeld[1], L. Burek[2], N. Rose[2], J. M. Souza[3], E. C. Santos[3], J. Graber[1] and I. Silva[4, 1]. *[1]Environmental Health Science, Johns Hopkins University Bloomberg School Public Health, Baltimore, MD, [2]JH Medical School, Baltimore, MD, [3]Evandro Chagas Institute, Para Belem, Brazil and [4]Institute Molecular Cell Biology, Porto, Portugal.*

Mercury (Hg) compounds have long been associated with autoimmunity in rodents, especially in inbread mouse strains carrying the H-2s locus. However, to date there has been little evidence to associate risks of autoimmunity in humans with occupational or environmental exposures to Hg. To further examine potential immunotoxic effects of Hg in humans, we have studied populations in Amazonian Brazil exposed to both inorganic Hg and methyl Hg *via* direct and indirect contamination resulting from the use of Hg in gold extraction. Using serum samples from cross sectional surveys carried out in three Amazon communities, we analyzed for antinuclear autoantibodies (ANA) and antinucleolar autoantibodies (ANoA) using the indirect immunofluorescence microscopy method (INOVA Diagnostics). Overall, there was an association between Hg exposures (inorganic and methyl) and increased prevalence of elevated serum ANA and ANoA. Stratifying by Hg in hair < or = 8 ppm, and by time in gold mine, we found a significant positive association with odds of serum ANoA detectable at dilutions of 1:10 or greater, and 1:40 or greater, respectively. There was an apparent interaction between Hg exposure and frequency of malaria infection in increasing the prevalence of autoantibody elevations. These are the first indications that Hg exposures may induce autoimmunity in human populations, similar to those demonstrated in murine models. Research support by CNPq (Brazil), NIEHS-Fogarty, PAHO, and Heinz Family Foundation

62 METABONOMIC EVALUATION OF IDIOSYNCRASY-LIKE LIVER INJURY IN RATS COTREATED WITH RANITIDINE AND LIPOPOLYSACCHARIDE.

J. F. Maddox[1], J. P. Luyendyk[1], G. N. Cosma[2], A. P. Breau[3], G. G. Harrigan[4], R. H. Bible[3], R. Goodacre[5], P. E. Ganey[1], G. H. Cantor[2], G. L. Cockerell[2] and R. A. Roth[1]. *[1]Pharmacology and Toxicology, Michigan State University, East Lansing, MI, [2]Investigative Toxicology, Pharmacia Corporation, Kalamazoo, MI, [3]Global Drug Metabolism, Pharmacia Corporation, Skokie, IL, [4]HTS Metabolic Profiling, Pharmacia Corporation, Chesterfield, MO and [5]Department of Chemistry, University of Manchester Institute of Science and Technology, Manchester, United Kingdom.*

Idiosyncratic liver injury occurs in a small fraction of people undergoing therapy with certain drugs, including the histamine-2 receptor antagonist, ranitidine (RAN). The cause of idiosyncratic hepatotoxicity is not known; however, it has been proposed that environmental factors such as concurrent inflammation initiated by bacterial lipopolysaccharide (LPS) or other inflammagens could increase an individual's susceptibility to drug toxicity. Recently, liver injury resembling human RAN idiosyncrasy was created in RAN-treated rats by cotreating them with a non-hepatotoxic dose of LPS. Biofluid metabonomics is showing promise in monitoring toxic liver injury. It has not, however, been used to segregate animals treated with a single drug from those coexposed to other agents. Rats were treated with RAN (30 mg/kg, iv) and/or LPS (44 x 10^6 EU/kg, iv, 2 h earlier) or the vehicles; and urine was collected for nuclear magnetic resonance (NMR)- and electrospray ionization mass spectrometry (ESI-MS)-based metabonomic analyses. Clinical chemistry and histopathologic analyses showed that rats cotreated with RAN and LPS developed hepatocellular necrosis, whereas those treated with either agent alone or with vehicles did not. Cluster analyses of the urine spectra by either NMR or ESI-MS revealed a clear separation of the rats treated with LPS/RAN from the other three groups. These findings support the potential use of a noninvasive metabonomic approach to identify individuals with toxic reactions to drug-inflammagen coexposure.

63 CALPASTATIN EXPRESSION: A NEW LINE OF DEFENSE AGAINST PROGRESSION OF TOXICANT-INDUCED INJURY.

P. Limaye[1], P. S. Palkar[1], University. M. Apte[1], J. C. Latendresse[2], S. Yu[3], P. Kashireddy[3], J. K. Reddy[3] and H. M. Mehendale[1]. *[1]Department of Toxicology, University of Louisiana at Monroe, Monroe, LA, [2]Pathology Associates Intl., NCTR, Jefferson, AR and [3]Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

We have previously demonstrated the role of calpain in the progression of acute liver injury initiated by a toxicant taking place even after the offending toxicant is long gone. It is known that stimulated tissue repair stops the progression of injury due to the resistance of the newly divided/dividing cells against the action of the death proteins such as calpain. However, the mechanism of such resistance is not known. We hypothesized that higher expression of calpastatin (CSTN), an endogenous inhibitor of calpain, in the newly divided/dividing cells provides resistance against calpain action. To test this hypothesis, hepatic expression of CSTN was studied over a time course after CCl_4 treatment of male S-D rats. Livers of the CCl_4-treated rats exhibited high expression of CSTN from 24 to 72 h after CCl_4 injection, coincident with extensive cell division. Immunohistochemical analysis of PCNA and CSTN on serial liver sections indicated that newly divided/dividing cells stained positive for CSTN. CSTN expression was further studied in two additional models of cell division: 70 percent partial hepatectomy (PH) and liver cell division in the newborn rats. The 20-day-old newborn rat pup livers extensively expressed CSTN coincident with marked cell division. In PH, hepatic expression of CSTN was intense up to 4 days, declining thereafter to normal by 7 days after PH. To test further whether overexpression of CSTN will provide protection from toxicant mediated injury, an adenoviral vector expressing CSTN has been generated which will be used to induce CSTN overexpression in the livers of male SW mice and in primary hepatocytes. These models will be used to study toxicant induced injury to confirm the role of CSTN expression in the resistance exhibited by newly divided/dividing cells. (Supported by Kitty DeGree Endowed Chair and LBRSF)

64 MECHANISMS OF DIFFERENTIAL HEPATIC TOXICITY BETWEEN TROGLITAZONE AND ROSIGLITAZONE.

H. M. Rhee[1], B. J. Song[2] and M. Bae[2]. *[1]Metabolic Endocrine Drug Products, Food and Drug Administration, Rockville, MD and [2]Lab. Membrane Biochemistry and Biophysics, NIH, NIAAA, Rockville, MD.* Sponsor: J. Colerangle.

Structurally troglitazone is a thiazolidinedione common to rosiglitazone and pioglitazone, which are classified as PPAR agonists. They have hepatic, renal, cardiac and bone marrow toxicities in animals, although they have strong antidiabetic activity. We hypothesize the various toxicities are due to formation of apoptotic cell cycle regulators. Methods: HepG2 and Chang liver human hepatoma cells were used. Cell *via*bility, cell cycle analysis, and apoptosis-related proteins were determined (Toxicology Letter 139: 67-75, 2003). HepG2 cells (2x106 cells/dish) were treated with control vehicle, troglitazone, or rosiglitazone at doses of 12.5, 25, 50 and 100 uM for 48 hours. Approximately 10, 000 cells from each group were analyzed for DNA histograms and cell cycle phase distribution using a FACS Calibur cytometer. The percentage of cells undergoing apoptosis were determined by CellQuest software program. Results: Troglitazone damaged HepG2 cell drug dose dependently while rosiglitazone did not, even at 100 uM concentration. The percentage of HepG2 cells in the sub-G1(apoptotic cells) phase significantly increased as the concentration of troglitazone increased. Sub-G1 cell populations after troglitazone exposure are remarkably different from that of control while 50 uM rosiglitazone did not change the G1 cell population from the DMSO-treated control. We also determined the levels of p53 and its downstream proteins such as Gadd45, p21, and p27

in HepG2 cells. Indeed, troglitazone specifically increased the levels of mRNA for various transcription factors or apoptosis-related genes such as c-Jun, Bax, and Bad including the cell cycle regulator proteins. In addition, troglitazone decreased the activities of cdk2, cdc2, and cyclin A kinases at 24 h post-treatment. All these facts indicate that the hepatotoxic mechanisms of troglitazone may be due to its detrimental effects on cell cycle intermediates, which will eventually lead to cell death.

65 GENOMIC AND PROTEOMIC INVESTIGATIONS INTO THE IDENTIFICATION OF SUSCEPTIBILITY FACTORS IN DRUG-INDUCED LIVER DISEASE (DILD).

K. Welch[1], T. Reilly[2], B. Wen[3], T. Hays[1], J. Brady[4], C. Masison[4], M. Radonovich[4], D. Goodlett[5], E. Yi[5], H. Lee[5], S. Nelson[3] and L. Pohl[1]. *[1]LMI, NHLBI/NIH/HHS, Bethesda, MD, [2]Bristol-Myers Squibb, Syracuse, NY, [3]University of Washington, Seattle, WA, [4]NCI/NIH/HHS, Bethesda, MD and [5]Institute for Systems Biology, Seattle, WA.*

DILD causes significant morbidity and mortality and impairs new drug development. Recent evidence suggests that DILD is a complex polygenetic disease. Previously, we reported on differences in susceptibility to acetaminophen (APAP)-induced liver disease using various strains of mice. Of the strains studied we found C57Bl/6 (B6) and SJL mice to be the most susceptible and resistant, respectively. Global hepatic gene expression profiling following APAP administration revealed an elevated mRNA expression of heat shock proteins (HSPs) 40 & 70 in SJL mice as compared to B6 mice. We have now further characterized susceptibility differences between SJL and B6 mice. SJLxB6-F1 hybrid mice developed an intermediate toxicity phenotype and intermediate expression profiles of HSPs 40 & 70 as compared with its parental strains. Further expression profile analyses revealed that several other potential hepatoprotective genes including HSPs 10, 86, and 105 and transcription factors involved in regenerative processes, such as c-jun, LRG-21, and Egr1, were more highly expressed in SJL and SJLxB6-F1 mice than B6 mice. Quantitative proteome analysis of livers from SJL and B6 mice before and after treatment with APAP, using isotope-coded affinity tag (ICAT) mass spectrometry, revealed that SJL mice have higher levels of several potentially important protective factors including glutathione peroxidase, GST Mu 1 & 2 and thioredoxin 2. There also appears to be a loss of many mitochondrial proteins from the livers of B6 mice, suggesting that the loss of functional mitochondria may indeed play a role in APAP-induced liver injury. These findings support the use of genomic and proteomic analyses of susceptible and resistant mouse strains in identifying susceptibility factors for DILD. (Support to SN from the UW NIEHS sponsored Center for Ecogenetics and Environmental Health: NIEHS P30ES07033).

66 IN SILICO PREDICTION OF HEPATOTOXICITY OF DRUGS IN HUMANS USING POST-MARKET DATA AND MCASE SOFTWARE.

E. J. Matthews, N. L. Kruhlak, R. D. Benz and J. F. Contrera. *USFDA, Rockville, MD.*

FDA/CDER is conducting a pilot study to determine whether in silico methods can predict the potential adverse effects of drugs in humans. The study is using several structure activity relationship (SAR) software programs and adverse drug reaction (ADR) reports in CDER's post-market Spontaneous Reporting System and Adverse Event reporting System databases, Agency archives, and drug labeling. This presentation describes results of an investigation to estimate hepatotoxicity adverse effects (AEs) of drugs in humans using MCASE software (MultiCASE, Inc.) and our ICSAS Adverse Effect database (see related abstract). MCASE reduces the molecular structures of organic chemicals to 2–10 atom fragments, compares fragments of active and inactive molecules, and identifies fragments only present in active molecules. The investigation used our database to construct 14 FDA hepatotoxicity database modules and employed 46, 497 hepatotoxicity ADR reports for 47 liver adverse effect (AE) endpoints. A significant hepatotoxicity AE required: ≥4 ADR reports/generic drug and >20, 000 cases shipped/first 5-year of marketing; an AE Index (AEI) ≥4.0; and ≥1 biologically significant hepatotoxicity decision alert present in ≥2 concordant hepatotoxicity endpoints. The AEI estimates drug usage and human exposure and is calculated as: (# ADR report)/(# 5-year units shipped) x (1, 000, 000). Results demonstrated that significant hepatotoxicity was restricted to a small subset of the drugs in our database (~20%) and only 6.7% of the drugs had concurrent liver enzyme, obstruction, and pathology AEs. Internal validation studies and an external validation study using 47 drugs being considered by the FDA/PhRMA Hepatotoxicity Workgroup showed that the MCASE program and FDA expert rules could be used to accurately predict hepatotoxicity of drugs. The experiments exhibited high positive predictivity (75–98%) and specificity (88–99%). This presentation also discusses the issues of low sensitivity of the current model (27–45%) and possible applications of the model.

67 GENOMIC ANALYSIS OF THE HEPATOPROTECTIVE ROLE OF N-ACETYL-L-CYSTEINE.

M. L. Adams[1], M. F. Radonovich[2], J. N. Brady[2] and L. R. Pohl[1]. *[1]Molecular and Cellular Toxicology Section, Laboratory of Molecular Immunology, National Heart Lung & Blood Institute, National Institutes of Health, Department of Health & Human Services, Bethesda, MD and [2]Virus Tumor Biology Section, National Cancer Institute, National Institutes of Health, Department of Health & Human Services, Bethesda, MD.*

N-acetyl-L-cysteine (NAC) is a well established therapeutic agent commonly used as a mucolytic as well as an antidote for acetaminophen-induced hepatotoxicity. More recently, NAC has been investigated as treatment for other forms of hepatic failure or prophylaxis for ischemia-reperfusion injury and radiocontrast media-induced nephropathy. Although the protective effects of NAC are believed to be mediated in part by its conversion into glutathione, the molecular basis of the pharmacological activity of NAC remains unclear. In an effort to help resolve this problem, the genome-wide changes in hepatic mRNA expression of wild type C57Bl/6J mice 3h after treatment with NAC (450 mg/kg, i.p.) was investigated with the use of Affymetrix Murine Genome U74v2 Set GeneChip® Array (36, 767 probe sets on three sub-arrays). The results were compared to the expression data obtained following PBS vehicle treatment. Using the microarray analysis program GeneSpring, a gene list of 1, 594 genes with either at least two fold increase (1, 103 genes) or decrease (491 genes) in expression level was generated. Several gene categories of interest, including cell cycle regulation, acute phase response, calcium binding proteins, cell adhesion molecules, chaperone proteins, and growth factors were effected by NAC treatment. Additionally, changes were observed in glutamate-cysteine ligase modifier subunit, caspase-I, glutathione peroxidase-3, hepatocyte growth factor, as well as metallothioneins 1, and 2. These findings suggest that the protective effects of NAC are likely due to its modulation of multiple networks involved in cellular homeostasis.

68 PATHWAYS OF FIBROSIS CHARACTERIZED *IN VITRO* WITH ORGAN SLICES FROM RAT AND HUMAN TISSUE.

A. E. Vickers[1], M. J. Saulnier[1], R. Fisher[2], E. Cruz[1], K. Rose[1] and P. Olinga[3]. *[1]Biomarker Development, Novartis Pharmaceuticals Corp, E Hanover, NJ, [2]Vitron Inc., Tucson, AZ and [3]Department of Pharmacokinetics & Drug Delivery, University of Groningen, Groningen, Netherlands.*

The extension of organ slice *via*bility and morphology for 4-10 days with rat and human tissue expands this *in vitro* model for studying pathways of fibrosis. The contributing factors to increase organ slice survival include the use of a preservation solution, like UW, to perfuse the organ and prepare the slices, use of a cellulose insert atop the titanium mesh roller-insert to support the slice, and an optimized culture medium which is replaced daily to mimic multiple dosing. The slices are metabolically active as indicated by the maintenance of ATP and glutathione, and the synthesis of glycogen. This *in vitro* methodology triggers fibrogenic pathways as shown by the activation of stellate cells, portal and random proliferation of myofibroblast-like cells, and collagen deposition after 48 hr. An activation of stellate cells was characterized by the increased expression of the known stellate cell marker genes, α-smooth muscle actin, collagen 1a1, desmin, and HSP47 using RT-PCR. Global gene expression profiling studies using Affymetrix gene chip arrays revealed an increased expression of many cytoskeleton genes including collagens and actins, as well as protein synthesis genes, while metabolic genes were repressed. The proliferation of myofibroblast-like cells was indicated by increased PCNA staining and immunohistochemical α-smooth muscle actin staining around the vessels initially and in the tissue by 72-96 hr. Increased collagen deposition was also demonstrated. The recent improvements in organ slice culturing methods, and the 3-dimensional cellular complexity of this system, allows its use as an *in vitro* model system to study pathways of fibrosis, and can potentially be extended to relate changes to the mechanisms of fibrotic liver diseases.

69 COMPLEMENTARY ROLES OF FARNESOID X RECEPTOR, PREGNANE X RECEPTOR, AND CONSTITUTIVE ANDROSTANE RECEPTOR IN PROTECTION AGAINST BILE ACID TOXICITY.

G. L. Guo[1], G. lambert[2], M. Negishi[3], J. M. Ward[4], H. Brewer[5], S. A. Kliewer[6], C. J. Sinal[1,7] and F. J. Gonzalez[1]. *[1]Laboratory of Metabolism, NCI\NIH, Bethesda, MD, [2]INSERM U539, Nantes, France, [3]Pharmacogenetics Section, Laboratory of Reproductive and Developmental Toxicology, NIEHS/NIH, Research Triangle Park, MD, [4]Veterinary and Tumor Pathology Section, NCI\NIH, Frederick, MD, [5]Molecular Disease Branch, NHLBI\NIH, Bethesda, MD, [6]University of Texas Southwestern Medical Center, Dallas, TX and [7]Dalhousie University, Halifax, NS, Canada.*

The nuclear receptors, farnesoid X receptor (FXR) and pregnane X receptor (PXR), are important in maintaining bile acid homeostasis. Deletion of both FXR and PXR *in vivo* by crossbreeding B6;129-Fxrtm1Gonz (FXR-null) and B6;129-

Pxrtm1Glaxo-Wellcome (PXR-null) mice revealed a more severe disruption of bile acid, cholesterol and lipid homeostasis in B6;129- Fxrtm1Gonz Pxrtm1Glaxo-Wellcome (FXR-PXR double null or FPXR-null) mice fed a 1% cholic acid (CA) diet. Hepatic expression of the constitutive androstane receptor (CAR) and its target genes were induced in FXR- and FPXR-null mice fed the CA diet. To test whether up-regulation of CAR represents a means of protection against bile acid toxicity to compensate for the loss of FXR and PXR, animals were pretreated with CAR activators, phenobarbital (PB) or 1, 4-bis[2-(3, 5-dichlorpyridyloxy)]benzene (TCPOBOP), followed by the CA diet. A role for CAR in protection against bile acid toxicity was confirmed by a marked reduction of serum bile acid and bilirubin concentrations, with an elevation of the expression of the hepatic genes involved in bile acid and/or bilirubin metabolism and excretion (CYP2B, CYP3A, MRP2, MRP3, UGT1A, and GSTalpha), following pretreatment with PB or TCPOBOP. In summary, the current study demonstrates a critical and combined role of FXR and PXR in maintaining not only bile acid, but also cholesterol, and lipid homeostasis *in vivo*. Furthermore, FXR, PXR and CAR protect against hepatic bile acid toxicity in a complementary manner, suggesting that they serve as redundant but distinct layers of defense to prevent overt hepatic damage by bile acids during cholestasis.

70 UROPORPHYRIA CAUSED BY ETHANOL IN HFE(-/-) MICE OF DIFFERENT GENETIC BACKGROUNDS.

P. Sinclair[1, 2], N. Gorman[1, 2], H. Trask[1, 2], W. Bement[1], A. Zaharia[1, 2], J. Szakacs[3], G. Elder[4], D. Balestra[2], J. Sinclair[1, 2] and G. Gerhard[5]. *[1]VA Medical Center, White River Junction, VT, [2]Dartmouth Medical School, Hanover, NH, [3]Pathology, University of Utah Medical School, Salt Lake City, UT, [4]Medical Biochemistry, University of Wales Medical Schl, Heath Park, Wales, United Kingdom and [5]Weis Ctr Research, Danville, PA.*

Two major risk factors for the development of porphyria cutanea tarda (PCT) are alcohol consumption and homozygosity for the C282Y mutation in the hereditary hemochromatosis gene (HFE). We previously demonstrated that Hfe(-/-) mice of 129S6/SvEvTac background accumulate hepatic uroporphyrin (URO) when fed 10 per cent ethanol in their drinking water for 6.5 months. Here we show that increasing the ethanol concentration from 10 to 15 per cent decreased the time to onset of the uroporphyria, and further increased hepatic 5-aminolevulinate synthase activity as well as CYP1A2-catalyzed activities. There were no increases in non-heme iron. Treatment of C57BL/6 Hfe(-/-) mice with 15 per cent ethanol for 6.5 months failed to produce uroporphyria. In these latter mice, hepatic non-heme Fe was increased by the ethanol, but the total amount was much less than in 129S6 mice. Ethanol treatment of Hfe(-/-) mice of both strains caused increases in diffuse iron staining of parenchymal cells with more staining in 129 than C57BL/6 mice. Iron staining of either strain was no greater with 15 than with 10 per cent ethanol. The increase in ALAS activity caused by 15 per cent ethanol was similar in Hfe knockout mice of either background. Administration of Fe dextran, but not of Fe carbonyl, to wild-type 129S6 mice treated with 10 or 15 per cent ethanol for 6-7 months, resulted in hepatic URO accumulation. Fe dextran only increased ALAS in wild-type S6 and Hfe(-/-) mice also treated with ethanol. In conclusion, the results of this study further support the hypothesis that the uroporphyrinogenic effect of ethanol is mediated by its effects on hepatic iron metabolism and localization. Other genetic factors than Hfe appear to modify host susceptibility to ethanol-mediated uroporphyria. This work was supported by the Department of Veterans Affairs.

71 PREDICTING THE CARCINOGENIC POTENTIAL OF PHARMACEUTICALS AND CHEMICALS USING MOLECULAR SIMILARITY, E-STATE INDICES AND MDL-QSAR SOFTWARE.

J. F. Contrera[1]. *[1]Office of Pharmaceutical Science, USFDA Center for Drugs, Rockville, MD, [2]Office of Pharmaceutical Science, USFDA Center for Drugs, Rockville, MD and [3]Office of Pharmaceutical Science, USFDA Center for Drugs, Rockville, MD.*

MDL QSAR software is one of several software systems under evaluation by the Informatics and Computational Safety Analysis Staff (ICSAS) of the FDA Center for Drug Evaluation and Research for regulatory and scientific decision support applications. MDL QSAR software contains an integrated set of tools for similarity searching, compound clustering and modeling of molecular structure related parameters that include 240 electrotopological E-state, connectivity and other descriptors. These molecular descriptors can be used to develop structure activity relationship (SAR) models that are statistically correlated with toxicological or biological endpoints. The goal of this research was to evaluate the feasibility of using MDL QSAR software and descriptors to develop SAR models to predict the carcinogenic potential of pharmaceuticals and organic chemicals. A validation study of 108 compounds that include 86 pharmaceuticals and 22 chemicals that were not present in a control rodent carcinogenicity learning data set of 1275 compounds demonstrated that MDL QSAR models had excellent coverage (92%) and good sensitivity (74%) and specificity (72%) for rodent carcinogenicity. The software correctly predicted 73 % of non-carcinogenic compounds and compounds with carcinogenic findings. E-state descriptors contributed to more than half of the SAR models used to predict carcinogenic activity. The recent addition of non-parametric discriminant analysis functions to MDL QSAR software has further improved predictive performance to the 90% range. We believe that electrotopological E-state descriptors and MDL QSAR software are promising new in silico approaches for modeling and predicting rodent carcinogenicity that has wider application for other toxicological endpoints. Preliminary results suggest that this approach can also be used to estimate the maximum therapeutic dose and no effect dose level.

72 A MODEL TO ASSESS THE TUMORIGENIC POTENTIAL OF NATALIZUMAB (NAT), A RECOMBINANT HUMANIZED ANTI-α4 INTEGRIN ANTIBODY.

J. V. Rutkowski[1], D. Lepage[1], D. Hutto[1], N. Wehner[2], Y. Maxuitenko[3], J. Heath[3], M. Koratich[3], C. Tenhoor[1] and J. Green[1]. *[1]Biogen, Cambridge, MA, [2]Elan Pharmaceuticals, San Diego, CA and [3]Southern Research Institute, Birmingham, AL.*

NAT binds to the α4 subunit of α4β1 and α4β7 integrins and blocks interaction with their receptors, fibronectin, VCAM-1 and MadCAM-1. α4 expression is present on a variety of human tumors including melanomas and lymphomas. An alternative approach to the assessment of potential carcinogenicity of this antibody was undertaken since NAT lacks cross-reactivity to rodent α4. Thus, the potential for NAT to either inhibit or potentiate human tumor cell growth and/or metastases was evaluated. A number of human tumor cell lines were screened for expression of α4 by IHC and flow cytometry and functionality was confirmed by an *in vitro* binding assay. Any cytotoxicity or induction of cell proliferation of the human tumor cells was evaluated by ^{3}H-thymidine incorporation. Cell lines for *in vivo* evaluation were selected based on the composite *in vitro* data. Either MOLT-4 or UACC-257 human tumor cells were implanted s.c. in the athymic nude or SCID mouse, respectively. Treatment with NAT or control material was initiated prior to or following establishment of a preformed tumor. In each instance, NAT was administered with a loading dose of 10 mg/kg and subsequent doses at 5 mg/kg, twice per week. To assess the effects of NAT on the growth of metastasis, one arm of the study design included a surgical excision of the tumors at an estimated size of 1 g from selected animals. All animals were sacrificed and evaluated for tumor regrowth and metastases by macroscopic and microscopic evaluation. Administration of NAT was well tolerated without deaths or body weight loss and did not affect tumor incidence or latency. Enhancement of xenograft growth rate was not evident. Extension of xenograft regrowth following excision was noted in the ventral abdominal skin. Metastasis or extension of the xenograft was not observed in any other tissue or organ. In summary, there was no evidence of exacerbation of regrowth or metastasis as a result of treatment with NAT.

73 ORAL TOXICITY AND ANGIOSTATIC POTENCY OF ANTI-VEGF DRUGS ZD-6474, ZK 222584, AND SU11248 IN MICE.

D. A. Badger[1], J. M. Holland[1], T. C. Malone[3], S. R. Vanapalli[1], J. L. Edelman[2] and G. W. DeVries[2]. *[1]Safety Evaluation, Allergan, Irvine, CA, [2]Biological Sciences, Allergan, Irvine, CA and [3]Medicinal Chemistry, Allergan, Irvine, CA.*

Based on their anti-VEGF receptor activity, ZD-6474 (ZD), ZK 222584 (ZK), and SU11248 (SU) are currently being developed for the treatment of cancer. This study determined if these structurally diverse drugs share common preclinical toxicity profiles. CD-1 mice (3 males/group) were given 50, 150, or 500 mg/kg/day ZD, ZK, or SU once daily by oral gavage (10 mL/kg/day in 2% carboxymethylcellulose) for 10 consecutive days. Viability, clinical observations, body weight, drug plasma concentrations, and histopathology were evaluated. Mortality associated with lethargy and dehydration was observed in mice given 500 mg/kg/day ZD, ZK, or SU. Mean drug plasma concentrations in mice given 150 mg/kg/day determined 1 hr post dose on day 11 were 4510 ng/mL ZD, 7680 ng/mL ZK, and 1260 ng/mL SU. Renal histopathology included tubular degeneration (ZD; ZK; SU) and glomerulonephropathy (ZK; SU). Heart lesions included coronary vasculitis (ZD; SU), myocardial necrosis (SU) and myocardial hemorrhage (ZK; SU). Other lesions included hepatic hypertrophy (ZK; SU), hepatic vacuolation (ZD; ZK), lymphatic atrophy (ZD; ZK; SU), thymic atrophy (ZD; ZK; SU) and myeloid atrophy (ZD; SU). In general, drug toxicology profiles correlated with pharmacologic potency. Anti-angiogenic activity, determined by epiphyseal thickness at day 10, showed rank order efficacy of SU>ZD>ZK. In contrast, rank order pharmacologic potency using *in vitro* (kinase inhibition) and *in vivo* (Miles assay, 2 hrs post-dose) assays was ZK>SU>ZD. The reduced toxicity and lack of anti-angiogenic activity observed with ZK in the current study may be explained by pharmacokinetic/pharmacodynamic differences among models (a similar lack of activity was observed in a chronic rat model of choroidal neovascularization). Overall, these results suggest that the effects observed are likely related to pharmacological activities, although some chemical specific toxicity might be occurring.

74 SUBCHRONIC ORAL TOXICITY/ENZYME MODULATION STUDY OF FARNESOL IN RATS.

T. Horn[1], L. Long[1], M. Cwik[1], W. Johnson[1], R. Morrissey[2], I. Kapetanovic[3] and D. McCormick[1]. *[1]IIT Research Institute, Chicago, IL, [2]Pathology Associates, Chicago, IL and [3]National Cancer Institute, Bethesda, MD.*

Farnesol is a naturally occurring phytochemical that inhibits cholesterol synthesis by suppressing 3-hydroxy-3-methylglutaryl CoA reductase. Farnesol has anticarcinogenic activity in animal models for human cancer, and is in preclinical development for cancer chemoprevention. The present study was performed to evaluate the toxicity of subchronic oral administration of farnesol in rats, and to determine the influence of farnesol on the activity of hepatic and renal drug metabolizing enzymes. CD rats (40/sex/group) received daily gavage exposure to farnesol (in corn oil) at doses of 0, 500 or 1000 mg/kg/day for 28 days; parallel groups of rats were necropsied on day 29 and on day 57 (after a 28-day recovery period). No deaths occurred during the study, and farnesol had no effects on body weight, food consumption, or hematology or coagulation parameters. Farnesol exposure was associated with statistically significant alterations in several clinical chemistry parameters; all clinical chemistry effects were reversible. At day 29, statistically significant increases in the absolute and relative weights of the liver and kidney were seen in farnesol-treated rats. These organ weight effects were not associated with any histopathologic alterations, and were not seen at day 57. Organ weight effects may be secondary to enzyme induction, as the activities of several drug metabolizing enzymes were elevated at day 29 in the liver (CYP2E1, glutathione reductase, NADPH quinone reductase) and kidney (glutathione-S-transferase). The influences of farnesol on the activity of drug metabolizing enzymes were reversible; enzyme activities did not differ from control after the 28-day recovery period. No evidence of limiting toxicity was observed at either farnesol dose used in the study. Subchronic administration of farnesol induced dose-related alterations in liver and kidney weights and increased drug metabolizing enzyme activities. These effects were reversible. (NCI-N01-25012)

75 A TOXICITY EVALUATION OF HUMANIZED ANTI-CD20 ANTIBODY PRO70769.

K. P. McKeever[1], T. Watson[1], J. Beyer[1], L. Nguyen[1], B. Wu[1], P. Fielder[1], K. Howell[1], F. Qureshi[1], D. Auyeung[2], H. Lowman[1] and Y. Vugmeyster[1]. *[1]Genentech, Inc., South San Francisco, CA and [2]CRL DDS Sierra Division, Sparks, NV.*

PRO70769 is a humanized monoclonal antibody against CD20 present on normal and malignant B cells. PRO70769 is being evaluated for treatment of B cell-mediated diseases. To assess the safety and pharmacology of PRO70769, cynomolgus monkeys were administered drug (iv) on Days 1 and 15 with 10, 50 and 100mg/kg of PRO70769 (total dose of 20, 100, and 200mg/kg) and euthanized 2 weeks after the 2nd dose. Animals from control and high dose groups were also assessed for reversibility, persistence, or delayed occurrence of toxicity after 12 weeks of recovery. Flow cytometric analysis of peripheral blood showed a dose dependent decrease in B cells by Day 2. Decreased absolute lymphocyte counts also reflected this change, as compared to baseline values. Due to maximal depletion of B cells prior to the second dose, there was no evidence of additional drug-related decreases in absolute lymphocyte counts on Day 15. On Day 29, B cells remained depleted at 6.2%, 2.2% and 1.5% of baseline. By Day 113, B cells from the high dose animals had recovered to an average of 19.8% of baseline. Flow cytometric analysis of lymphoid tissues on Day 29 revealed that B cell numbers were also reduced, as compared to the control group: 39-52% in the bone marrow; 2-14% in the spleen; 13-34% in the inguinal lymph nodes; and 5-21% in the mandibular lymph node. Histologically, lymphoid atrophy was apparent in the spleen and lymph nodes. Depletion of B cells in these organs was confirmed by immunohistochemistry. There were no apparent PRO70769-related changes in clinical signs, physiological indices, physical examinations, or clinical chemistry parameters. Furthermore, there were no changes in gross pathology or non-lymphoid organ weights related to the administration of PRO70769. The results of this study demonstrate the ability of PRO70769 to decrease CD20+ B cells in the blood and lymphoid tissues, with pharmacologic depletion of CD20-expressing cells being the only treatment-related effect.

76 EFFECTS OF CHRONIC PERTUZUMAB-MEDIATED HER2 PATHWAY INHIBITION.

K. M. Towndrow[1], N. Dybdal[1], L. Nguyen[1], D. Allison[1], F. Qureshi[1], L. Bernier[2] and K. P. McKeever[1]. *[1]Genentech, Inc., S. San Francisco, CA and [2]Covance, Inc., Vienna, VA.*

Human epidermal growth factor receptors (HER) are important mediators of cell growth, survival, and differentiation. Pertuzumab, a monoclonal antibody, blocks dimerization of HER2 and other HER family members, including epidermal growth factor receptor (EGFR), HER3, and HER4. Since pertuzumab is being developed as a cancer therapeutic, the effects of chronic exposure of monkeys to 15, 50, or 150 mg/kg (weekly i.v.) pertuzumab for 26 weeks, followed by an 8-week recovery period, were assessed. No drug-related changes in body weight, organ weight, body temperature, blood pressure, respiration rate, electrocardiograms, serum testosterone or serum troponin values were observed after pertuzumab administration. In addition, no drug-related histomorphologic findings occurred after either the 26-week treatment or following an 8-week recovery period and no positive antibody titers to pertuzumab were detected. An increased incidence of diarrhea occurred in all treatment groups, including control animals. Diarrhea was more frequent, but not dose-dependent, in pertuzumab-treated monkeys. All monkeys survived until scheduled necropsy except for one 50 mg/kg group female that was euthanized on Day 126 due to morbidity that was likely the result of dehydration caused by persistent diarrhea, unresolved with basic supportive care. Furthermore, no drug-related findings were observed during necropsy and histopathology examinations of this animal. One 15 mg/kg group male exhibited poor health during Week 12 and had recurring, transient episodes of diarrhea throughout the study; both of which required supportive care. Similar clinical observations were noted for one 150 mg/kg group female during Week 26. While there was a trend towards pertuzumab-related increases in diarrhea and dehydration, a direct relationship could not be determined or excluded. Aside from incidences of diarrhea and dehydration, chronic pertuzumab-mediated inhibition of the HER2 pathway was generally well tolerated in monkeys.

77 PRECLINICAL SAFETY ASSESSMENT OF A HUMAN LYMPHOTOXIN BETA RECEPTOR IMMUNOGLOBULIN FUSION PROTEIN IN CYNOMOLGUS MONKEYS FOLLOWING REPEATED INTRAVENOUS AND SUBCUTANEOUS DOSING.

C. Sachs[1], G. Beattie[2], J. Gommerman[1], C. Chan[2], J. Browning[1], W. Meier[1], P. L. Martin[1] and J. D. Green[2]. *[1]Biogen, Cambridge, MA and [2]Charles River DDS Sierra Division, Sparks, NV.*

Lymphotoxin αβ, a TNF family member, plays a crucial role in the maintenance of the lymphoid microenvironment. Antagonism of this pathway has been efficacious in several animal models of immune disease. Cynomolgus monkeys (3-7/sex/group) were administered human lymphotoxinβ immunoglobulin fusion protein (hLTBR-IgG) weekly at doses of 0, 0.8, 8 and 40 mg/kg by slow bolus intravenous (IV) injection or at 8 mg/kg by subcutaneous injection for 4 consecutive weeks. Necropsies were performed after 4 weeks of dosing, and 8 and 12 weeks after cessation of dosing. To evaluate functional follicular dendritic cell (FDC) activity (trapping of immune complexes) a subset of animals was administered an IV injection of goat peroxidase-anti-peroxidase immune complexes (PAP) 24 h prior to necropsy. There were no LTBR-IgG-related changes in clinical signs, body weight, clinical chemistry, coagulation, electrocardiographic, or occular evaluations. No target organs of toxicity were identified in routine histopathology. Immunohistopathology revealed LTBR-IgG-related decreases in FDC marker staining and PAP staining in the spleen after 4 weeks of treatment consistent with down regulation of FDC networks and function (immune complex trapping). Despite these changes in the FDC network, the primary immune response to keyhole limpet hemocyanin was not impaired. Recovery of staining for CD35 and PAP was observed during the 12-week treatment-free period. All doses were well tolerated and produced no evidence of toxicity. The results of this study supported the initiation of clinical trials.

78 SAFETY AND BIODISTRIBUTION OF A MULTIPLE STRAIN EBOLA GENE DNA PLASMID VACCINE (VRC-EBODNA012-00-VP) IN THE NEW ZEALAND WHITE RABBIT.

T. S. Manetz[1], J. Stein[3], R. Sheets[2], G. Wolfe[1], C. Duffy[4] and P. Gomez[2]. *[1]Gene Logic, Gaitherburg, MD, [2]Vaccine Research Center of NIH/NIAID, Bethesda, MD, [3]Consultant to the Vaccine Research Center, Ann Arbor, MI and [4]Althea Technologies, Inc., San Diego, CA.*

These studies outline the pre-clinical safety studies for a candidate Ebola vaccine that is a recombinant multi-DNA plasmid vaccine (VRC-EBODNA012-00-VP). Previously published studies by Nabel and colleagues of the VRC showed protection in non-human primates to a prime/boost regimen of DNA prime and adenoviral vector boost. These studies evaluated the intramuscular safety and biodistribution of VRC-EBODNA012-00-VP in New Zealand White Rabbits. For the biodistribution study, rabbits received control or VRC-EBODNA012-00-VP by single Biojector 2000® (Biojector) injection on Study Day (SD) 1 and were necropsied for biodistribution analysis on SD 8, 30, and 60. For the safety study, rabbits received PBS or VRC-EBODNA012-00-VP by Biojector injection on SD 1, 22, 43, and 64, with necropsy on SD 66 and 78. Parameters evaluated include

mortality, clinical and Draize observations, body weights, food consumption, ophthalmology, immunogenicity, clinical pathology, organ weights, bone marrow, gross pathology, and histopathology. Biodistribution analysis determined the test article was present in injection site muscle and subcutis. Frequency and/or copy number progressively decreased over time, suggesting plasmid clearance. All animals treated with VRC-EBODNA012-00-VP had increased antibody titers to the Ebola vaccine epitopes, demonstrating vaccine immunogenicity. Increased recoverable Draize findings at the injection site were found in VRC-EBODNA012-00-VP-treated animals following the third and fourth injections. These likely resulted from the injection procedure and expected immune response against the transgene. Therefore, under these study conditions, repeat intramuscular administration of VRC-EBODNA012-00-VP to New Zealand White Rabbits did not exhibit any specific signs of systemic toxicity but resulted in increased levels of reversible Draize observations following the third and fourth injections.

79 A LONG-TERM IMMUNOGENICITY AND SAFETY STUDY OF AN IMMUNONEUTRALIZING VACCINE TARGETED TO INHIBIT CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) IN RABBITS.

L. J. Thomas, M. D. Picard, E. G. Linnetz, K. M. Borrelli, R. A. Hammond and C. W. Rittershaus. *AVANT Immunotherapeutics, Needham, MA.*

CETP is a plasma glycoprotein that facilitates the transfer of neutral lipids and phospholipids between lipoproteins and contributes to the regulation of the plasma concentration of HDL. An immunoneutralizing vaccine targeted against CETP (designated CETi-1) was developed to reduce the lipid transfer function of CETP by eliciting antibodies to bind to CETP. CETi-1 is a synthetic chimeric peptide, including residues 461-476 of human CETP and residues 830-843 of tetanus toxoid, formulated with an alum adjuvant. CETi-1 has been shown to raise HDL, decrease LDL and reduce atherosclerotic lesions in a cholesterol-fed rabbit model. This vaccine could be administered to patients periodically over extended periods of time as a preventive measure or as a therapy for atherosclerosis. Consequent to its intended use, a study was conducted to determine the levels and duration of the immunogenicity of this vaccine when administered repeatedly to rabbits over more than four years. Male NZW rabbits were primed with a single injection of either 0.1ug, 1.0ug, 10ug, 100ug, or 1000ug of CETi-1 vaccine on week 1. The animals were boosted with the same dose of vaccine on weeks 67, 109, 135, 164, and 195. Blood samples were taken periodically up to the end of the study on week 223. Blood samples were evaluated by ELISA for antibodies to CETP and endpoint titers were determined. As expected, anti-CETP antibody titers in all rabbits were low following the first immunization, with none exceeding 1/200. For animals in each dose group, antibody titers increased with successive boosts. Upon repeated boosting the vaccine did not lose its ability to induce an immune response. Antibody titers were generally dose-dependent and returned to baseline levels over time. This data would support the concept that this vaccine would maintain, or improve, its immunogenicity when repeatedly administered. Furthermore, there were no observations of toxicity attributable to prolonged autoreactive anti-CETP antibody titers in these animals.

80 EFFECT OF OIL COMBUSTION PARTICLE BIOAVAILABLE CONSTITUENTS ON *EX VIVO* VASCULAR FUNCTION OF AORTAE RECOVERED FROM HEALTHY AND EARLY TYPE 2 DIABETIC RATS.

K. Dreher[1], S. E. Kelly[2], S. D. Proctor[2] and J. C. Russell[2]. [1]*USEPA, Research Triangle Park, NC and* [2]*University of Alberta, Edmonton, AB, Canada.*

Epidemiological studies have reported statistical associations between air particulate pollution exposure and alterations in cardiovascular function particularly in individuals with recent myocardial infarctions or Type 2 diabetes. However, the physicochemical properties of particles and biological mechanisms responsible for these observations are not known. This study examines the ability of soluble constituents of air pollution particles to alter vascular function in arteries recovered from healthy and Type 2 diabetic JCR:LA-cp rats. Aortic rings recovered from healthy and diabetic rats were maintained in oxygenated Krebs Henseleit buffer at 37°C. Contractile force was measured for each aortic ring following exposure to various doses of a particle-free leachate of residual oil fly ash (ROFA-L) (1.5 to 12.5 μg/ml) and phenylepherine (PE) (10^{-9} to 10^{-3} M). ROFA-L exposure produced a dose dependent increase in PE-mediated vascular contractility in the aortae from both healthy and diabetic rats. However, at the lowest concentration of ROFA-L (1.5 μg/ml) diabetic aortae displayed a greater PE-induced contractile response when compared to aortae recovered from healthy rats. Interestingly, a very strong ROFA-L induced vascular contractile response was observed only in diabetic aortae following the initial ROFA-L exposure with PE stimulation. Finally, acetylcholine-mediated vascular relaxation was inhibited to a greater extent in diabetic aortae when compared to healthy aortae following exposure to higher ROFA-L doses (6.25 and 12.5 μg/ml). Our results demonstrate that: 1) bioavailable constituents of oil combustion particles can directly impair vascular function; 2) exposure history and disease status maybe critical to this response; and 3) aortae from Type 2 diabetic rats were found to be more sensitive to alterations in vascular responsiveness following exposure to bioavailable constituents associated with oil combustion particles. (This abstract does not reflect EPA Policy)

81 VANADIUM EXPOSURE ALTERS SPONTANEOUS BEAT RATE AND GENE EXPRESSION OF CULTURED CARDIAC MYOCYTES.

D. W. Graff[1], R. B. Devlin[1], L. A. Dailey[1] and W. E. Cascio[2]. [1]*NHEERL, USEPA, Research Triangle Park, NC and* [2]*Cardiology, University of North Carolina, Chapel Hill, NC.*

Ambient air pollution particulate matter (PM) exposure is associated with increased morbidity and mortality. Recent toxicological studies report PM-induced changes in a number of cardiac parameters, including heart rate variability, arrhythmias, repolarization, and internal defibrillator discharges. In this study we identify cellular processes that might contribute to cardiac dysfunction after PM exposure. Isolated neonatal rat ventricular myocytes were cultured for 11 days and then exposed to different concentrations of vanadium (V), a common soluble PM component. A 30 min exposure to V decreased spontaneous myocyte beat rate by 10% compared to baseline, an effect that persisted at 1, 2, 4, and 24 hrs. To account for this decrease in beat rate, we used real-time PCR to test for changes in gene expression of several ion channels that contribute to repolarization, gap junction proteins (mediators of intercellular communication), and markers of inflammation following 6 and 24 hrs of V exposure. A 6 hr exposure did not affect connexin or ion channel gene expression but increased expression of IL-6, IL-1a, and HSP70 1.5-2-fold. A 24 hr exposure produced 1.5-2-fold increases in gene expression of the gap junction protein connexin 43, IL-6, and IL-1a, and a 50% decrease in the K+ channel coded for by the gene KvLQt1. These data suggest that soluble metals found in air pollution particles could contribute to PM-associated cardiac morbidity and mortality by affecting the function of cardiac myocytes. These effects may be triggered by an inflammatory response or by the remodeling of proteins associated with cellular communication and/or repolarization. This abstract does not necessarily reflect EPA policy.

82 SUBCHRONIC HEALTH EFFECTS OF CONCENTRATED AMBIENT PARTICULATE MATTER (CAP).

L. Chen[1], J. Hwang[1, 2], C. Nadziejko[1] and M. Lippmann[1]. [1]*Environ Med., NYUSOM, Tuxedo, NY and* [2]*Statistical Science, Academia Sinica, Taipei, Taiwan.*

Several epidemiologic cohort studies indicate that people who live in areas with elevated PM2.5 experience significantly reduced longevity in addition to acute effects. In this study, we hypothesized that subchronic exposure of normal and compromised mice to CAPs causes cumulative adverse effects on the respiratory and cardiovascular systems. A particle concentrator was used to expose mice to filtered air (FA) or CAPs for 6h/d, 5d/w, for up to 6 months to the regional background aerosol in Tuxedo NY. A cohort of C57 mice (n=17/group) was used to investigate respiratory effects. In addition, C57 (n=6/group) and ApoE knockout (n=9/group) mice, implanted with ECG transmitters, were used to investigate cardiovascular effects. A separate cohort of double knockout mice (DK, ApoE and LDLr knockout, n=6/gender/group) were also included to investigate histopathological changes and gene expression patterns of the cardiopulmonary systems. Preliminary results showed that CAPs (mean: 110±79 ug/m3, median: 89 ug/m3) had no effect on the lung lavage parameters in any of the mouse strains. In contrast, there were long term cumulative changes in heart rates (HR) in both C57 and ApoE after a 5 month exposure to CAPs. The pattern of alterations in HR was dependent on the strain of mice, and on the time of day when HR was measured. For example, by the end of the 5 months of exposure, for midnight to 4 am, there was a 28 beats/min reduction in HR in the ApoE, whereas the HR was increased by 22 beats/min in C57. There were also daily concentration-dependent changes in HR both during and post the CAPs exposure period in both strains of mice. In addition, several deaths occurred in both the CAPs and FA exposed DK mice. However, a log-rank test showed that CAPs did not affect the end of study survival in these mice. Taken together, our data showed that subchronic exposure to CAPs produced significant daily changes in the cardiovascular system in both healthy and health compromised animals and the effects appeared to be cumulative. Supported by: EPA (R827351) and NIEHS (ES00260).

83 EFFECTS OF INSTILLED EMISSION PARTICULATE MATTER (EPM) ON ELECTROCARDIOGRAPHIC INDICES AND HEART RATE VARIABILITY (HRV) IN SPONTANEOUSLY HYPERTENSIVE (SH) RATS.

L. Wichers[1], J. P. Nolan[2], W. H. Rowan[2], M. J. Campen[3], T. P. Jenkins[4], D. L. Costa[2] and W. P. Watkinson[2]. *[1]SPH, UNC, Chapel Hill, NC, [2]ORD/NHEERL/ETD/PTB, USEPA, Research Triangle Park, NC, [3]LRRI, Albuquerque, NM and [4]Brody SOM, ECU, Greenville, NC.*

Epidemiological studies have linked increased levels of ambient particulate matter (PM) with increased morbidity and mortality, noting higher correlations in persons with cardiovascular disease. To further investigate this phenomenon, we examined the effects of EPM (an "ambient-like" emission particle) in SH rats (a susceptible disease model). Rats were implanted with radiotelemeters capable of monitoring electrocardiogram (ECG), heart rate (HR), blood pressure (BP), and core temperature (T_{co}). Animals (n=109) were divided into four groups and exposed *via* intratracheal instillation (IT) to suspensions of EPM (0.0, 0.83, 3.33, 8.33 mg/kg) in acidified saline vehicle. Telemetered rats were monitored continuously for 96h post-IT and ECG waveforms were acquired at 10–min intervals throughout the study. Ventilatory function was examined (Buxco Electronics) concurrently for 6h/day on postexposure Days 1–4 while subsets of rats underwent bronchoalveolar lavage (BAL) at 24h, 96h, and 192h post-IT. ECG abnormalities (rhythm disturbances, premature ventricular contractions) were observed primarily in the high dose group. Detailed analyses of ECG intervals and indices of HRV showed evidence of immediate (<2 hours) increases in HRV followed by a delayed (>2 days) return to control levels. Additionally, exposure to EPM induced dose-related decreases in HR, BP, and T_{co}, that persisted for up to 72h. Significant increases in BAL indices of pulmonary injury were also noted. These studies demonstrate substantial deficits in cardiopulmonary function in SH rats after IT exposure to EPM. The observed changes in cardiopulmonary function in moderately compromised rodents support the results of previous epidemiological studies and may implicate similar biologically-plausible mechanisms in humans. (Abstract does not represent USEPA policy. This research was supported in part by EPA CT826513.)

84 ASSESSMENT OF TOXICITY OF OIL COMBUSTION EMISSION EXPOSURE IN NORMAL AND HYPERTENSIVE RATS.

M. I. Gilmour[1], University. Kodavanti[1], K. Dreher[1], M. Daniels[1], M. Schladweiller[1], Q. krantz[1], W. P. Linak[2], C. Miller[2] and D. L. Costa[1]. *[1]NHEERL, USEPA, Research Triangle Park, NC and [2]NRMRL, USEPA, Research Triangle Park, NC.*

It has been suggested that the increased morbidity and mortality associated with exposure to airborne particulates (PM) is related to materials derived from combustion sources such as gasoline, diesel, oil, wood and coal. In these studies hickory oil # 6 was burned in a residual oil combustor previously shown to produce approximately 150 mg/m3 of PM. The particles were then passed through a 2.2 mm cyclone and diluted with clean ambient air. The resulting PM was directed to a Hinners animal exposure chamber equipped for continuous monitoring of O2, CO, CO2, NOx, SO2, as well as total particle concentrations. Mean PM 2.5 concentrations ranged between 2 and 3 mg/m-3 with SO2 denuded to 2-3 ppm and 20-30 ppm NOx. Sprague-Dawley and spontaneously hypertensive (SHR) rats were exposed to the combustion atmosphere for 4 hrs for one or three days and immediately and 20 hr later assessed for changes in systemic and pulmonary markers of injury and inflammation. Exposure to the emission atmosphere caused an increase in neutrophils and BAL lysozyme in both rat strains, but no significant changes in other markers of lung injury such as protein and LDH. Increased vanadium levels detected in the plasma of CEAS-exposed Sprague-Dawley rats were associated with an elevated platelet count and decreased white blood cell numbers. Analysis of systemic biomarkers in SHR rats is in process. We conclude that these acute exposures resulted in modest levels of pulmonary inflammation in the two rat strains, and that the biological effects may be more prominent in the systemic circulation. Future studies will determine the relative contribution of gases versus particles on these responses, and will test whether animals with cardiopulmonary disease are further compromised by such exposures. (This abstract does not necessarily reflect EPA policy.)

85 MYOCARDIAL AND CARDIOVASCULAR EFFECTS FOLLOWING PULMONARY EXPOSURE TO ZINC.

P. S. Gilmour[1, 2], A. Nyska[2], M. C. Schladweiler[3], A. D. Ledbetter[3] and University. P. Kodavanti[3]. *[1]CEMALB, UNC, Durham, NC, [2]NIEHS, Research Triangle Park, NC and [3]PTB, USEPA, Durham, NC.*

Exposure to environmental particulate matter (PM) is associated with cardiovascular (CV) health effects. Although the mechanisms have yet to be determined, particle-associated metals are thought to play a role. Recent interest has focused on zinc, a major constituent of ambient and combustion particles, however the roles of pulmonary zinc exposure on coagulation factors and inflammation in the heart are unknown. We have previously shown that pulmonary exposure to zinc-containing PM causes myocardial lesions in rats. Therefore, we hypothesized that pulmonary exposure to zinc causes lung and heart thrombosis, and cardiac inflammation and injury. Adult male Sprague-Dawley and Wistar-Kyoto (WKY) rats were intratracheally (IT) instilled with 0, 1 or 2 µmol/kg of zinc sulfate (ZnSO4). Inflammation, blood parameter changes, tissue plasminogen activator (tPA), thrombomodulin (TM), and tissue factor (TF) mRNA (PCR) in heart and lung tissue were analyzed post-ZnSO4 IT. Lung and cardiac injuries were evaluated 1-96 hr post-IT. Myocardial lesions were characterized by immunohistochemical staining for troponin and apoptosis in cardiac sections. Significant increases in bronchoalveolar lavage fluid neutrophils, protein, and LDH occurred in the zinc-exposed SD rats and not in controls. TF mRNA was significantly increased in the lung of zinc-exposed SD rats. The expression of tPA and TM was significantly decreased in heart tissue of zinc-exposed SD rats. Instilled zinc took 24 hr to clear during which time the liver Zn increased while there was only a modest increase in cardiac zinc. WKY rats exposed to Zn demonstrated cardiac lesions which consisted of multi-focal myocardial degeneration, acute-active inflammation, and myocardial necrosis. WKY rats exposed to air only contained no cardiac lesions. These data suggest that zinc has significant inflammatory and procoagulative effects in the lung and heart, and may play a critical role in PM-induced CV effects. (This abstract does not reflect USEPA policy. Supported in part by #CR829522 between EPA and UNC.)

86 ULTRAFINE PARTICLE (UFP) EFFECTS ON EXPERIMENTAL THROMBOSIS: THE EAR VEIN MODEL.

V. M. Silva, N. Corson, A. Elder, R. Gelein and G. Oberdorster. *Environmental Medicine, University of Rochester, Rochester, NY.*

Photodynamic excitation of rose bengal (RB) is commonly used to study thrombosis in the femoral vein. It generates singlet oxygen leading to endothelial damage and thrombus formation. Factors affecting this response include plasma RB concentration, time post RB administration and total excitation time. Also, manipulation of the vessel itself can affect RB-induced endothelial responses. We avoided this by adapting the RB-thrombosis model to the rat ear vein. Furthermore, initial experiments were aimed at optimizing RB plasma levels and keep them stable for an extended period of time. The animal was placed on an inverted microscope stage such that ear blood vessels and flow could by visualized. After RB was given, an ear vein was illuminated (532nm, 20mW) for 5 to 120 sec and thrombosis was captured with a digital camera. Animals received either intraperitoneal (ip, 200mg/kg), intravenous (iv, 10mg/kg) or iv infused (24mg/kg/hr) doses of RB in separate experiments. Blood was collected at different times to determine the kinetics of RB concentration up to 2 hrs. Our data indicate that the iv bolus resulted in a plasma RB concentration that decreased rapidly 5 min after injection, while the ip dose caused a continuous increase in plasma even 60 min after RB administration. This change in blood concentration produced by both routes of administration affected RB-induced endothelial damage irreproducibly. In contrast, a steady state RB level of 45ug/ml plasma was reached after 30 min of continuing infusion and a consistent reaction was observed after 10 sec illumination of the ear vein. Administration of iv ultrafine polystyrene particles 30 min after start of the RB infusion shortened the laser exposure time to 5 sec. This data suggest that continuous iv infusion leads to more consistent plasma RB levels and that UFP can affect endothelial function directly. Furthermore, the advantages of using the ear model include: no manipulation of the vessel; and the availability of several veins on each ear make possible to perform several studies in the same animal.

87 TCDD FROM AGENT ORANGE IS CURRENTLY AS ELEVATED IN VIETNAM AS DURING SPRAYING 30-40 YEARS AGO: A CASE STUDY ILLUSTRATING THE PERSISTENCE OF POPS.

A. Schecter[2], H. T. Quynh[1], O. Paepke[3], R. Malisch[4], J. D. Constable[5], M. Pavuk[2] and K. Tung[2]. *[1]Cancer Research Center, Hanoi, Viet Nam, [2]Environmental Sciences, University of Texas School of Public Health, Dallas, TX, [3]ERGO Research Laboratory, Hamburg, Germany, [4]State Laboratory for Chemical & Veterinary Analysis, Freiburg, Germany and [5]Massachusetts General Hospital, Harvard Medical School, Boston, MA.*

The persistence of POPs, persistent organic pollutants, is among their least desirable characteristics. Over 400 pounds of one of the POPs, TCDD, a contaminant of the herbicide Agent Orange, was sprayed in Vietnam between 1962-1971. Between 1970 and 2003, we collected over 3, 200 Vietnam human, food, and environmental samples. In approximately a dozen locations elevated TCDD was

found in southern Vietnamese. In several of these dioxin hot spots, we did extensive sampling of humans, food, and the environment. In 1970 samples, human milk lipid from an Agent Orange sprayed area contained up to 1, 850 ppt of TCDD. The 1970 sampling also found fish TCDD levels as high as 810 ppt, wet weight. Recently, in one hot spot, Bien Hoa city, we found ducks, chickens, one (of 5) fish and one toad to have high TCDD levels, 7-331 ppt wet weight, similar to the 1970 findings. In 1988, we found elevated TCDD in pork, beef, snake and a turtle from a village near Bien Hoa. Typical values in the USA and in Vietnam for fish are currently less than 0.1 ppt wet weight TCDD. Levels in soil from this area, usually less than 1 ppt, were as high as 1, 100, 000 ppt, dry weight. In 2003 we obtained samples of blood and food from two potential dioxin hot spots and compare these data with the earlier findings. The findings of substantially elevated TCDD in humans, food, and the environment for up to 4 decades after initial contamination provides a striking example of the persistence of these compounds.

88 PBPK MODELED CHANGES IN TCDD BASED ON ESTIMATED COMMUNITY EXPOSURES THROUGH SEAFOOD CONSUMPTION: A CASE STUDY IN PUBLIC HEALTH ASSESSMENT.

D. B. Moffett[1, 2] and H. A. El-Masri[1]. *[1]Computational Toxicology Laboratory/Division of Toxicology, CDC/ATSDR, Atlanta, GA and [2]United States Public Health Service, Atlanta, GA.* Sponsor: B. Fowler.

Physiologically based pharmacokinetic modeling of data from a hazardous waste site was used to estimate long-term body burden of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) in people who may be eating dioxin contaminated fish/shellfish as part of their diet. PBPK modeled levels of TCDD were estimated after 5-70 years of regular consumption of fish/shellfish from a contaminated estuary. The estimated daily intakes of TCDD from contaminated fish/shellfish consumption were compared to expected daily intake in the average US diet. The impact of replacing fish/shellfish consumption for protein sources containing less TCDD was also examined. For the exposed population, PBPK model simulations were run for a 5 year exposure period and a lifetime 70 year exposure using a consumption rate of 8 fish/shellfish meals per month. The results were 4 parts per trillion (ppt) dioxin in adipose tissue after a simulated 5 year exposure and 19 ppt dioxin in adipose tissue after a simulated 70 year chronic lifetime exposure. The predicted levels for the chronic exposure to dioxin from fish contribution only are higher than the reported average US population fat tissue value from all dioxin sources which ranges between 0.98-15.1 ppt with a mean value of 6.5 ppt. In addition, using the ATSDR Minimal Risk Level (MRL) of 1 pg/kg/day for an oral chronic exposure to dioxin, we obtained a predicted adipose tissue value of 17.5 ppt. A comparison of the modeled case study adipose tissue values and the modeled dioxin MRL adipose tissue value shows the case study results exceed the health based guidance value for chronic exposure but are below the known effect levels. This approach goes beyond traditional health assessment by examining the effects of increased dietary intake of TCDD on body burden to better understand the potential for adverse health effects due to a chronic exposure scenario.

89 THE INFLUENCE OF VARIABLE ELIMINATION RATE AND BODY FAT MASS IN A PBPK MODEL FOR TCDD IN PREDICTING THE SERUM TCDD CONCENTRATIONS FROM VETERANS OF OPERATION RANCH HAND.

C. Emond[2], M. J. DeVito[1], L. S. Birnbaum[1] and J. E. Michalek[3]. *[1]ORD/NHEERL/ETD, USEPA, Research Triangle Park, NC, [2]NRC, NAS, Washington, DC and [3]HEDB, AFRL, Brooks City-Base, TX.*

Epidemiological and experimental studies suggest that the elimination rate of TCDD is influenced by the body burden and adipose tissue mass. Previous PBPK models for rodent and humans assumed a constant elimination rate. The aims of this study were 1) to compare PBPK models for TCDD that had used either a constant or variable elimination of TCDD and 2) study the influence of adipose tissue mass on the T½ in humans. A PBPK model was developed for TCDD in rats and then extrapolated to humans. This model was described with three diffusion-limited compartments (liver, fat and rest of the body). Ah receptor-mediated induction of CYP1A2 was also described. Parameters used in this model came from the literature. The variable elimination rate was described based on the dose response for induction of CYP1A2. TCDD serum concentrations were determined in over 300 Veterans of Operation Ranch Hand. This cohort was divided in two groups. One was used for the optimization of the model and the other was used for model validation. In an initial analysis, body fat mass was set at 26%. The results suggest that T½ could vary from <6 months in those with high initial body burdens (89 ng/kg) to over 20 years for those with background body burdens. However, adipose tissue mass also plays a role in the elimination of TCDD. Using the example above, changing the adipose mass to 50%, the T½ varies from <1 to 30 years, depending on the body burden. This analysis indicates that the T½ of TCDD is dependent upon the body burden and the adipose tissue mass and that a variable elimination rate is critical for accurate model prediction of human exposures. This work aids in understanding the biological mechanisms of the variability for the T½ of TCDD in humans. (This abstract does not represent USEPA policy. Funded by in part by a cooperative agreement with NRC and EPA (CR 828790) and an interagency agreement between the US Air Force and EPA (FQ7624-00-YA085)).

90 SERUM 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) LEVELS AND SLEEP DISORDERS IN US AIR FORCE VETERANS OF THE VIETNAM WAR.

Y. Liu[2, 1], J. E. Michalek[3] and L. T. Frame[1]. *[1]Pharmacology and Neuroscience, Texas Tech Health Sciences Center, Lubbock, TX, [2]The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX and [3]Air Force Research Laboratory, US Air Force, Brooks City-Base, TX.*

TCDD is the contaminant of concern in the defoliant, Agent Orange. Recently, TCDD was shown to cause circadian disturbances in rodents, suggesting a mechanistic framework for understanding sleep disorders in TCDD-exposed humans. In this study, data were re-analyzed from an existing cohort study of 953 USAF Vietnam veterans involved in Operation Ranch Hand, exposed to Agent Orange between 1962-1971, and 1278 comparison veterans who were not involved with spraying herbicides. Participants were asked questions regarding sleep problems during an in-person interview in 1992. Exposure was assessed by serum TCDD levels measured in 1987 or 1992 and extrapolated initial serum TCDD levels. A first-order elimination model assumed a 7.6 year biological half-life. Each veteran was assigned to 1 of 4 exposure categories, which included Comparison and Ranch Hands with background, low, or high exposure to TCDD. Risk ratios of sleep disorders in Ranch Hands relative to Comparisons were estimated by logistic regression. Overall sleep problems were increased among all Ranch Hands (RR = 1.26, 95% CI 1.11, 1.42). Risk of insomnia, as identified by reports of trouble falling asleep, waking up during the night, or waking up early and being unable to go back to sleep, was significantly increased in the High TCDD subgroup (RR=1.30, 95% CI 1.12, 1.51). Adjustment for other covariates did not reduce this estimate (RR = 1.78, 95% CI 1.27, 2.51). A greater proportion of Ranch Hand veterans in the High TCDD subgroup (RR=1.43, 95%CI 1.09, 1.90) complained of waking up unrefreshed as compared with the comparison group. In the adjusted analysis, this estimate was not reduced (RR=1.58, 95%CI 1.13, 2.22) and showed significant TCDD-dose dependence (RR for Ranch Hand Background=1.35, Ranch Hand Low=1.38, Ranch Hand High=1.60). These findings support a link between TCDD exposure and circadian dysregulation.

91 LOW DOSE *IN VIVO* EXPOSURE TO 2, 3, 7, 8 TETRACHLORODIBENZO-P-DIOXIN (TCDD OR DIOXIN) ALTERS EXPRESSION OF THE CLOCK-ASSOCIATED PROTEIN, PERIOD, IN THE SUPRACHIASMATIC NUCLEUS (SCN) AND LIVER OF C57B6 MICE.

W. Li, R. L. Dickerson and L. T. Frame. *Pharmacology and Neuroscience, Texas Tech Health Sciences Center, Lubbock, TX.*

Halogenated arylhydrocarbons are present in the environment, bioaccumulate in the food chain, and have biological half-lives that can be measured in years. The most potent member of this class of compounds is TCDD. Low-dose TCDD exposure is associated with a spectrum of adverse health effects in humans, including disorders of sleep, lipid and glucose metabolism, behavior, and endocrine function. This suggests a TCDD-associated global circadian disorder. The master circadian clock is located in the suprachiasmatic nucleus (SCN) of the hypothalamus and drives a network of peripheral circadian clocks in other tissues. Circadian expression of the clock-associated protein, mPER, correlates well with locomotor activity and core body temperature, reaching a peak in the SCN during early subjective night. In this series of experiments, C57B6 mice were used to test the hypothesis that adverse health effects associated with TCDD exposure are linked through a common toxicological mechanism targeting the circadian system. Immunohistochemistry was used to quantify the time-course of expression for the clock-associated protein, mPer1, in SCN slices of corn oil- and TCDD-dosed animals. Western blot analyses were used to monitor the corresponding mPer1 expression in liver. In parallel experiments, surgically-implanted radiotelemetry probes (Mini-Mitter) were used to continuously monitor circadian rhythms of locomotor

activity and core body temperature. Low-dose exposure to TCDD (100ng/kg, i.p.) was associated with rapid and transient phase-shifts in the circadian expression of mPer1 in both the SCN and liver, which was followed within 5-7 days by stable resetting of circadian rhythms. Higher TCDD doses (>100 ng/kg i.p.) were associated with prolonged inhibition and pronounced disturbances in circadian rhythms. These results support the idea that diverse toxicities associated with TCDD exposure are mediated *via* effects on the central biological clock.

92 EFFECT OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON POSITIVE AND NEGATIVE SELECTION OF T CELLS IN THE THYMUS.

M. Fisher, M. Nagarkatti and P. S. Nagarkatti. *Virginia Commonwealth University, Medical College of Virginia Campus, Richmond, VA.*

The thymus is highly sensitive to the toxic effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). The purpose of the thymus is to generate a diverse T cell repertoire capable of MHC-restricted antigen recognition, while eliminating autoreactive T cells (positive and negative selection). In the current study, we investigated whether TCDD alters positive and/or negative selection using the HY-TCR mouse model. These mice bear a transgenic TCR that recognizes the male HY-antigen in the context of MHC class I H-2Db. Thymocytes from male HY-TCR mice (which have the HY-Ag) undergo negative selection, while those from female HY-TCR mice (lacking the HY-Ag) are positively selected. Adult male and female HY-TCR mice were exposed to 10-50 μg/kg body weight TCDD. After 48-72 hrs, we measured thymic cellularity, T cell subsets and apoptosis. We found that male HY-TCR mice had greater thymic atrophy following TCDD exposure relative to HY-TCR females. In addition, HY-TCR males exhibit significant changes in T-cell subsets, while HY-TCR females did not. This was due to increased induction of apoptosis in the HY-TCR male thymus following TCDD exposure when compared to HY-TCR females. The difference in apoptosis was specific to immature T cell subsets, suggesting that TCDD induces apoptosis in the thymus in part by increasing the numbers of thymocytes that are negatively selected. Exposure to TCDD also induced the expression of the HY-TCR and MHC class I molecules, suggesting that TCDD might alter the dynamics of thymic selection by increasing the expression of molecules integral to the selection process. Finally, TCDD-induced alterations in T-cell subsets in the HY-TCR males were mirrored in the periphery, so that the effects of TCDD on the thymus may have functional consequences. Together, the current study suggests that TCDD alters the T cell selection process by inducing apoptosis and interfering with the signaling molecules (Supported by NIH grants RO1ES09098, F32ES011732).

93 *IN VIVO* MOLECULAR MECHANISMS OF TCDD-INDUCED CELL DEATH IN THE THYMUS: EVIDENCE FOR AHR-DEPENDENT APOPTOSIS VIA ACTIVATION OF MITOCHONDRIAL AND DEATH-RECEPTOR PATHWAYS.

I. A. Camacho[1], M. Nagarkatti[1] and P. S. Nagarkatti[2]. *[1]Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA and [2]Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA.*

The current study investigated the mechanisms of apoptosis induced by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) in murine thymocytes, particularly addressing the role of AhR. AhR knock-out (KO) mice showed resistance to TCDD-induced thymic atrophy and apoptosis when compared to the wild-type AhR +/+ mice following exposure to 50 μg/kg TCDD. Microarray analysis revealed that TCDD induced the expression of several apoptotic genes including FasL in wild-type but not AhRKO mice. To further address the mechanisms of apoptosis, all experiments were performed in wild-type mice. TCDD treatment increased the expression of FasL on thymic stromal cells at the mRNA and protein level. Upregulated FasL induced by TCDD on Thy 1.2+ thymic stromal cells was able to kill untreated Fas+ Thy 1.1+ thymocytes. However, this effect was absent when TCDD-treated thymic stroma from FasL-defective mice was mixed with wild-type thymocytes or when TCDD-exposed wild-type stromal cells were mixed with Fas-deficient thymocytes. Also, the thymic stromal cell-induced apoptosis was significantly inhibited following addition of anti-FasL Abs to cultures. TCDD treatment *in vivo* caused cleavage of caspase-8, caspase-9, caspase-10, caspase-3 and Bid in thymocytes. However, Bid KO mice treated with TCDD showed thymic atrophy and susceptibility to TCDD-induced apoptosis, suggesting that Bid was not crucial for TCDD-induced apoptosis. Furthermore, TCDD treatment caused loss of mitochondrial membrane potential, release of cytochrome C and increased Bax protein. Taken together, these studies demonstrate that TCDD-induced apoptosis in thymocytes is regulated through the AhR involving death receptor and mitochondrial pathways, and that thymic stroma plays a critical role in this process. (This work was funded in part by grants from NIH: R01ES09098, R01 AI053703, R01 DA016545, F31ES11562, R21DA014885 and R01HL058641).

94 CHRONIC TOXICITY AND CARCINOGENICITY OF DIOXIN-LIKE COMPOUNDS IN FEMALE HARLAN SPRAGUE-DAWLEY RATS.

N. J. Walker[1], A. Nyska[1], C. Alden[1], A. E. Brix[2], L. T. Burka[1], J. R. Hailey[1], J. K. Haseman[1], M. R. Hejtmancik[4], M. P. Jokinen[3], D. P. Orzech[1], D. Sells[4], C. S. Smith[1], M. E. Wyde[1] and J. R. Bucher[1]. *[1]NIEHS, Research Triangle Park, NC, [2]Experimental Pathology Laboratories, Research Triangle Park, NC, [3]Pathology Associates -A Charles River Company, Durham, NC and [4]Battelle Columbus, Columbus, OH.*

The dioxin Toxic Equivalency Factor (TEF) methodology has been developed as a mathematical tool to assess the health risk posed by mixtures of structurally related dioxin-like compounds that bind to the aryl hydrocarbon receptor and exhibit biological actions similar to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). To address the adequacy of the TEF methodology for predicting the potency of different compounds to induce a variety of chronic responses, we conducted a series of kinetic and 2-year rat toxicity and carcinogenicity studies of dioxin-like compounds. Female Harlan Sprague-Dawley rats were treated by oral gavage five times per week with either TCDD, PCB126, 2, 3, 4, 7, 8-pentachlorodibenzofuran, or a mixture of these for up to 2 years with doses ranging up to 100 ng TCDD equivalents/kg. Interim necropsies were carried out at 14, 31, and 53 weeks. Dose-dependent increases in hepatic CYP1A1 and CYP1A2 activity were observed at all interim time points in all studies. In addition alterations in thyroid hormone homeostasis were observed in all studies. There were significant dose and time dependent increases in the incidence and severity of hepatotoxicity and increased incidences of non-neoplastic effects in multiple organs notably in the lung, adrenal gland, oral mucosa, pancreas, kidney, heart, thymus and thyroid gland. In addition increased incidences of neoplasms in several sites were seen in all studies notably in the liver, lung and oral mucosa. This pattern of neoplastic activity is consistent with a prior 2-year dosed-feed study of TCDD conducted in Spartan Sprague-Dawley rats. Qualitatively these data support one of the key assumptions in the use of the TEF methodology; that individual, and mixtures of dioxin-like compounds have similar site-specificity in terms of potential adverse responses following chronic exposure.

95 HEPATIC GENE EXPRESSION PROFILING OF HAHS AND THE IDENTIFICATION OF NOVEL DIOXIN-RESPONSIVE GENES.

B. J. Ovando[1], R. J. Foxenberg[1], C. M. Vezina[2] and J. R. Olson[1]. *[1]Pharmacology and Toxicology, University at Buffalo, Buffalo, NY and [2]Pharmacy, University of Wisconsin, Madison, WI.*

Comparative hepatic gene expression profiling was conducted in female Sprague-Dawley rats exposed to dioxin and structurally related halogenated aromatic hydrocarbons (HAHs) in order to better understand mechanisms for the toxicity of this class of agents. Rats were treated for 14 weeks with toxicologically equivalent doses (based on the respective toxic equivalency factors, TEFs) of TCDD (100 ng/kg/day), 2, 3, 4, 7, 8-Pentachlorodibenzofuran (PeCDF; 200 ng/kg/day), 3, 3, 4, 4, 5-Pentachlorobiphenyl (PCB126; 200 ng/kg/day), and the non-AhR ligand 2, 2, 4, 4, 5, 5-hexachlorobiphenyl (PCB153; 1000 ug/kg/day). Hepatic RNA from each animal was subjected to DNA microarray analysis on Affymetrix RGU34A GeneChips. The hepatic gene expression for toxicant-treated animals was compared to control animals and principal components analysis (PCA) was employed to compare the global expression profiles of each toxicant and filtered to identify genes that were differentially regulated by TCDD, PeCDF, and PCB126, but not PCB153. The gene expression profile for the non-AhR ligand PCB153 was substantially different from that of the AhR ligands TCDD, PeCDF, and PCB126, supporting their common receptor-based mechanism of action. Several genes were co-regulated by treatment with the AhR ligands TCDD, PeCDF, and PCB126, including classical AhR target genes and some novel genes such as cell-cell adhesion molecule 4 (CCAM-4) and adenylyl cyclase associated protein 2 (CAP2). Real Time RT-PCR analysis confirmed the dramatic up regulation of CCAM-4 (4-18 fold) and CAP2 (49-169 fold), not only following subchronic exposure to TCDD, but at 24 and 72 hr following a single po dose of 5 ug TCDD/kg. Thus, these novel dioxin-responsive genes are up regulated as initial events and are not secondary to hepatic injury. Together, these finding may help to expand current knowledge of the AhR signaling cascade and its role in the toxicity of these agents. (Supported in part by NIEHS ES09440, SOT, and University at Buffalo).

96 MOLECULAR STRUCTURE-BASED PREDICTION OF THE STEADY-STATE BLOOD CONCENTRATIONS OF INHALED ORGANICS IN RATS.

M. Beliveau and K. Krishnan. *Occupational and Environmental Health, Universite de Montreal, Montreal, QC, Canada.*

Environmental exposures to volatile organic chemicals (VOCs) often lead to steady-state condition. The establishment of quantitative relationships between steady-state blood concentrations (SS-BC) and molecular structures of such chemicals can be potentially useful. The objective of this study was therefore to investigate the relationship between the SS-BC in rat and the molecular structures of a series of VOCs that belong to multiple chemical families (alkanes, haloalkanes, haloalkenes and aromatics). The overall approach consisted of developing quantitative relationships between molecular fragments (CH3, CH2, CH, C, C=C, H, Cl, AC, H_AC) in various alkanes, haloalkanes, haloethylenes, and aromatic hydrocarbons, as well as their SS-BC (per micromolar inhalation exposure) according to an additive model of Gao. This modeling approach implies that each fragment in the structure of a chemical has an additive and constant contribution to its SS-BC. A multilinear regression was performed using a commercially-available statistical software package (SPSS for Windows v10.0.7, SPSS Inc., Chicago, IL), and the results obtained were essentially the contributions associated with each of the nine structural fragments. This molecular structure vs SS-BC relationship was then validated using an external dataset on SS-BC for a series of aliphatic hydrocarbons obtained from the literature (hexane, heptane, octane, nonane, decane, 2-methyl heptane, 2-methyl octane, 2-methyl nonane, 1-octene, 1-nonene, and 1-decene; 100 ppm). The ratio of experimental to predicted SS-BC for these chemicals ranged from 0.6 to 1.5. The results of this study suggest that steady-state blood concentrations of inhaled VOCs can be predicted using structure-activity type models.

97 TISSUE DISTRIBUTION OF NUCLEOSIDE TRANSPORTERS IN MALE AND FEMALE RATS AND MICE.

H. Lu, C. Chen and C. Klaassen. *University of Kansas Medical Center, Kansas City, KS.*

Concentrative nucleoside transporters (CNT) and equilibrative nucleoside transporters (ENT) have essential physiological functions and are important in disposition of many anticancer and antiviral nucleoside analogs. Various CNTs and ENTs have different but overlapping substrate specificity; however, knowledge of their tissue distribution in rodents is incomplete. Thus, the purpose of this study was to determine distribution of CNT1, CNT2, CNT3, ENT1, ENT2, and ENT3 mRNA transcripts in 19 tissues (heart, thymus, spleen, brain, pituitary, muscle, kidney, liver, blood vessel, duodenum, jejunum, ileum, large intestine, stomach, lung, testis, prostate, ovary, and uterus) of male and female Sprague-Dawley rats and C57BL/6 mice. The 6 CNT and ENT transcripts were determined using branched DNA signal amplification assay. Expression of CNT1 was highest in small intestine, intermediate in kidney, and lower in testis of both species. CNT2 was also highest in small intestine of rats and mice, intermediate in liver of rats but not mice, and somewhat lower in thymus and spleen of both species. The expression of CNT3 was markedly different in rats and mice; expression was highest in lung of rats, but highest in uterus of mice. ENT1 was highly expressed in testis and lung of both species, as well as liver and pituitary of mice. ENT2 was expressed at high levels in testis and brain of both species. Skeletal muscle expression of ENT2 was moderate in mice and rats, in contrast to the very abundant expression previously reported in humans. ENT3 expression was highest in kidney, followed by testis, in both rats and mice. These studies demonstrate that in general, tissue distribution of CNT and ENT is similar in rats and mice. However, a few differences do exist, such as with CNT2 in liver (high in rats; low in mice), CNT3 in lung (high in rats; low in mice), and ENT1 in pituitary (low in rats; high in mice). Such species differences could be responsible for some species differences in disposition and toxicity of xenobiotics that are substrates for these transporters. (Supported by NIH grants ES-09649 and ES-09716).

98 METABOLISM AND DISPOSITION OF [2-^{14}C]-2-METHYL-1, 3-PROPANEDIOL (MPDIOL® GLYCOL) IN SPRAGUE-DAWLEY RATS FOLLOWING ORAL GAVAGE ADMINISTRATION.

R. J. Boatman[1], H. B. Lantum[1], J. C. English[1], M. Thomas[2], W. D. Faber[4] and M. I. Banton[3]. *[1]Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY, [2]Lyondell Chemical Company, Maidenhead, United Kingdom, [3]Lyondell Chemical Company, Houston, TX and [4]WFTC, LLC, Victor, NY.*

The absorption, distribution, elimination, and metabolic fate of 2-methyl-1, 3-propanediol (MPDiol® Glycol) were determined in female Sprague-Dawley (SD) rats following oral gavage administration of [2-^{14}C]-MPDiol® Glycol at either 1000 or 100 mg MPDiol® Glycol/kg. Absorbed radioactivity was eliminated rapidly, with the majority recovered by 12 h as either expired CO_2 or in urine (combined urine and cage wash). Total amounts recovered for these two routes of excretion were similar at the high and low dose levels, but the relative proportions for the routes differed. At 1000 mg/kg, radioactivity in urine and expired CO_2 accounted for 45.4% and 42.0%, respectively, of the administered dose; whereas at 100 mg/kg, the respective values were 31.3% and 57.5%. Only about 1% of the administered radioactivity was recovered in feces and 5% was present in combined tissues and carcasses. A total of 93.1% and 95.3% of the administered radioactivity was recovered from the 1000 and 100 mg/kg exposure groups, respectively, by 7 d. Based on residual radioactivity remaining in the body at each sampling point, the half-lives for the elimination of [2-^{14}C]-MPDiol® Glycol from rats were calculated to be 3.57 h and 3.88 h, respectively, at the 1000 and 100 mg/kg dose levels. HPLC analysis of urine metabolites indicated the majority of the radioactivity was associated with two components. After conversion to the trimethylsilyl derivatives and GC/MS analysis, these were identified as 3-hydroxyisobutyric acid (3-HIBA) and unchanged MPDiol® Glycol. Stereospecific analysis by GC/MS of the 3-HIBA metabolite indicated the predominant urinary stereoisomer to be the (R)-(-)-enantiomer. It is proposed that MPDiol® Glycol may undergo metabolism in the rat predominantly by the pathway responsible for valine metabolism.

99 RAT MULTIDRUG RESISTANCE PROTEIN 4: MOLECULAR CLONING AND CHARACTERIZATION OF REGULATION.

C. Chen, A. L. Slitt, M. Z. Dieter and C. D. Klaassen. *University of Kansas Medical Center, Kansas City, KS.*

Multidrug resistance protein 4 (MRP4/ABCC4) belongs to a group of primary active transporters for organic anions. Human MRP4 has been cloned and shown to transport many chemicals, such as dehydroepiandrosterone-3-sulfate, cAMP/cGMP, methotrexate and antiviral nucleoside analogs. However, information on MRP4 regulation is limited. The aim of this study was to clone rat Mrp4 and characterize its expression under various conditions at mRNA and/or protein levels by branched DNA and immunoblot analyses, respectively. The deduced amino acid sequence of cloned rat Mrp4 cDNA had 1325 amino acids and was 83% identical to human MRP4. In Sprague-Dawley (SD) rats, Mrp4 mRNA was detected in a number of tissues examined: kidney and gonads had the highest levels, whereas the expression in liver was the lowest. Mrp4 expression in kidney was higher in males than females, and was low at birth but steadily increased between 20 to 30 days of age. Mrp4 expression in liver and kidney was not consistently inducible by prototypical Ah receptor ligands, CAR activators, PXR ligands, PPAR? ligands, EpRE activators, or CYP2E1 inducers. Mrp4 mRNA levels in kidney of transport-deficient (TR-) rats that lack Mrp2, were 100% higher than those in normal Wistar rats. Similarly, a 2-fold increase in Mrp4 protein was also seen in kidney of the mutant rats as compared to the normal rats. Furthermore, in kidney, the levels of Mrp4 transcript, but not protein, were increased up to 100% 1 and 3 days after bile-duct ligation in SD rats. In conclusion, the cloned rat Mrp4 encodes a protein that is orthologous to human MRP4. Mrp4 renal expression exhibits gender-specific and developmental patterns. Although Mrp4 is not readily induced by xenobiotics, its relative abundance in kidney, together with its up-regulation in kidney of TR- rats, suggests that Mrp4 is an important transporter for the urinary excretion of organic anions, and may be regulated by endogenous substrates for Mrp2. (Supported by NIH grants ES-09716 and ES-07079)

100 DISPOSITION AND METABOLISM OF 1-BROMOPROPANE IN RATS.

C. Garner, J. Davis, J. Burgess, Y. Yeuh, A. Jeffcoat, T. Fennel and J. Mathews. *Drug Metabolism/Pharmacokinetics, RTI, Research Triangle Park, NC.*

Experiments were conducted to determine the effect of dose on the disposition and metabolism of 1 [14C]bromopropane (1-BrP) in F-344 rats. Male rats were administered iv doses of 1-BrP at 5, 20, and 100 mg/kg and radiochemical content of expired breath volatiles (VOC), expired CO2, urine, feces, and selected tissues was measured. By 48 hours post dose, radioactivity in VOC accounted for ca. 25, 31, and 60% of administered dose at 5, 20, and 100 mg/kg, respectively. Radioactivity expired as 14CO2 was 28, 31, and 8% of those same doses. Urinary radioactivity was 17, 19, and 11% of recovered radioactivity. Excretion in feces was negligible. Radioactivity recovered in tissues accounted for 11, 11, and 2% of administered dose for the respective levels. The molar ratio of 14CO2 to bromide released decreased as dose increased, suggesting that the proportion of 1-BrP metabolized *via* oxidation relative to conjugative pathways is dose dependent. This was supported by a similar pattern in the ratios of CO2 to total metabolized dose. At 20 mg/kg

ca.17% of the administered radioactivity was excreted in urine, distributed between 10 metabolites. Two separate groups of 4 rats were pretreated with either buthionine sulfoximine (BSO), an inhibitor of GSH synthesis or with 1-aminobenzotriazole (ABT), a potent general P450 suicide inhibitor, prior to receiving an iv dose of 1-BrP (20 mg/kg). Relative to controls, ABT pretreatment resulted in profound changes in the proportion of radioactivity appearing in urine (-40%), VOCs (+45%), and CO2 (-80%). BSO pretreatment had no effect. Following inhibition of oxidative metabolism with ABT, urine metabolites were reduced to a single metabolite, N-acetyl-(propyl)-S-cysteine which accounted for >90% of total radioactivity. Overall, the data suggest that oxidation by P450 plays a dominant role in 1-BrP metabolism and disposition, but diminishes as dose decreases.

101 COMPARATIVE DISPOSITION OF 2-METHYL-TETRAHYDROFURAN (MTHF) IN MALE F344 RATS AND B6C3F1 MICE.

R. F. Henderson, M. W. Gurule, J. D. McDonald, D. A. Kracko, B. M. Hedtke, K. Ghanbari and K. J. Dix. *Lovelace Respiratory Research Institute, Albuquerque, NM.*

MTHF is a component of P-series fuels and is used as a specialty solvent and reactant. Little information on MTHF metabolism and disposition is available in the literature. We report *in vivo* studies of MTHF disposition in rodents. Rates and routes of excretion, tissue distribution, and metabolism of MTHF were assessed after oral exposure of rats and mice to [^{14}C]MTHF (1, 10, and 100 mg/kg). In rats, pulmonary excretion in the form of $^{14}CO_2$ was the major route of elimination (40 to 56%) and was inversely related to dose. This was followed by urinary excretion (ca. 24%), which was independent of dose. Recovery of radioactivity as exhaled volatile organics increased 28-fold with dose. The major compound in the exhaled breath at the high dose was identified by LC/MS/MS as the parent compound. This suggests a possible saturation of one or more metabolic pathways. Clearance of the administered dose was rapid, with approximately 80% of the dose excreted within 12 hours at all doses. In mice, urinary excretion was the major route of elimination (ca. 65-70%) followed by $^{14}CO_2$ (ca. 35%) and volatile organics. As with the rats, there was a large (at least a 17-fold) increase in the amount of radiolabel recovered as volatile organics at the high dose compared to the low dose. Excretion was rapid, with over 90% of all doses excreted within 12 hr. Fecal excretion was greater for the high relative to lower doses in both species, and terminal tissue burdens were 7% to 21%. All data collected to date are consistent with saturation of one or more processes at the high dose. (Supported by the National Institute of Environmental Health Sciences, Contract No. N01-ES-25483.)

102 EFFECT OF NASAL ADMINISTRATION OF NICOTINE ON THE BRAIN DELIVERY THROUGH THE OLFACTORY BULBS IN RATS.

H. J. Kim[1], S. H. Chang[2] and H. S. Kim[2]. *[1]Preventive Medicine, Daegu Haany University, Daegu, South Korea and [2]Preventive Medicine, Keunkook University, Chungju, South Korea.*

Previously we have demonstrated that nasal administration of ganciclovir, a nucleoside analogue with antiviral activity, enhances brain delivery through the olfactory bulbs. In this study we explored the possibility of intranasal (*in*) administration of nicotine to provide an enhanced access to the brain through the olfactory pathway by determining the extent of systemic absorption and uptake of [^{3}H]- nicotine in the brain after intravenous (*iv*) or *in* administration. Male CD rats were given 20 μCi/kg [^{3}H]- nicotine by *iv* or *in* administration into the right nostril. Serial plasma samples were collected over 60 min and total radioactivities were analyzed. Olfactory bulbs, cerebrum, midbrain and cerebellum were also collected at 15 and 60 min post-dosing and analyzed. Nicotine was absorbed rapidly from the nasal cavity; peak radioactivity was reached within 2 min after *in* dosing. The absolute bioavailability of intranasally administered nicotine was close up to 1. The uptake of nicotine total radioactivity into the cerebrum, midbrain, and cerebellum did not show any significant difference after *iv* or *in* dosing and between left and right sides. The uptake into the right olfactory bulb reveals a statistically significant difference at 60 min post-dosing ; 784 cpm (*iv*, mean) and 1364 cpm (*in*, mean), whereas the uptake into the left olfactory bulb was 1039 cpm (*iv*) and 1082 cpm (*in*), respectively. Also, 37% increase of nicotine uptake into the right olfactory bulb was observed 15 min after *in* dosing. In summary, we have demonstrated that the brain delivery of nicotine through the olfactory pathway was not dramatic in the same manner as ganciclovir. However, 34–37% increase of uptake into the right olfactory bulb by *in* administration implys that small quantity of nicotine may transport into the brain through the olfactory bulbs. Furthermore, nicotine was well absorbed through the nasal epithelium and transported into systemic circulation.

103 PRELIMINARY TOXICOKINETIC STUDY AND BIOLOGICAL SAMPLE ANALYSIS METHOD DEVELOPMENT/VALIDATION FOR DICHLOROACETIC ACID.

J. D. Johnson[1], S. W. Graves[1], D. Emmerling[1], B. Burback[1], J. Merrill[1] and C. Smith[2]. *[1]Toxicology, Battelle, Columbus, OH and [2]NIEHS, NIH, Research Triangle Park, NC.*

Dichloroacetic acid (DCA) has been detected in drinking water as a result of chlorine disinfection and is a hepatocarcinogen in rodents. A method utilizing derivatization of DCA followed by gas chromatography with electron capture detection was developed and validated for use in rodent plasma. Male F344 rats and female B6C3F1 mice were given a single intravenous (IV) administration at doses of 40 (R) and 100 (M) mg/kg or a single gavage administration at doses of 10 or 100 (R) and 20 or 200 (M) mg/kg. Dosing volumes were 2 (R) and 4 (M) mL/kg for IV and 5 (R) and 10 (M) mL/kg for gavage. Serial blood samples were collected after dosing and analyzed by the validated method. Analytical standards were prepared (range 0.1 to 20 ug/mL in plasma). A quadratic regression equation weighted 1/y2 was calculated, thereby relating the response (y) to the concentration (x) of the standards. The concentration of each sample was calculated using its individual response and the regression equation. IV plasma concentration time profiles were monophasic. For the rats, the Cmax, ke, and AUCinf were 91 ug/mL, 0.05 min-1, and 2650 min.ug/mL and, for the mice, were 122 ug/mL, 0.12 min-1, and 1900 min.ug/mL, respectively. Following gavage administration, the Cmax, Tmax, ke, and AUCinf values were 0.4 ug/mL, 20 min, 0.03 min-1, and 21 min.ug/mL at 10 mg/kg (R); 53 ug/mL, 90 min, 0.004 min-1, and 15, 600 min.ug/mL at 100 mg/kg (R); 2.5 ug/mL, 5 min, 0.20 min-1, and 18 min.ug/mL at 20 mg/kg (M); and 67 ug/mL, 10 min, 0.10 min-1, and 1, 030 min.ug/mL at 200 mg/kg (M). The results from this preliminary study provided the information necessary to design a definitive toxicokinetic study for DCA.

104 EFFECT OF DIFFERENT DOSING PARADIGMS ON THE BODY BURDEN OF CHLORPYRIFOS (CPF) IN NEONATAL SPRAGUE-DAWLEY RATS.

J. Y. Domoradzki[1], M. S. Marty[1], S. C. Hansen[1], C. Timchalk[2] and J. L. Mattsson[3]. *[1]The Dow Chemical Co., Midland, MI, [2]Battelle, Richland, WA and [3]Dow AgroSciences, LLC, Indianapolis, IN.*

In some circumstances direct neonatal dosing has been recommended in EPA developmental neurotoxicity studies; however, there is a paucity of data on neonatal systemic exposure using different dosing paradigms. Male rats at postnatal day 5 were dosed with CPF (1 mg/kg) using different routes of exposure, or vehicles, and by single vs. divided dose. Blood concentrations of CPF and trichloropyridinol (TCP) were measured at multiple times through 24 h. Groups included: bolus vs. divided gavage doses in corn oil (3 times in 24 h), bolus vs. divided gavage doses in rat milk, and single sc injection in DMSO. Data were compared with lactational exposure of pups from dams exposed to CPF in the diet (5 mg/kg/day). In dams, blood CPF was 2-4 ng/g (limit of detection, 1 ng/g). In a published study, 5 mg/kg by gavage in corn oil resulted in maternal blood CPF levels of ~14 ng/g. CPF was not detected in blood of nursing pups from dietary maternal exposure while TCP blood concentrations ranged from 49-71 ng/g. In dams, TCP ranged from 281-1263 ng/g. In pup direct-dosing paradigms, the concentration of CPF in blood was non-quantifiable or usually <10 ng/g. Peak blood concentrations of TCP were 320, 112, 195, 100 and 171 ng/g for the bolus and divided corn oil, bolus and divided milk, and the sc DMSO groups, respectively. With all direct-dosing paradigms, higher blood levels of CPF and TCP were reached than blood levels in neonates exposed to CPF *via* nursing. With respect to vehicle, corn oil yielded higher peak TCP levels than milk, particularly with bolus dosing, and resulted in higher systemic exposure to CPF. Divided doses yielded lower peak TCP blood levels than a single dose. Contrary to expectations, sc injection of CPF in DMSO did not result in rapid and complete absorption. To be meaningful for risk assessment, neonatal studies require attention to the exposure scenario, since route, vehicle and frequency of administration result in different systemic exposure to a chemical and its metabolites.

105 MORE RENAL AND LESS INTESTINAL ADDUCTION BY THE NSAID DICLOFENAC IN MRP2-DEFICIENT RATS.

M. Treinen-Moslen[1], L. Kaphalia[1], L. Lemley[1], B. A. Rampy[1], C. R. Atchison[2] and M. F. Kanz[1]. *[1]Pathology, University of Texas Medical Branch, Galveston, TX and [2]HQ's USAMRMC, Fort Detrick, MD.*

Background: Diclofenac and many of its structurally related carboxylic acid NSAIDs are converted to acyl glucuronide with sufficient electrophilic reactivity to form adducts with proteins in multiple tissues. When compared to the responses of

wild-type animals to diclofenac treatment, rats with a genetic deficiency in the hepatic canalicular MRP2 exporter have been found to show several pronounced differences in response, notably a marked decreases in biliary secreting of the acyl glucuronide metabolite with faster urinary clearance, less enteropathy, and less adduction of liver proteins. **Goal and Approach:** Experiments addressed unknowns about the influence of MRP2-deficiency on the fate of diclofenac using a well characterized antibody that recognizes drug-protein adducts as a biomarker of tissue exposure to reactive diclofenac entities. Specifically, we hypothesized less adduction of intestine and more adduction of kidney. MRP2- deficient TR -rats and wild type Sprague-Dawley were treated with diclofenac, 50 mg/kg orally. Blood and urine were collected for biochemical indices of renal dysfunction. Tissues were collected for histopathology and adduct characterization chiefly the semi-stable acyl glucuronide metabolite. **Results:** Consistent with their known impairment in biliary secretion of diclofenac acyl glucuronides, MRP2- deficient animals showed more intense adduction of the kidney with meager adduction in the biliary tract and intestine as well as greatly reduced enteropathy. However after drug treatment, wild type but not MRP2-deficient rats had mild kidney-dysfunction associated changes in serum Na, K, BUN and in urine K, glucose and uric acid. **Conclusion:** This is the first observation of more prominent adduction by an NSAID known to form reactive acyl glucuronides in the kidney of MRP2-deficient rats. Supported by NIH grants DK 34806, DK 56494, CA 101621, ES 06348, ES 00267 and ES T32-07254

106 COMPARISON OF PARTITION COEFFICIENTS FOR A MIXTURE OF VOLATILE ORGANIC COMPOUNDS IN RATS AND HUMANS AT DIFFERENT LIFE STAGES.

D. A. Mahle[1], C. C. Grigsby[2], R. J. Godfrey[1], J. M. Gearhart[1], H. A. Barton[3], J. C. Lipscomb[4] and R. S. Cook[2]. *[1]ManTech Environmental Technology, Inc., Wright-Patterson AFB, OH, [2]AFRL/HEST, Wright-Patterson AFB, OH, [3]USEPA/ORD/NHEERL, Research Triangle Park, NC and [4]USEPA/ORD/NCEA, Cincinnati, OH.*

Pharmacokinetic differences in child, adult and elderly populations remain ill-defined. Partition coefficients (PC) are an integral component of pharmacokinetic models, and determining differences in tissue partitioning of volatile organic chemicals across life stages can help reduce the uncertainty in risk assessment. Control male adult and aged (18 month) Sprague-Dawley (S-D) rats (n = 10) and control male and female S-D pups (n = 10 litters) at postnatal day 10 were sacrificed, and blood, liver, kidney, fat, muscle and brain were taken. Unused blood from male and female human pediatric and adult patients was collected for determination of age-specific PC. PC for methylene chloride, methyl ethyl ketone, chloroform, benzene, trichloroethylene and perchloroethylene were determined using a *vial* headspace equilibration method and analysis by gas chromatography. Human adult and pediatric blood:air PC were similar regardless of sex. Values for the solvents ranged from 7.6 ± 2.3 (adult female, benzene) to 192.7 ± 6.0 (pediatric male, methyl ethyl ketone). Liver:air methylene chloride PC for adult male rats averaged 20.4 ± 4.8 compared to 12.7 ± 1.0 for male rat pups. By quantifying the pharmacokinetic variances in PC for young, adult and elderly populations, biologically based models can be improved to reduce uncertainties in risk assessment.

107 ETHANOL PHARMACOKINETICS ARE ALTERED BY PREGNANCY AND CALORIC INTAKE IN FEMALE RATS.

M. HIdestrand[1], K. Shankar[1], L. Humphrey[2], R. Haley[2], M. Zipperman[2], T. M. Badger[3, 2] and W. D. McGuinn[4]. *[1]Pharmacology & Toxicology, University of Arkansas for Med. Sciences, Little Rock, AR, [2]Arkansas Children's Nutrition Center, Arkansas Children's Hospital, Little Rock, AR, [3]Physiology, University of Arkansas for Med. Sciences, Little Rock, AR and [4]NA, Columbia, MD.*

We have previously shown that the when ethanol is infused into rats intragastrically as part of a system of total enteral nutrition (TEN), blood and urine ethanol concentrations (BECs and UECs) do not achieve steady state but instead pulse from almost 0 to over 500 mg/dl every 6-7 d. During pregnancy the amplitude of the UEC pulses is significantly reduced in rats fed optimal levels of calories and the pregnancy effect is abolished by under nutrition. In the current study, we examined the pharmacokinetics of ethanol clearance in these animals. BECs were followed in chow fed 300 g pregnant and virgin rats following a single oral gavage of 3 g/kg ethanol at gestational day (GD) 8, 13 and 20. In each case, the area under the BEC time curve was reduced by the same amount in the pregnant animals ($p<0.05$). In a second experiment, pharmacokinetics of ethanol clearance was followed in chow fed pregnant rats at GD 13 and weight matched cycling rats following a single oral gavage of 3 g/kg or an IV bolus of 0.75 g/kg. Initial non-compartmental estimates of clearance and t1/2 were 0.014 vs 0.011 L/kg/min and 58 and 68 minutes for pregnant vs cycling rats respectively after an IV dose. For gavaged rats, these values were 0.023 vs 0.010 L/kg/min and 46 vs 100 min for pregnant vs cycling rats. Area under the BEC time curves was lower for pregnant rats administered ethanol by either route ($p<0.05$) but the pregnancy effect was significantly greater when ethanol was administered orally suggesting the involvement of a first pass component in addition to increased hepatic clearance. Caloric restriction in pregnant rats fed TEN diets at 220 or 154 kcal/kg3/4/d resulted in reduced ethanol clearance at GD13 when ethanol was administered either orally or IV ($p<0.05$). Thus pregnancy accelerates ethanol clearance and this effect is impaired by under nutrition. Supported in part by AA12819 (M.R.)

108 DOSE-DEPENDENCY OF ASPIRIN-TRICHLOROETHYLENE INTERACTION.

K. Kim, S. Muralidhara, S. Lee and J. Bruckner. *Department of Pharmaceutical and Biomedical Sciences, The University of Georgia, Athens, GA.*

1, 1, 2-Tricholoethylene (TCE), a rodent carcinogen and potential human cancer risk, is metabolically activated by cytochrome P450 2E1 (CYP2E1) to chloral hydrate (CH), trichloroacetic acid (TCA), dichloroacetic acid (DCA) and trichloroethanol (TOH). Acetylsalicylic acid (ASA) has been previously reported to enhance hepatic CYP2E1 activity. The therapeutic dose for analgesic and antipyretic effects of ASA is 325 mg ~ 1.0 g orally every 3 or 4 h for adults. The current study was conducted to: 1) characterize the dose- and time-dependency of CYP2E1 induction in rats by ASA, and 2) learn whether the significant induction of CYP2E1 would affect the metabolism of high and low doses of TCE differently. Groups of control and ASA (450 mg/kg bw)-treated male Sprague-Dawley rats of about 150 g were gavaged with one of a graded series of oral TCE doses 12 h after ASA treatment, the time of peak CYP2E1 induction by the drug. CH, TCA, DCA and TOH blood levels were monitored over time by headspace gas chromatography. The time-course profiles revealed that ASA markedly enhanced the metabolism of high doses of TCE, but had relatively little influence on lower, more environmentally-relevant doses. (Supported by DOE: DE-FC02-02CH11109)

109 BIO-DISTRIBUTION OF BISPHENOL A IN THE NEUROENDOCRINE ORGANS OF FEMALE RATS.

H. M. Luu[2], W. Johnson[1], J. C. Hutter[2], C. S. Kim[1], I. A. Ross[1] and P. P. Sapienza[1]. *[1]Toxicology, US Food and Drug Administration, Laurel, MD and [2]Radiological Health, USFDA, Rockville, MD.*

The bio-distribution of 14C Bisphenol A (BPA) in plasma and neuroendocrine organs was determined in the Fischer 344 female rats following three oral doses (0.1, 10, or 100 mg/kg). The distribution and pharmacokinetics between the plasma and neuroendocrine organs were compared. Within 25 minutes of dosing, the maximum concentrations of 14C- BPA were found to be 16.99 ?g/ml in plasma, 14.73 ?g/g in pituitary, 8.73 ?g/g in uterus/gonads, 6.96 ?g/g in hypothalamus and 7.12 ?g/g in the rest of the brain at 48 h after100 mg/kg dose, respectively. Plasma areas-under-the-curve (AUC) were found to increase with dose levels (0.06, 5.37, and 53.83 ?g-hr/ml at 48 h after 0.1, 10 or 100 mg/kg dose, respectively). However the AUCs of the pituitary gland and uterus/gonads were 16-21 % higher than that of plasma at 48 h after100 mg/kg dose. The AUCs of hypothalamus and the rest of the brain were 43.7 % and 77% of the plasma AUCs, respectively. In the brain tissue, the exposure increased linearly with the oral dose, as the dose was increased from 0.1 to 10 and 100 mg/kg; the exposure in the brain relative to the plasma increased by factors of 1, 1.19 and 1.24. The increases of the uterus/gonads relative to the plasma were 1, 1.07 and 1.04. This indicates that the brain barrier systems do not limit the access of lipophillic BPA to the brain.

110 PRELIMINARY TOXICOKINETIC STUDY AND BIOLOGICAL SAMPLE ANALYSIS METHOD DEVELOPMENT/VALIDATION FOR 2-METHYLTETRAHYDROFURAN.

B. L. Burback[1], L. Fomby[1], B. Harritos[1], G. W. Steven[1] and S. S. Cynthia[2, 3]. *[1]Battelle Memorial Institute, Columbus, OH, [2]NIH, Research Triangle Park, NC and [3]NIEHS, Research Triangle Park, NC.*

2-Methyltetrahydrofuran (MTHF) is a component of novel P-series alternative fuels and is being evaluated in toxicological studies. Methods utilizing a heated headspace gas chromatography with selected ion monitoring (SIM) mass spectroscopy for low concentration range and flame ionization detection for high concentration range were developed and validated for use in rodent plasma and brain tissue. Biological samples for validating the method were obtained by conducting a preliminary toxicokinetic study with male and female F344 rats and B6C3F1 mice given a single intravenous or gavage administration of MTHF and collecting samples at specified time points (one animal/species/sex/dosage level). Untreated animals were used to obtain samples for preparing matrix matched plasma or brain samples. Analytical standards were prepared (range 4.3 to 860 ng/mL in plasma for

the low concentration range and 0.645 ug/mL in plasma or for the high concentration range). A quadratic regression equation weighted 1/x was calculated relating the response (y) to the concentration (x) of the standards. The concentration of each sample was calculated using its individual response and the regression equation. Following a single intravenous dosage of 40 mg/kg, the maximum plasma and brain concentrations were similar between species and were approximately 29, 000 ng/mL and 24, 000 ng/g, respectively. After a single gavage dosage (400 mg/kg), the maximum plasma and brain concentrations in rats were approximately 269, 000 ng/mL and 334, 000 ng/g, respectively. Maximum plasma and brain concentrations in mice after gavage administration of 400 mg/kg were approximately 207, 000 ng/mL and 222, 000 ng/g, respectively. The validation of an analytical method for MTHF together with the preliminary toxicokinetic study results will be used to design a definitive toxicokinetic study in order to determine toxicokinetic parameters after MTHF administration.

111 DISPOSITION OF DODECAMETHYLCYCLOHEXASILOXANE (D6) IN FISCHER 344 RATS FOLLOWING A SINGLE ORAL DOSE.

K. P. Plotzke, J. Durham, M. L. Jovanovic and J. M. Regan. *Dow Corning Corporation, Midland, MI.*

The disposition of ^{14}C-D6 was evaluated in male and female F344 rats following a single oral administration of 1000 mg of ^{14}C-D6 in corn oil/kg of body weight. Animals (N=4/sex) were housed in glass metabolism cages for collection of excreta and expired volatiles. At 168 hr post-dose, animals were sacrificed and selected tissues and remaining carcasses were collected. All samples were analyzed for radioactivity content. Feces and expired volatiles were also analyzed for parent D6 concentration. A separate group of animals, cannulated *via* the jugular vein, (N=6/sex) was used to determine radioactivity and parent D6 concentration in blood at predetermined time points. Whole-body autoradiography (WBA) was used for qualitative *in vivo* assessment of tissue distribution of radioactivity. The majority of administered radioactivity (>80%), regardless of sex, was eliminated in the feces. Total absorption (radioactivity recovered in urine, expired volatiles, expired CO_2, tissues and carcass) in males and females was 11.88% and 11.83%, respectively. Both sexes showed similar patterns of disposition (Urine: 0.38% and 0.32%; Expired volatiles: 11.20% and 11.21%; Expired CO2: 0.13% and 0.09%; Tissues: 0.03% and 0.04%; Carcass: 0.14% and 0.17% for males and females, respectively). The entire radioactivity in the expired volatiles was attributed to parent D6. Metabolic profile evaluation showed that the entire radioactivity in the urine consisted of polar metabolites, whereas in the feces the majority was parent D6 with a trace of nonpolar metabolite. The WBA data also showed that the majority of D6 remained in the GI tract and was excreted in feces within 48 hr. Low levels of radioactivity were systemically available and distributed to organs and tissues such as liver, brown fat and bone marrow. Statistical analysis of the blood curves indicated the presence of small levels of metabolites in the blood (approximately 117 $_{\mu}$g equivalent D6*hr/g of blood). In summary, approximately 12% of ^{14}C-D6 delivered in corn oil appeared to be absorbed after a single oral administration to F344 rats.

112 PHARMACOKINETIC INTERACTION BETWEEN BUPRENORPHINE AND DESMETHYLFLUNITRAZEPAM IN RATS.

S. Pirnay[1, 2], B. Megarbane[2], S. Bouchonnet[3], C. Monier[2], P. Risede[2], I. Ricordel[1] and F. J. Baud[2]. *[1]Laboratoire de Toxicologie de la Prefecture de Police, Paris, France, [2]INSERM U26, Hopital Fernand Widal, Paris, France and [3]DCMR, Ecole Polytechnique, Palaiseau, France.*

Recently, a retrospective study reported fatalities linked to buprenorphine (BUP), in France from June 1997 to June 2002 (1). It showed evidence that buprenorphine is misused and administered by the intravenous route. Our *in vivo* experimental studies using a high dose of BUP and flunitrazepam (FNZ) confirmed significant respiratory depression, only when co-administered (2). The objective is to determine if the reported toxicity of FNZ with BUP is caused by a pharmacokinetic interaction. The study was realized on Sprague-Dawley male rats (Iffa-Credo, France). The day before the study, animals were intraperitoneally anesthetized with ketamine (70 mg/kg) and xylazine (10 mg/kg) and catheterized in the femoral vein (for administration of drugs) and artery (for collecting blood). Drugs plasma kinetics were performed in two groups (control group receiving the BUP solvent then FNZ 40mg/kg, study group receiving BUP 30 mg/kg then FNZ 40 mg/kg). FNZ and metabolites (desmethylflunitrazepam (DMFNZ) and 7-aminoflunitrazepam (7AMF)) dosages were performed by GC-MS (Agilent 5973). There was no statistically significant effect of BUP pretreatment on FNZ and 7AMF kinetic parameters. However, this study shows clearly, that concentrations, and AUC of DMFNZ are significantly doubled (p=0.05), when BUP is associated. There is no pharmacokinetic interaction of BUP (30 mg/kg) on plasma kinetics of FNZ, but a significant interaction of BUP (30 mg/kg) on DMFNZ concentrations. The pharmacokinetic interaction process is unknown yet, but may be related either to the distribution or the elimination of DMFNZ. 1. Pirnay S, Tourneau J, Ricordel I. Death associated to treatments of substitution. Retrospective study of 1600 postmortem toxicological cases. Le courrier des addictions. 2002;4:156-158. 2. Megarbane B, Pirnay S, Risede P, et al. Respiratory effects of the interaction between buprenorphine and norbuprenorphine. Congress of SRLF, La Defense, France, January 2003.

113 DETERMINATION AND QUANTITATION OF ANTHRAQUINONE URINARY METABOLITES.

S. Graves[1], S. Runyon[1] and C. S. Smith[2]. *[1]Toxicology Columbus, Battelle, Columbus, OH and [2]NIEHS, NIH, Research Triangle Park, NC.* Sponsor: M. Hejtmancik.

A metabolism study was conducted using male Fischer 344 rats in which they were fed formulations of 4 lots of anthraquinone, produced by three different synthetic routes, with concentrations of 938, 3750 and 7500 ppm and a control diet containing no anthraquinone in irradiated NTP 2000 feed for seven consecutive days. One of the lots had been previously used to conduct subchronic and chronic rodent toxicity studies in feed. Ten animals were used per group. The formulations were prepared using anthraquinone with particle sizes smaller than 80 mesh and consistent in distribution for each lot. All animals were placed in individual metabolism cages following dosing and urine was collected for 24 hours. The urine of all animals from each group was pooled. The purpose of this study was to evaluate any difference in absorption and metabolism of the anthraquinone. A high performance liquid chromatographic method with ultraviolet absorbance detection (HPLC/UV) was developed to analyze the urine samples for 1- and 2-hydroxyanthraquinone, metabolites of anthraquinone. The method consisted of extracting 2 mL of urine with three 2-mL aliquots of ethyl acetate, combining them, evaporating, and reconstituting in 25% water:75% acetonitrile. The reconstituted extracts were analyzed using a C18 reverse-phase column, a mobile phase starting at 75% water:25% acetonitrile, remaining there for 5 minutes and then going to 25%water:75% acetonitrile over 20 minutes with a linear gradient, and a detection wavelength of 260 nm. This method was validated and found to have acceptable linearity, specificity, sensitivity, accuracy, precision, recovery, and ruggedness. Analysis of the samples found that the metabolic profiles and concentrations were consistent for each source of anthraquinone at a given dose level. 1- and 2-hydroxyanthraquinone and anthraquinone were found in all samples from the dosed animals. Within a given sample the concentrations of 2-hydroanthraquinone and anthraquinone were similar and the concentration of 1-hydroxyanthraquinone was approximately 2% of the other two.

114 *IN VIVO* PERCUTANEOUS ABSORPTION OF DECAMETHYLCYCLOPENTASILOXANE (D5) IN FISCHER 344 RATS.

M. L. Jovanovic, J. McMahon and K. P. Plotzke. *Dow Corning Corporation, Midland, MI.*

The percutaneous absorption of ^{14}C-D5 was evaluated in female F344 rats (N=4) when applied topically at 10 mg/cm^2 of skin for 24 hr. Animals were housed in glass metabolism cages for collection of excreta and expired or escaped volatiles associated with the test article. A 24 hr exposure group was used to evaluate disposition of the residual D5 six days after a soap and water wash. Additionally, four animals previously euthanized were dosed in order to differentiate expired volatiles from ^{14}C-D5 that escaped from the skin depot. All animals were exposed in a semi-occluded manner using an aluminum skin depot with a charcoal basket for collection of volatilized test article. At the end of exposure, rats were sacrificed, skin was washed, tape stripped, excised, and remaining carcasses were collected. All samples were analyzed for radioactivity content. Total radioactivity in the expired volatile traps was compared to the amount of unchanged D5 determined by GC-MS analysis. Most (>80%) of the test article evaporated from the skin surface and was trapped in a charcoal basket placed above the exposure site. Data from the euthanized animal group suggested that the amount of radioactivity found in the expired volatile and CO_2 traps could be attributed to leakage from the skin depot or background, and could be excluded from the total absorption. Overall, more than 99% of the recovered dose was found in the charcoal baskets and charcoal tubes combined for the animals exposed for 24 hr. The percent dose absorbed was determined as the amount of radioactivity in carcasses, feces, urine, skin dosing sites and cage rinses. Six days after the skin was washed, absorption of ^{14}C-D5 (0.089 $\pm$ 0.0302% of applied dose) was significantly lower ($p < 0.05$) than that seen immediately after 24 hr of exposure (0.243 $\pm$0.0259% of applied dose). These results demonstrated that the portion of D5 that remained in the skin after 24 hr of exposure, and was considered part of the absorbed dose, migrated to the skin surface and continued to evaporate, significantly decreasing apparent absorption of the D5.

115 DISPOSITION OF DERMALLY ADMINISTERED 5-AMINO-O-CRESOL (AOC) IN FEMALE F344 RATS.

K. J. Dix and B. M. Hedtke-Weber. *Lovelace Respiratory Research Institute, Albuquerque, NM.*

AOC is used almost exclusively as a coupler in oxidative (permanent) hair dyes. Hence, there is potential human exposure to AOC *via* dermal absorption, particularly in women. *In vitro* studies with pig skin and *in vivo* studies in rats, hairless guinea pigs, and humans have shown that AOC permeates the skin. Although AOC is an aromatic amine, a class of compounds with a structural alert for mutagenicity, evidence for AOC-induced mutagenicity is equivocal. AOC is relatively nontoxic; the dermal LD50 in rabbits is >5000 mg/kg (Loyd et al., 1977, Food Cosmet Toxicol, 15:607-610) and the oral LD50 is 2928 and 4355 in female and male rats (CIR Expert Panel, 1989, J Am College Toxicol, 8:569-587). Given the aromatic amine structural alert, the NTP is considering chronic carcinogenicity testing of AOC by the dermal route. In those studies, rodents would be exposed daily to AOC by skin-painting and the dose site accessible for grooming, which could result in oral and dermal doses of AOC. We have conducted dermal disposition studies of [14C]AOC (2 and 20 mg/kg) in female F344 rats in which the dermal dose site was protected or unprotected from oral grooming. Doses were prepared in ethanol the day prior to dosing and AOC in the formulation was ca. 90% radiochemically pure on dose day. When the dose site was accessible to grooming, much more radioactivity was absorbed as evidenced by urinary excretion; 33-55% of the applied dose was excreted in urine when the dose site was not protected from grooming compared with 5-7% when the dose site was protected. Less than 1% of the dose was excreted by all other routes. The remainder of the absorbed radioactivity was found in tissues. When the dose site was protected, the majority of the applied dose was recovered in dose-site washes and the protective appliance. We continue to evaluate AOC metabolism and disposition in female rats and B6C3F1 mice after oral administration to evaluate possible species-specific disposition of AOC to aid the NTP in designing toxicity and carcinogenicity studies to assess potential human health risk(s) from AOC exposure.

116 MOGCHOAECK REDUCES ETHANOL CONCENTRATION ELEVATED BY ALCOHOL INGESTION IN RATS.

T. W. Jeon[2] and H. J. Kim[1]. [1]*Preventive Medicine, Daegu Haany University, Daegu, South Korea and* [2]*Toxicology, Youngnam University, Daegu, South Korea.*

This study was designed to investigate the effect of mogchoaeck on the ethanol concentration elevated by alcohol ingestion. Male Sprague-Dawley rats weighing 200 g were given mogchoaeck (10, 5 and 2.5 ml/kg, p.o.) and administered ethanol at a dose of 3 g/kg bw (25% in distilled water) by gavage 30 min after. To evaluate the effect of mogchoaeck on ethanol pharmacokinetics, blood was collected from caudal artery at 0.25, 0.5, 1, 2, 4, 8 and 12 hr after ethanol gavage. A dose of 10 ml/kg mogchoaeck significantly decreased the ethanol levels in blood; maximum concentration of 24.3±5.1 mg/dl (mean±S.E.) vs. 72.6±3.6 mg/dl in control rats. The administration of mogchoaeck significantly decreased the area under the serum alcohol concentrations-vs.-time curves; 502±50 (control) vs. 148±33 and 329±51 mg·hr/dl (10 and 5 ml/kg). With increasing doses of mogchoaeck, AUC decreased in a dose-dependent manner, -12, 35 and 71% reduction of control, respectively. These results clearly demonstrated that the intake of mogchoaeck reduces the ethanol concentration after alcohol ingestion.

117 STUDIES OF THE UPTAKE, DISTRIBUTION, AND ELIMINATION OF ^{14}C IN SPRAGUE-DAWLEY RATS GIVEN ^{14}C-LABELED N-ACETYL-L-CYSTEINE BY ORAL GAVAGE.

A. E. Jung, A. J. Bobb, A. R. Thitoff, E. W. Johnson, S. L. Lohrke and D. P. Arfsten. *Naval Health Research Center Toxicology Detachment, Wright-Patterson AFB, OH.*

N-acetyl-L-cysteine (NAC) is being studied as a possible chemoprotectant agent against chemicals of military significance. NAC is thought to provide protection against chemical toxicity by several mechanisms including free radical quenching, enhancement of tissue glutathione (GSH) synthesis, and possibly by modulating tissue glutathione-S-transferase (GST) concentration/activity. The uptake, distribution, and elimination of ^{14}C-NAC following oral intake has not been completely studied. SD rats, 8 weeks of age, were dosed once by oral gavage at a dose rate of 600 or 1, 200 mg NAC/kg containing 6 microCi/gram of ^{14}C-NAC. Animals were sacrificed at 0.5, 1, 2, 4, 6, and 12 hours post dosing. All major tissues were separated, homogenized, and tissue ^{14}C counts were collected with a Packard Tricarb Scintillation Counter. Radioactivity levels for urine and feces were both significantly elevated at all time points as compared with controls. Radioactivity levels were also significantly elevated for heart, lungs, thymus, spleen, liver, brown fat, muscle, and skin. As expected, radioactivity counts were elevated for organs in the GI tract (stomach, large and small intestine, the liver) and for the kidneys. The amount of radioactivity present in each of the tissues at each time point was dependent on dose for most of the tissues; radioactivity levels remained elevated in many tissues for up to 12 hours post-administration. Brain tissue counts were not elevated at any time point which is consistent with the findings from previous studies involving intravenous administration of NAC. The finding that radioactivity levels were elevated for some tissues at up to 12 hours post-administration supports the hypothesis that oral NAC is probably utilized by certain tissues as a source of cysteine. These preliminary findings suggest that NAC oral supplementation may provide some benefit to tissues that are often a site of chemical exposure (e.g., skin, lungs) and possibly may benefit immune function.

118 A PHARMACOKINETIC STUDY OF CJC-1131, A NOVEL GLP-1 ANALOGUE, IN RATS USING DUAL ISOTOPE LABELING DEMONSTRATES A LONG ELIMINATION HALF-LIFE.

B. Lawrence[1], S. Wen[1], S. Wilson[2], V. Iordanova[1] and J. Castaigne[1]. [1]*ConjuChem, Montreal, QC, Canada and* [2]*Milestone Biomedical Associates, Frederick, MD.*

CJC-1131 is an anti-diabetic compound designed by applying the Drug Affinity Complex (DAC) technology to the short acting glucagon-like peptide-1 (GLP-1). This chemical modification with maleimidoproprionic acid (MPA) allows CJC-1131 to covalently bond to circulating albumin, after parenteral administration, thus prolonging its half-life. In order to study the pharmacokinetic (PK) and tissue distribution profile of CJC-1131, the compound was either labeled with [^{14}C] on the MPA or with [^{3}H] on histidine at position 1 (His^{1}) of the GLP-1 moiety. Rats were dosed subcutaneously (SC) with the formulated [^{14}C/^{3}H]-CJC-1131 at a target dose of 2.25 mg/kg. Quantifiable levels of [^{14}C] and [^{3}H] were observed in both plasma and blood up to 336 h post dose. Two blood and plasma peaks were observed between 40-50 minutes post dose and between 18-24 h post-dose. The elimination half-life ($t_{1/2el}$) in blood and plasma was found to be long for both isotopes, ranging between 41-49 h for [^{14}C] and 121 h for [^{3}H]. The radioactivity of both isotopes was distributed in all tissues with the concentrations of [^{3}H] being slightly greater than [^{14}C] except in the kidneys. The highest concentration of radioactivity was in the kidneys, injection site, underlying muscle, and the skin surrounding the injection site, and the lowest levels in the brain and adipose tissue. Less than 1% of the injected dose remained at injection sites by 96 hours. Excretion was predominantly in the urine with <10% excreted in the feces. The mean mass balance of radioactivity for both isotopes at 336 h post dose was > 100%. In conclusion, the dual isotope labeling has provided a method to analyze the PK and distribution of the protein-conjugated CJC-1131, showing distribution within all examined tissues and a long plasma $t_{1/2el}$ of 49-121 h.

119 EFFECT OF DOSE AND ROUTE OF EXPOSURE ON THE TOXICOKINETICS OF 1, 1-DICHLOROETHYLENE (DCE) IN RATS.

C. Hines, C. White, S. Muralidhara, C. Dallas and J. Bruckner. *University of Georgia, Athens, GA.*

The purpose of these experiments was to characterize the effects of route of exposure and dose on the bioavailability and toxicity of DCE in rats. Sprague-Dawley, fasted male rats received either 10 or 30 mg/kg of DCE by IV bolus, oral bolus (gavage) or gastric infusion (GI) over 2 hours. Other rats inhaling 100 or 300 ppm DCE for 2 hr retained 10 and 30 mg/kg DCE, respectively. DCE was quantitated by gas chromatography. Toxicity was contrasted in the 30 mg/kg (gavage and GI) and 300 ppm inhalation groups by measuring plasma sorbitol dehydrogenase (SDH) and urinary N-acetyl-B-glucosaminidase (NAG) and gamma-glutamyl-transpeptidase (GGT) activitites. The half-life (113 vs. 120 min), clearance (0.06 vs. 0.06 L/min-kg) and volume of distribution (9 vs. 10 L/kg) of DCE were not significantly different between the 10 and 30 mg/kg IV dose groups, indicating linear kinetics in this dosage range. Clearance was not significantly different among routes of administration. However, the bioavailability of DCE was increased approximately 2-fold when the oral dose increased from 10 to 30 mg/kg (oral bolus and GI), indicating saturation of first-pass metabolism. Additionally, bioavailability of DCE was consistently higher with oral bolus administration than with GI. Rats inhaling 100 or 300 ppm DCE had significantly greater bioavailability than their respective oral groups. SDH levels, an index of liver damage, were significantly higher than controls in all 3 dosage groups at 4 and 24 hr and were most pronounced in the gavaged rats. The pattern of kidney injury was quite different. GGT and NAG excretion in the urine were markedly elevated in rats that inhaled 300 ppm DCE. No significant differences in GGT and NAG from controls were observed in the rats dosed orally (bolus and GI) with 30 mg/kg DCE. These findings reveal that route and rate of administration substantially influence the toxicokinetics and systemic toxicity of DCE. Support: DOE #DE-FC01-01CH11109

120 BIOAVAILABILITY OF PHENANTHRENE FROM SOIL: CORRELATION BETWEEN A RAT MODEL AND A PHYSIOLOGICALLY BASED EXTRACTION TEST.

X. Pu[1], G. P. Carlson[1], R. Galinsky[3] and L. Lee[2]. *[1]School of Health Sciences, Purdue University, West Lafayette, IN, [2]Department of Agronomy, Purdue University, West Lafayette, IN and [3]Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, IN.*

Recently, a physiologically based extraction test (PBET) was developed to predict the bioavailability of soil-bound organic chemicals. There are limited data relating it to *in vivo* animal models. The bioavailabilities of phenanthrene (PA) from soils with varying characteristics were determined using a rat model and a PBET test to examine this relationship. Four soils varying in organic carbon, clay content and pH were spiked with 200 and 400 mg/kg PA. In the *in vivo* assay, soil suspensions containing 0.25 g soil/ml were administered by gavage. Equivalent doses were given by iv. PA blood concentrations at time points after administration were analyzed by HPLC-FD. Area under the blood concentration versus time curves for each soil was compared to the iv injection to calculate the absolute bioavailability of PA from soil. In the PBET tests, 1 g samples of soils were extracted by artificial saliva, gastric juice, duodenum juice and bile. The fraction of PA mobilized from each soil was calculated. The AUCs of all soils were significantly lower than those of iv injection, indicating that soil matrix could reduce the bioavailability of PA from soil. There was a trend that the higher the OC content, the lower the bioavailability of PA. A good correlation was observed between the fraction of PA mobilized from soil and its bioavailability at 400 mg/kg soil. At 200 mg/kg soil, there was a weaker, but suggestive correlation. These results suggest that PBET assay is a useful alternative to predict bioavailability of soil-bound organic chemicals. (Supported by a Purdue Research Foundation grant.)

121 TISSUE DISTRIBUTION OF 2, 2′, 4, 4′-TETRABROMODIPHENYL ETHER FOLLOWING SINGLE AND MULTIPLE DOSES TO MALE F344 RATS.

J. M. Sanders[1, 2], M. L. Cunningham[1] and L. T. Burka[1]. *[1]LPC, NIEHS, Research Triangle Park, NC and [2]Toxicology, N.C. State University, Raleigh, NC.*

Polybrominated diphenyl ethers (PBDE's), widely used as flame retardants, are subsequently found as environmental contaminants and are present in mammalian tissues. 2, 2′, 4, 4′-Tetrabromodiphenyl ether (BDE-47) makes up 24-38% of a commercial PBDE mixture used mainly in polyurethane foam and is a major PBDE congener found in the environment. BDE-47 is highly lipophilic and has some structural similarity to dioxins, PCB's, and thyroxine. In preparation for future toxicity studies of PBDE's by the National Toxicology Program, the present work has investigated distribution of BDE-47 in tissues of male F344 rats following single and repeated exposure to the compound. Dose concentrations closer to human exposure levels than those of published studies were used. Rats received either 1, 5, or 10 consecutive daily doses of 0.1 µmol ^{14}C BDE-47/kg by gavage in corn oil. Tissue distribution of ^{14}C was determined 24 hr following the last daily dose to all rats (n=5/treatment group). Adipose tissue was the major depot of ^{14}C, containing up to 50% of each daily dose. BDE-47-derived ^{14}C accumulated extensively in skin, thymus, adrenal, and thyroid and to a lesser extent in liver, brain, blood, and muscle. The disposition of BDE-47 was further investigated following oral administration of single doses from 1-100 µmol/kg in corn oil. Analysis of major tissues and excreta indicated that both absorption of BDE-47 and tissue distribution of ^{14}C were dose-proportional in this range. Cumulative 24 hr excretion was ca. 0.5 and 35% total dose in urine and feces, respectively. A comparison of oral and iv excretion data indicated that ca. 70% of an orally administered BDE-47 dose was absorbed from the gut. However, absorption of BDE-47 apparently increased when delivered by gavage in an aqueous-based vehicle. In summary, these studies confirm bioaccumulation of BDE-47 in mammalian tissues at low exposure concentrations and provide tissue dosimetry data for design of future toxicity studies.

122 DISPOSITION OF 2, 2′, 4, 4′, 5-PENTABROMODIPHENYL ETHER IN MALE F344 RATS.

E. H. Lebetkin, J. M. Sanders and L. T. Burka. *LPC, NIEHS, Research Triangle Park, NC.*

2, 2′, 4, 4′, 5-Pentabromodiphenyl ether (BDE-99) is the most abundant of the three major polybrominated diphenyl ethers (PBDE) in the flame-retardant DE-71. DE-71 is used as an additive in plastics, electronic equipment, textiles, and building materials. It is subsequently found in the environment, accumulates in mammalian tissues, and is present in human milk. PBDE′s are of interest because of their structural similarity to PCB′s and dioxins. Some of the intermediate molecular weight PBDE′s are structurally similar to thyroxin. A disposition study of BDE-99 at a single 14.5 µmoles/kg dose has been reported. Toxicity studies being proposed by the National Toxicology Program are at lower doses. Therefore the disposition of ^{14}C-BDE-99 was studied in male F344 rats in single doses at 0.1 to 10 µmoles/kg. Based on the radioactivity excreted in feces (and present in the large intestine at termination) after oral and IV administration, 80-90% of the oral dose is absorbed. After an oral dose, BDE-99 concentrates in fat (17-23% of the total dose after 24 hours) and is excreted in the feces (35-38%), with less than 2% in urine. There is no dose effect on tissue distribution between 0.1 and 10 µmoles/kg. The liver/fat ratio is about 0.2 indicating that sequestration in liver, typical of dioxin-like chemicals is not occurring (for example, 3, 3′, 4, 4′, 5-PCB, has a liver/fat ratio of about 20 after 24 hr). BDE-99 concentration after an oral dose was higher in all tissues than in blood, with tissue/blood ratios over 10 in liver, kidney, skin, fat, thymus, and adrenal glands. The amount of BDE-99-derived radioactivity in thyroid is not significantly different from other tissues, such as muscle and lung, and is about an order magnitude less than the amount of PBE-47-derived radioactivity found in thyroid following the same molar dose of PBE-47. Over 70% of the BDE-99 in the blood was in the plasma. In summary, the tissue distribution of BDE-99 is typical of a lipophilic, polyhalogenated, aromatic chemical, but does not have high liver to fat ratio typical of dioxin-like chemicals.

123 INFLUENCE OF DIET RESTRICTION ON THE TOXICOKINETICS OF THIOACETAMIDE.

J. Chilakapati[1], K. Shankar[2], M. korrapati[1] and H. M. Mehendale[1]. *[1]Toxicology, University of Louisiana at Monroe, Monroe, LA and [2]UAMS, Little Rock, AR.*

Thioacetamide (TA) is bioactivated by CYP2E1 to TA sulfoxide (TASO), and further to sulfone, a reactive metabolite that initiates hepatic necrosis by covalent binding. Studies have shown that despite higher injury of TA by enhanced bioactivation, moderate diet restriction (DR) protects rats from a lethal dose (600 mg/kg, ip). It is known that DR induces CYP2E1. Low dose of TA (50 mg/kg) produces a 6-fold higher injury, whereas a lethal dose produces only 2.5-fold higher and delayed injury in DR rats compared to ad libitum (AL) fed rats. The objective of present work was to determine if lack of dose response for injury in AL rats and higher injury in DR rats could be explained by altered toxicokinetics. Male SD rats were 35% diet restricted and on day 22, AL and DR rats received 50 or 600 mg TA/kg. TA and TASO were quantified in plasma, liver and urine. In the AL rats, with increasing doses, half-lives of both TA and TASO were increased, revealing that TA bioactivation exhibits zero order kinetics. Greater elimination of TA and TASO was seen in urine with increasing doses. These results explain the lack of appreciable increase in bioactivation-based liver injury with the high dose in AL rats. Covalent binding of ^{14}C-TA to macromolecules was higher for the low dose compared to the lethal dose further strengthening this notion. DR rats exhibited significantly lower plasma levels of TA at the 600 mg/kg dose compared to the AL rats. Hepatic TA levels were higher in the DR rats at that dose. Also there was an increase in TASO levels in the DR group, consistent with augmented liver injury. With regard to the low dose, there was a rapid and steep increase in TASO in the DR group at 60 min., which promptly declined thereafter, further explaining the six-fold increase in liver injury. Higher hepatic levels of TA in DR in combination with induced CYP2E1 explain higher injury. The lack of dose response may be attributed to saturation of bioactivation at the higher dose. (Supported by ES09870)

124 PRELIMINARY DISTRIBUTION, EXCRETION AND PHARMACOKINETICS OF POLYACRYLAMIDE NANOPARTICLES IN MALE RATS AFTER A SINGLE I.V. INJECTION.

R. J. Schneider[1], R. Reddy[2], T. Kropp[1], B. Martin[1], M. Sherman[3] and M. Philbert[1]. *[1]Environmental Health Sciences, University of Michigan, Ann Arbor, MI, [2]Molecular Therapeutics, Inc., Ann Arbor, MI and [3]MPI Research, Inc., Mattawan, MI.*

Polymer based nanoparticles are currently being developed for biomedical applications including drug delivery and magnetic resonance imaging (MRI). Despite the composition of the different nanoparticle matrices, pharmacokinetic data has shown consistent clearance from the blood *via* the mononuclear phagocytic cells that comprise the reticular endothelial system. This study was designed to determine the distribution, excretion and pharmacokinetics of PEGylated (surface-modified) and non-PEGylated polyacrylamide nanoparticle (PAA-PEG & PAA, respectively) suspensions. Preparations of nanoparticles with mean diameters of 60nm were administered as a single I.V. dose in the tail vein of healthy male rats. Plasma, tissue and excreta were collected between 0.083-120 hours. Preliminary data from these studies suggest PAApeg nanoparticles remain in plasma up to 48hr after administration while the non-PEGylated nanoparticles are removed as early as 8hr. Furthermore, preliminary tissue distribution and excretion analyses show accumulation of PAA and PAApeg nanoparticles in the liver, while urine analyses suggest renal clearance was not a major route of excretion. The difference in kinetics of the two suspensions was further investigated by analyzing the composition and quantity of proteins adsorbed onto the surface of the nanoparticles using SDS-PAGE and mass spectroscopy. Preliminary protein binding experiments show nanoparticle interactions with albumin, Apo A-1, Alpha 1-Antitrypsin and various light and heavy immunoglobulin chains. Supported by NIH grants CA85878 and F003747.

125 BLOOD KINETICS AND PULMONARY RESPONSE OF INHALED ETHANOL IN RATS.

J. D. Field[1, 2], R. Tardif[1] and J. Nash[2]. *[1]Environmental and Occupational Health, University of Montreal, Montreal, QC, Canada and [2]DMPK, CTBR BioResearch, Senneville, QC, Canada.*

In much of the industrialized world, ethanol is added to gasoline in an attempt to reduce carbon monoxide emissions from automobile exhaust. This has led to speculation that the increased use of ethanol and inhalation of ethanol vapour during automobile refuelling may cause toxicity in some individuals. While the adverse health effects of ingested ethanol are well documented, toxicity *via* the inhalation route is unclear, especially in individuals deficient in alcohol dehydrogenase (ADH) and/or acetaldeyhyde dehydrogenase (ALDH). This preliminary study was conducted to investigate the toxicokinetics of ethanol and acetaldehyde in blood, and to assess the pulmonary inflammatory response of Long-Evans rats challenged with ADH and ALDH inhibitors prior to inhalation exposure of ethanol. Differences in ethanol metabolism between Long Evans (LE) and Sprague-Dawley (SD) rats were also evaluated. Following up to 4 hours of inhalation exposure to approximately 1300 ppm ethanol, (current exposure limit = 1000 ppm), blood ethanol (BETOH) and acetaldehyde (BACET) levels in rats treated with either metabolic inhibitors (4-MP or DDTC) or controls showed evidence that LE rats are rapid metabolizers of ethanol, compared to SD rats. Cmax and AUC parameters for both BETOH and BACET were consistent with the inhibition of ADH by 4-MP, but also inhibition of ALDH, which was stronger than by DDTC alone. In addition, results showed that the expression of ADH and/or ALDH may be different between genders and suggested that catalase may play a larger role in ethanol metabolism than previously believed. No evidence of a pulmonary inflammatory response could be established, although white blood cell counts appeared to decrease for animals with higher BETOH and BACET levels. While this appeared to have no immediate toxicological significance, it remains unclear if this decrease would limit an immune response over the long term for subjects with compromised ethanol metabolism, especially since acetaldehyde has been shown to be highly reactive.

126 DETERMINATION OF DICHLOROACETIC ACID (DCA) BY LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY (LC/MS) IN RATS DOSED WITH TRICHLOROETHYLENE (TCE).

A. M. Dixon[1], D. C. Delinsky[1], S. Muralidhara[1], J. W. Fisher[2], J. V. Bruckner[1] and M. G. Bartlett[1]. *[1]Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA and [2]Environmental Health Sciences, Univesity of Georgia, Athens, GA.*

DCA is a compound commonly found in drinking water as a chlorination disinfection by-product. In addition, DCA is a metabolite of TCE, a VOC also found in many drinking water sources as a result of its widespread use as a metal degreaser and dry cleaning agent. As DCA has been shown to cause liver cancer in rats and mice, there is concern that DCA and TCE in drinking water may present a cancer risk to humans. Interestingly, no one has definitively shown that TCE is metabolized to DCA *in vivo*. The current study was designed to determine whether DCA could be detected in tissues and blood of rats dosed with TCE. Rats (n=3) were dosed with 2 g/kg of TCE. Liver, lung, kidney, and blood samples were then collected in a scintillation *vial* at the following post dosing times: 0, 0.5, 1, 2, 4, and 8 hours. Liquid nitrogen was added to each scintillation *vial* in order to stop metabolism. Each tissue sample was homogenized and protein precipitated by adding 100 µL of acetonitrile (ACN) to each sample. After centrifugation at 16060 x g for 5 minutes, the supernatant was removed from each sample and diluted to a volume of 1 mL. Solid phase extraction was then performed on all samples. The sample eluate was dried under liquid nitrogen and reconstituted in 60:40 ACN:water prior to LC/MS analysis. Preliminary results show 2-20 times higher levels of DCA compared to blanks in all tissue and blood samples 0.5 - 8 hours after administration of TCE. These data indicate that TCE is metabolized to DCA by rats. Further studies using the LC/MS method described here will better characterize the extent of DCA formation in rats and mice. (Funded by DOE DE-FC02-02CH11109)

127 *IN VIVO* PHARMACODYNAMICS AND SUB-CELLULAR DISTRIBUTION OF A 2′-MOE MODIFIED ANTISENSE OLIGONUCLEOTIDE (ASO), ISIS 116847, TARGETING PUTATIVE PROTEIN TYROSINE PHOSPHATASE (PTEN) MNRA IN MICE AND RATS.

R. Yu, E. J. McArdle, R. Gina, K. Hoc, K. C. Nishihara, T. A. Watanabe, S. Bhanot and R. S. Geary. *ISIS Pharmaceuticals, Carlsbad, CA.*

Purpose: To study and compare *in vivo* pharmacodynamics for a 2′-MOE ASO targeting to PTEN and to explore the differences in suborgan and subcellular distributions of ASO in the liver of these two species. Methods: Subcutaneous injections of anti-PTEN ASO were given to mice and rats, respectively, at doses of 0 (control saline), 5, 10, 25 or 50 mg/kg twice a week for two weeks. Animals were sacrificed for sample collection 2 days after the last dose following two weeks of treatments. Liver samples from all groups were collected for the measurement of ASO concentrations, PTEN mRNA, and PTEN protein. Suborgan sample collection was performed for the 0 mg/kg and 25 mg/kg dose group for both species. For suborgan sample collection, liver was perfused in situ with collagenase buffer to isolate hepatocytes. PTEN ASO concentrations in whole liver, hepatocyte and subcellular fractions were quantitated by CGE or ELISA method. PTEN mRNA and protein were measured using RT-PCR and Western blot, respectively. Results: Body weight increased 5% and 30 % for mouse and rat, respectively, over the two week treatment period. ISIS 116847 concentrations in liver increased from 43 to 293µg/g and 57 to 388 µg/g in mouse and rat, respectively, as dose increased from 5 to 50 mg/kg. *In vivo* EC50 in whole liver, estimated by an inhibitory Emax model, was 157 µg/g and 105 µg/g in mouse and rat, respectively. Suborgan and sub-cellular distribution study showed that uptake by hepatocytes as well as subcellular fractions was nearly doubled in mouse compared to rat, yet equivalent message knock down was achieved. Conclusion: This study showed similar pharmacology for the ASO targeting to PTEN in mouse and rat at the level of whole organ, which correlates with whole organ pharmacokinetics. At suborgan level, we confirmed hepatocyte and nuclei had less ASO accumulation in rats, but achieved similar pharmacology compared to mice.

128 STRUCTURAL FACTORS THAT INFLUENCE THE PERFORMANCE OF MULTICASE IN THE ASSESSMENT OF MUTAGENICITY.

B. J. Braunstein, G. Mandakas, F. M. Goodsaid, R. D. Snyder and I. Y. Rosenblum. *Molecular Toxicology, Schering-Plough Research Institute, Lafayette, NJ.*

MCASE is a SAR package for the prediction of mutagenicity based on a statistical analysis of biophores and deactivating fragments derived from a learning set of chemical structures. We have characterized the output of MCASE versions defined by learning sets assembled from different combinations of reference structure databases and have challenged these MCASE versions in a matrix with structure sets not included in the specific learning set for each version. Structures for these learning sets were compiled from the original MCASE A2I database with the addition of compounds from databases populated with either environmental (UPSAL) and/or pharmaceutical (SCH (Schering-Plough) and PDR (Physicians Desk Reference)) compounds. The sensitivity and specificity of the different MCASE versions were only weakly dependent on the composition of their learning sets when challenged by any specific structure set. The specificity for all structure sets ranged between 89 and 94%. The sensitivity for the pharmaceutical structure sets ranged from 45-63% for SCH and PDR but up to 78-80% for UPSAL. Deactivating fragments were found in less than 20% of the true negative UPSAL set but in over 50% of true negative SCH and PDR sets and were also more frequent in true negative molecules than in false negative molecules within a set. Differences in molecular size between environmental and pharmaceutical molecules may account for the latter observations. An alternative explanation is that such differences are artifactual, based on the fragment size constraint (as short as 2 carbons) defined by the MCASE software. Finally, the sets of biophores identified for true and false positive molecules showed little overlap with each other, inferring that discreet subsets of biophores correlated with incorrect positive calls by MCASE. The results of this analysis suggest that changes in how MCASE identifies biophores and deactivating fragments from its learning sets may be required for improved sensitivity with pharmaceutical molecules.

129 UTILIZATION OF SAR FILTERS, CHEMICAL SIMILARITY AND HT SAFETY SCREENS TO ENHANCE COMPOUND SELECTION FROM COMBINATORIAL LIBRARIES.

N. Greene[1], J. Aubrecht[1], J. J. Osowski[1] and D. L. Grossman[2]. *[1]Safety Sciences - Groton, Pfizer Global Research and Development, Groton, CT and [2]Department of Biology, Cedar Crest College, Allentown, PA.*

While technology in combinatorial chemistry, molecular biology, and automation are flourishing, there is an increased demand for the development of high throughput screening to evaluate the safety of drugs in the early stages of drug development. In principle, Structure-Activity Relationships (SAR or structural alerts), and measures of chemical similarity could be used to predict the mutagenic behavior of a compound. Here we describe the use an SAR filter, DEREK, in combination with Selector, part of the SYBYL molecular modeling suite of programs; to aid in compound selection from a commercially-available library. A total of 274 compounds were selected, 150 compounds that did not contain a structural alert according to DEREK, and 124 compounds that did contain an alert on the basis of its structural similarity to a compound predicted negative. The Bioluminescent Ames assay was

used to assess the mutagenic activity of selected compounds and thus explore the utility of the selection process. The results of the study showed that DEREK was effective at reducing the number of mutagenic compounds in a compound selection thus potentially minimizing the potential safety concerns at a much earlier stage in the drug discovery process.

130 CONTROLLED VOCABULARY DEVELOPMENT FOR GENETIC TOXICITY TO MAXIMIZE INFORMATION INTEGRATION OF DATABASES.

D. Benz[1], C. Yang[3], G. Hollingshaus[2], W. Johnson[3], G. Myatt[3] and E. Zeiger[4]. *[1]Center for Drug Evaluation and Research, USFDA, Rockville, MD, [2]E.I. DuPont de Nemours & Co., Newark, DE, [3]Leadscope, Inc., Columbus, OH and [4]Errol Zeiger Consulting, Chapel Hill, NC.* Sponsor: D. Johnson.

As the prediction of toxicity becomes more important for understanding chemically induced toxicity, the quality and availability of chemical structures and data become critically important. The synergistic relationship between the quality of data and accuracy of prediction models demands integration of data among disparate formats and in physically different locations. Translation and transformation of data such that multiple sources of information and structures can be pooled is in fact a pre-requisite for data mining and building prediction models. An XML standard based on a controlled vocabulary optimized for genetic toxicity has been developed and is presented here. Using this schema, a sample database for genetic toxicity was prepared for data mining and building predictive models. This sample database was used to demonstrate the effectiveness of the controlled XML vocabulary and the advantages of enriching chemical space by integration of databases.

131 DETECTION OF *N*-METHYL-*N'*-NITRO-*N*-NITROSOGUANIDINE-INDUCED MUTATIONS IN GILL AND HEPATOPANCREAS OF *rpsL* TRANSGENIC ZEBRAFISH.

K. Amanuma, T. Nakamura and Y. Aoki. *National Institute for Environmental Studies, Tsukuba, Japan.*

We established a transgenic zebrafish line carrying a shuttle vector plasmid (pML4) for detecting mutagens in aquatic environments (Amanuma et al. Nat Biotech 2000). The plasmid contains a target gene (the wild-type allele of *rpsL* of *E. coli*) for detection of mutations and the kanamycin-resistance gene for rescue of the plasmid from the zebrafish chromosomal DNA. Using fish embryos, we detected mutations induced by several compounds. However, adult fish may be more useful for long-term exposure and detecting mutations in target organs. Therefore, to evaluate the mutation assay system in adult fish, we exposed female adult transgenic fish to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), and examined the effect of concentration of MNNG and the effect of rearing time after exposure on mutations induced in *rpsL*. Fish were exposed to 0, 15, or 30 mg/L MNNG (dissolved in water) for 2 hours and killed after 2 weeks. The shuttle vector was successfully recovered from genomic DNA prepared from the gill and hepatopancreas. In the gill, MNNG induced a concentration-dependent increase in mutant frequency of the *rpsL* transgene (9, 18, 51 x 10^{-5}, respectively). In the hepatopancreas, only the group treated at 30 mg/L showed an increase in the mutant frequency. DNA sequencing revealed that the most predominant type of mutation was G:C to A:T transition, which is a typical effect of MNNG. Next, we examined the effect of rearing time after exposure. Fish treated at 15 mg/L were kept in MNNG-free water for 2 or 3 weeks. In both the gill and the hepatopancreas, a higher mutant frequency of *rpsL* was observed at 3 weeks than at 2 weeks, suggesting that a period of at least 3 weeks is preferable for showing effects. These results indicate that the gill and hepatopancreas in the fish are target organs of MNNG, and that *rpsL* transgenic fish may provide a useful *in vivo* assay for detecting waterborne mutagens.

132 FINDING THE OPTIMUM APPROACH FOR GENETIC TOXICOLOGY SCREENING.

J. Kitching[1] and G. Barker[2]. *[1]Experimental Biology, Huntingdon Life Sciences, Huntingdon, United Kingdom and [2]Gentronix, Manchester, United Kingdom.* Sponsor: C. Atterwill.

20-25% of compounds test positive in *in vitro* regulatory genetic toxicology testing, leading to delays and compound attrition. To reach quality decisions on candidate compound selection reliable data needs to be made available from, in some cases, sub-mg quantities of substance. Meaningful genetic toxicology data is required as early as possible in this process. Huntingdon and Gentronix have compared the data obtained from the SOS/umu and GreenScreen? assays on over 100 compounds with the corresponding regulatory genetox results, where available. The umu assay uses two S.typhimurium strains in which lac-Z is under the control of the SOS repair genes. TA1535/pSK1002 is the standard strain and NM2009 has high o-acetyl transferase activity to make it more sensitive to aromatic amines. The assay detects a wide range of mutagens, having a particularly good correlation with the regulatory Ames test (92% in this comparison). GreenScreen? is a yeast DNA repair reporter assay and is also sensitive to a wide range of mutagens, and clastogens. Compounds were tested up to approximately 2 mM in the umu and 1 mM in the GreenScreen? Although lower than the recommended regulatory upper concentration the assays are sensitive enough for this not to affect the prediction rate. Overall both assays had a similar prediction rate for positives (about 60%). The yeast assay, as expected, was able to predict more clastogens and the bacterial more of the Ames positives. The GreenScreen? assay, at present, cannot be used with S9 activation and although the yeast does have some p450 enzymes of its own the umu assay detected more of the *in vitro* with S9 only positives. As a result, when both assays were combined it led to a positive prediction rate of about 75%. It is concluded that a screen using both assays will provide a very sensitive prediction tool, leading to accurate ranking of candidate compounds and better designed regulatory testing strategies.

133 THE UTILITY OF THE DEL ASSAY IN SACCHAROMYCES CEREVISIAE FOR DETECTION OF CHROMOSOME ABERRATIONS *IN VITRO.*

Z. Kirpnick[1, 2, 3], N. Howlett[4], M. Repnevskaya[1, 2, 3], M. Homiski[5], E. Rubitski[5], J. Aubrecht[5] and R. H. Schiestl[1, 2, 3]. *[1]Pathology, David Geffen School of Medicine at UCLA, Los Angeles, CA, [2]Radiation Oncology, David Geffen School of Medicine at UCLA, Los Angeles, CA, [3]Environmental Health Sciences, UCLA School of Public Health, Los Angeles, CA, [4]Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA and [5]Pfizer Inc., Groton, CT.*

The DEL Assay for chromosomal rearrangement in the yeast S. cerevisiae is able to detect a wide range of carcinogens. In a study with approximately 60 compounds of known carcinogenic activity, the DEL assays detected 86% correctly whereas the Ames Salmonella assay detected only 30% correctly. Since the DEL assay is highly inducible by DNA double strand breaks, we examined the utility of the DEL assay for detecting chromosome aberrations. We have selected 10 model compounds with known genotoxic effects for testing using the DEL assay . The *in vitro* micronucleus assay in CHO cells, a commonly used tool for assessment of chromosomal aberrations, was performed on the same compounds and the results of the two assays were compared. The compounds tested were: Actinomycin D, Camptothecin, Methotrexate and 5-Fluorodeoxyuridine, which are anticancer agents, Noscopine and Furosemide are theraputics, Acradine and Methyl Acrylate are industrial chemicals, Diazinon is an insecticide and Resoricnol is a dye. Our data indicate that the DEL assay provides highly concordant data with the standard *in vitro* MN assay. The DEL assay might have a potential application as a robust, fast, and economical screen for detecting chromosome aberrations *in vitro.*

134 THREE-COLOR LABELING SCHEME FOR FLOW CYTOMETRY-BASED SCORING OF RODENT AND HUMAN PERIPHERAL BLOOD MICRONUCLEATED RETICULOCYTES.

S. D. Dertinger[1], D. Torous[1], M. Bishop[2], Y. Chen[3], R. K. Miller[4], C. Tometsko[1] and J. T. MacGregor[5]. *[1]Litron Laboratories, Rochester, NY, [2]FDA-NCTR, Jefferson, AR, [3]Radiation Oncology, University of Rochester Medical Center, Rochester, NY, [4]Obstetrics and Gynecology, University of Rochester Medical Center, Rochester, NY and [5]FDA-NCTR, Rockville, MD.*

Micronucleated reticulocyte (MN-RET) frequencies represent an index of recent cytogenetic damage. This report describes a modification to a well characterized flow cytometric scoring procedure for measuring the incidence of MN-RET in rodent and human blood samples. In the "standard" system, differential staining of young and mature erythrocytes, with and without micronuclei, is accomplished by combining the immunochemical reagent anti-CD71-FITC with a nucleic acid dye (propidium iodide plus RNase). The modification described here incorporates a fluorescent anti-platelet antibody into the labeling procedure (anti-CD61-PE and anti-CD42b-PE for rodent and human samples, respectively). This 3-color scheme was directly compared to the standard (2-color) procedure with serial blood samples that were obtained from rats before and over the course of daily vinblastine treatment (0 or 0.125 mg/kg/day). While the 3-color system did not add significant value to analyses of terminal day blood samples (obtained *via* heart puncture), it did improve the reliability of measurements for preceding day blood samples that were obtained *via* tail vein nick. The resulting dataset suggests that accuracy of flow cytometric MN-RET measurements can be maintained with the 3-color procedure, even when platelet-platelet and platelet-cell aggregates are present. In addition to these rodent blood analyses, human MN-RET measurements, for samples collected before and over the course of fractionated radiotherapy, are presented. Results of these analyses suggest that human samples benefit from the inclusion of a fluorescent anti-platelet immunochemical reagent.

135 OXYFLOW: DETECTION OF OXIDATIVE DNA DAMAGE TO HEMATOPOIETIC CELLS RESULTING FROM *IN VITRO* AGENT ADMINISTRATION USING FLOW CYTOMETRY.

I. N. Rich[1], M. Shaw[2] and C. G. Kilty[2]. [1]*HemoGenix LLC, Colorado Springs, CO and* [2]*Biotrin International, Ltd., Dublin, Ireland.* Sponsor: M. McKenna.

Oxidative injury is indicated in a wide range of diseases. Damage occurs by the release of free radicals caused by agents, injury or normal metabolism. Free radicals can react with DNA and lead to mutation, carcinogenesis, teratogenesis or cell death. Oxidative damage can occur at any time during hematopoesis. The most susceptible cells are the stem and progenitor cells, since these are responsible for maintaining hematopoietic steady-state conditions. Damage to stem or lineage-specific progenitor cells can result in drastic perturbation of the system and even blood malignancy. The effect of agents that result in free radical formation and oxidative DNA damage can be detected by the presence of 8-oxoguanine, an oxidized nucleotide of 8-oxyguanosine and is the commonest DNA adduct produced as a result of exposure of DNA to oxygen free radicals. DNA mispairing and misreading can also occur. A fluorescein isothiocyanate (FITC)-conjugated binding protein with high avidity and specificity to 8-oxoguanine was used to detect oxidative DNA damage in cells from human peripheral blood, bone marrow or umbilical cord blood and hematopoietic cells from other mammalian species. Target cells were first permeabilized and fixed and then incubated with the FITC-conjugated binding protein. The target cells were resuspended and analyzed by flow cytometry using an Epics XL/MCL. Using different fluorochromes conjugated to antibody membrane markers, oxidative damage could be detected in specific hematopoietic subpopulations using multiparameter gating analysis. To standardize and validate the procedure, control cells were treated with methylene blue and light in a dose-dependent manner. At concentrations of methylene blue above 10μM, there was a displacement to the right indicating increased fluorescence intensity due to binding of the FITC-conjugated binding protein to oxidised DNA. This new technique can be applied to toxicity testing, pharmacological and chemical high-throughput screening and occupational and environmental biomonitoring.

136 INCREASED FREQUENCIES OF MICRONUCLEATED RETICULOCYTES AND 8-OHDG LEVELS IN ALDH2 KNOCKOUT MICE.

N. Kunugita[1], T. Isse[2], T. Oyama[2], K. Kitagawa[3], M. Ogawa[2], T. Yamaguchi[2], R. Suzuki[2], T. Kinaga[2], A. Yoshida[4], I. Uchiyama[5] and T. Kawamoto[2]. [1]*School of Health Sciences, University of Occupational and Environmental Health, Kitakyushu, Japan,* [2]*Department of Environm Health, University of Occupational and Environmental Health, Kitakyushu, Japan,* [3]*First Department of Biochemistry, Hamamatsu Medical University, Hamamatsu, Japan,* [4]*Beckman Research Institute of the City of Hope, Duarte, CA and* [5]*Graduate School of Engineering, Kyoto University, Kyoto, Japan.*

Aldehyde dehydrogenase-2 (Aldh2) metabolizes acetaldehyde produced from ethanol into acetate and plays a major role in the oxidation of acetaldehyde *in vivo*. In order to investigate whether the induction on micronuclei by ethanol is associated with genetic polymorphisms of Aldh2 gene, the flow cytometric micronucleus (MN) assay were performed in Aldh2 knockout (Aldh2 KO) mice and wild type (Aldh2 WT) mice. The mice were given 24h free access to a 1% ethanol for alcohol group or water for control group for 20 weeks. We also estimated the 8-hydroxy-2′-deoxyguanosine (8OHdG) levels in mice urine by ELISA. In results, frequencies of MN in reticulocytes and erythrocytes significantly increased by ethanol drinking in Aldh2 KO mice, but not in Aldh2 WT mice. In addition, frequencies of MN and 8OHdG levels were significantly high in Aldh2 KO mice than in Aldh2 WT mice even in control groups. These results indicate that Aldh2 plays a major role not only in acetaldehyde detoxification but also in oxidative stress in mice.

137 QUANTITATIVE LONG PCR ANALYSIS OF DNA DAMAGE INDUCED BY PROSTAGLANDIN H2 SYNTHASE FORM-2: NORMALIZATION OF REPLICATION BY AN INTERNAL CONTROL.

H. Kim[1, 2], D. J. Kaplan[1], Y. Yuan[1], D. A. Putt[1] and B. Zhang[1]. [1]*Detroit R&D, Inc., Detroit, MI and* [2]*Institute of Environmental Health Sciences, Wayne State University, Detroit, MI.*

Human PGHS-2 was expressed in DNA damage repair-deficient XPA human fibroblasts. Genotoxicity mediated by prostaglandin H2 synthase form-2 (PGHS-2, COX-2) was quantitated by measurement of a lack of PCR replication with damaged DNA. The cells were treated with tert-butyl hydroperoxide (t-BOOH, a substrate of PGHS-2) and/or benzo(a)pyrene-7, 8-dihydrodiol (BPD, a known PGHS-activated DNA damaging agent). The 8.9 kb mitochondrial DNA fragments were replicated by 14 cycles of quantitative long PCR (QLPCR) with biotinylated and non-biotinylated primers and quantitated by chemiluminescent and real-time PCR analyses, respectively. Levels of the 8.9 kb QLPCR products were normalized using levels of 6.9 kb internal controls added to each PCR reaction mixture. The 6.9 kb PCR controls containing intact primer binding sites were produced by deleting a 2 kb fragment from the 8.9 kb fragment. For chemiluminescent analysis, replicated DNA fragments were electroblotted to membrane and visualized by incubation of the blot with chemiluminescent substrates of horseradish peroxidase conjugated with streptavidin. For real-time PCR analysis, QLPCR products were quantitated using purified 8.9 kb standards ranging from 0.01 to 1, 200 pg. The detection limit of the method is lower than 0.01 pg. Both chemiluminescent and real-time PCR quantitation revealed that, whereas levels of the QLPCR-replicated mictochondrial DNA fragments failed to decrease after treatment of cells with 5 μM BPD, the PCR products dramatically decreased after treatment of the cells with 0 to 15 μM BPD in the presence of 0.5 mM t-BOOH. These results suggest that 0.5 mM t-BOOH treatment damaged DNA in PGHS-2-expressing cells and BPD treatment damaged DNA in the cell only in combination with t-BOOH. Sponsored by the NIEHS SBIR grant R44 ES11251 & the EHS Center grant P30 ES06639.

138 TIME COURSE OF *CII* GENE MUTANT FREQUENCIES AND MUTATION SPECTRA IN THE BONE MARROW OF *N*-ETHYL-*N*-NITROSOUREA-TREATED TRANSGENIC MICE.

J. Wang[1, 2], N. Mei[1], X. Liu[1], M. M. Moore[1, 2] and T. Chen[1]. [1]*DGRT, NCTR, Jefferson, AR and* [2]*Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR.*

The *in vivo* transgenic mutation assays allow the mutation detection in multiple tissues. But the time required to reach the maximum mutant frequency (MF) is tissue specific. To identify the optimal sampling time, a time course study of *cII* gene mutant manifestation in the bone marrow of *N*-ethyl-*N*-nitrosourea (ENU) treated Big Blue mice was conducted. Six-month old female mice were treated i.p. with a single dose (125 mg/kg) ENU, then the bone marrow was sampled and assayed for *cII* gene MFs 1, 3, 7, 15, 30 and 120 days later. Compared with the MFs for concurrent controls [$(34\pm14)\times10^{-6}$], all the MFs of ENU-treated mice (253, 374, 277, 235, 238 and 189×10^{-6}) were elevated greatly. The maximum MF (day 3) was also significantly different from other MFs. To explain the differences, mutation spectra for day 1, 3 and 120 were analyzed. At day 1, the predominant mutation type is G:C to A:T transitions (32%), which can both result from unrepaired o^6-ethylGuanine and spontaneous mutation. A:T to T:A and A:T to G:C, the typical ENU-induced mutations, were 25% and 8%. At day 3, G:C to A:T were reduced to 26%, while A:T to T:A and A:T to G:C were elevated to 32% and 23%. At day 120, the three types were 26%, 34% and 26%, respectively. The spectrum of day 1 is significantly different from that of day 3 and 120, while the latter two are similar. The results suggest that the optimal sampling time is day 3 and transit cells in bone marrow are more sensitive to ENU than stem cells. While the replacement of immutable differented cells with mutated transit cells explains the MF elevation from day 1 to 3, DNA repair and spontaneous mutation contribute to their spectra difference. The similarity of the mutation spectra of day 3 and 120 suggests that their MF difference resulted from the lower sensitivity of stem cells to ENU, compared with the transit cells. The MF decline after day 3 is consistent with the loss of mutated transit cells, while mutated stem cells are responsible for the MF plateau.

139 AGE-DEPENDENT SENSITIVITY OF BIG BLUE TRANSGENIC MICE TO THE MUTAGENICITY OF ETHYLNITROSOUREA (ENU) IN LIVER.

N. Mei, J. Wang, R. H. Heflich, M. M. Moore and T. Chen. *Division of Genetic and Reproductive Toxicology, NCTR/FDA, Jefferson, AR.*

There are rising trends in the reported incidence of childhood cancer and recent evidence suggests an association between childhood cancer and environmental exposure to genotoxins. In addition, the liver of infant mice is extremely sensitive to tumor induction by carcinogens. In the present study, the Big Blue transgenic mouse model was used to determine whether specific periods in early life represent special windows of vulnerability to genotoxins. Groups of mice were treated with single doses of 120 mg ENU/kg body weight or vehicle control transplacentally at 3 days before birth or at postnatal days (PNDs) 1, 8, 15, 42 or 126, and the animals were sacrificed 6 weeks after the treatment. The *cII* mutation assay was performed to determine the mutant frequencies (MFs) in the livers of the mice. Liver *cII* MFs for both sexes were dependent on the age at which the animals were treated. Perinatal treatment with ENU (either transplacental treatment 3 days before birth or i.p. injection at PND 1) induced relatively high MFs. ENU treatment at PNDs 8 and 15, however, resulted in the highest mutation induction. The lowest mutation induction occurred in those animals treated as adults (PNDs 42 and 126). There was a significant difference ($P<0.05$) between the MFs in the infant animals

and those in the other treatment groups. For instance, the MF for the PND 8 female group was 646 x 10^{-6} while the MF for female adults was only 145 x 10^{-6}, a more than 3-fold difference. Our mutagenicity results correlate well with the age-dependent sensitivity to carcinogenicity in mice and indicate that infancy is the period at which mouse liver is the most sensitive to mutation and tumor induction by genotoxins.

140 EFFECT OF OVARIECTOMY ON MUTATIONS INDUCED BY 7, 12-DIMETHYLBENZ(*A*)ANTHRACENE (DMBA) IN THE LIVER *CII* GENE OF BIG BLUE TRANSGENIC RATS.

T. Chen[1], R. C. Hutts[2], N. Mei[1], M. E. Bishop[1], S. Shelton[1], M. G. Manjanatha[1] and A. Aidoo[1]. [1]*Division of Genetic and Reproductive Toxicology, NCTR/FDA, Jefferson, AR and* [2]*Center for Toxicology and Environmental Health, Little Rock, AR.*

Hepatocellular carcinoma (HCC) is one of the most common cancers. Women develop HCC with much less frequency and better prognosis for the disease than men. In rodent models, carcinogen-induced and spontaneous HCC also occurs at a lower incidence in females than in males. Although sex hormones have been implicated as an important factor associated with the development of HCC, the effects of estrogens on chemical mutagenesis in liver are presently unknown. To evaluate the role of endogenous estrogens in chemical hepatomutagenesis, 2 groups of 10 Big Blue female rats were treated with 80 mg DMBA (a multi-organ carcinogen)/kg body weight or vehicle, and 5 animals from each group were ovariectomized two weeks later. At 16 weeks after DMBA treatment, mutant frequencies (MFs) and types of mutations were determined in the liver *cII* gene of the overiectomized (OVX) and intact (INT) rats. The MFs were 203 x 10^{-6} for the DMBA-treated OVX rats and 135 x 10^{-6} for the DMBA-treated INT animals, compared to the untreated control MFs: 67 x 10^{-6} for the OVX group and 44 x 10^{-6} for the intact animals. DMBA significantly increased the MFs in both the OVX and INT rats. The MF in the DMBA-treated OVX rats was significantly higher than that in the DMBA-treated INT animals although there was no significant difference between the MFs in the untreated OVX and INT rats. Molecular analysis of the mutants showed that ovariectomy did not significantly alter the types of mutations in the DMBA-treated or untreated animals. However, a significant difference was found between spectra in the treated animals and the untreated controls. G:C → T:A transversion was the most common type of mutation in both the treated OVX and INT groups, whereas G:C → A:T transition was the predominant mutation in both control groups. These results suggest that ovarian hormones inhibit mutation induction in the liver by carcinogens.

141 IDENTIFICATION OF *IN VIVO* MUTATION FOR THE ΦX174 TRANSGENIC MUTATION ASSAY USING THE FORWARD MUTATION ASSAY OF GENE *A*.

C. R. Valentine[1], J. L. Raney[1] and R. R. Delongchamp[2]. [1]*Division of Genetic and Reproductive Toxicology, NCTR, USFDA, Jefferson, AR and* [2]*Division of Biometry, NCTR, USFDA, Jefferson, AR.* Sponsor: J. Valentine.

In vivo transgenic rodent mutation assays have high spontaneous mutant frequencies relative to induced mutant frequencies. The ΦX174 transgenic mutation assay has a unique method for distinguishing *in vivo* from *in vitro* mutations: single burst analysis requires that a burst of phage from a single bacterium exceed a minimum size, indicating that the mutation was fixed before entering the bacterial cell. Bursts are identified by separating electroporated bacterial suspensions into many aliquots before plating on selective bacteria. We have conducted DNA sequencing of multiple mutants from aliquots of different plaque count to characterize the distribution of mutations from each. As expected, aliquots with larger plaque counts (>100) had multiple plaques with the same mutation, indicating progeny from a single, *in vivo* mutation. Aliquots with small plaque count had a greater variety of mutation, indicating their independent origin. Large bursts, from ENU-treated animals, fit the spectrum of ENU-induced mutation. The optimal plating density (μg DNA / aliquot) was reduced relative to the reversion assay in gene *E* in order to separate *in vitro* bursts because of the larger number of target sites for the forward assay. We have also determined the mutant spectrum in *E. coli* of the ΦX174 gene *A* forward assay. The mutant spectrum from control animals of mutations contributing <60 PFUs per aliquot was not different from that of ΦX174 grown in *E. coli*, which included two hotspot sites that dominate the spectrum, 4225A→G, C, or T and 4218G→A or C. Therefore, the majority of mutant plaques from control animals was fixed in *E. coli*, not the animal. Single burst analysis allows the measurement of *in vivo* mutant frequencies below the spontaneous *E. coli* frequency. We are currently evaluating the frequency of *in vivo* bursts from control and ENU-treated animals treated with 40 mg/kg of ENU by the same protocol used to evaluate the *lacI* transgene in order to compare the relative sensitivity of the two assays.

142 POTENTIAL FOR PHENOL TO DISRUPT THE SPINDLE APPARATUS AS EVALUATED IN THE MOUSE BONE MARROW MICRONUCLEUS TEST (MNT).

P. J. Spencer[1], V. A. Linscombe[1], J. G. Grundy[1], B. B. Gollapudi[1], J. M. Waechter[1] and S. S. Dimond[2]. [1]*Toxicology & Environmental Research & Consulting, The Dow Chemical Company, Midland, MI and* [2]*Health and Environmental Safety, GE Plastics, Huntersville, NC.*

High doses of phenol cause significant hypothermia in treated mice. In the mouse, micronuclei (MN) were induced in the bone marrow polychromatic erythrocytes concomitant with phenol-induced hypothermia. Earlier attempts to distinguish direct vs. secondary mechanisms of phenol-induced MN by maintaining normal body temperature in phenol-treated mice were unsuccessful because of inadequate restoration of thermoregulatory homeostasis. In order to gain further insight into the mode of action responsible for phenol-induced micronuclei, the etiology of MN in phenol treated mice was investigated by staining the MN with antikinetochore antibody. Six male mice/group were administered phenol (300 mg/kg), the vehicle (saline) or the positive control for inducing kinetochores (vinblastine (VB), 4 mg/kg) as a single i.p. dose. Body temperature was monitored at 2 and 5 hrs post-dosing and the formation of MN and kinetochore-positive (K+) MN were evaluated at 24 hr. Phenol at 300 mg/kg induced a marked and prolonged hypothermia as well as a significant increase in MN formation. VB induced a significant increase in K+MN (78% of MN cells were K+). Phenol also induced a significant but smaller increase in K+MN (16%) over the negative controls (0%). The presence of kinetochores in the MN from phenol-treated mice provides evidence for a mode of action that involves, at least in part, disruption of the spindle apparatus which could be linked to hypothermia. (Sponsored by the Phenol Panel of the American Chemistry Council, Arlington, VA).

143 INHIBITION OF DNA REPAIR AS A MECHANISM OF ARSENIC CARCINOGENESIS.

S. Liu, E. Kopras, M. Medvedovic, G. G. Oakley and K. Dixon. *Environmental Health, University of Cincinnati, Cincinnati, OH.*

Arsenic is a metalloid that is widely distributed in the environment. Exposure to arsenic has been associated with an increased risk of a variety of cancers, including skin, liver, bladder, kidney and lung cancer. However attempts to prove arsenic carcinogenesis in animals have been unsuccessful. A hypothesis has been proposed that arsenic might act as a co-carcinogen instead of a carcinogen itself. Recently Rossman's group demonstrated that arsenic enhanced the induction of skin tumors by UV irradiation in hairless Skh1 mice (Rossman, 2001). Consistent with its co-carcinogenic activity, arsenic by itself does not seem to induce point mutations in most of the bacteria and mammalian systems tesed. Instead, it can potentiate the mutagenicity of other mutagens, such as UV (Hartmann, 1996), Benzo(a)Pyrene (Maier, 2002) and N-methyl-N-nitrosourea (NMU)(Li, 1989). The mechanism of this co-mutagenicity remains unknown. The most tempting hypothesis is that arsenic can inhibit DNA repair. We have used arsenite at low concentrations in cultured human cells to test this hypothesis, in the hope of shedding light on the mechanism of arsenic mutagenesis and co-mutagenesis. Our study shows that (1) Arsenic at 1 uM is not mutagenic, but induces deletion/insertion mutations at 2.5 uM. Arsenic synergistically enhances UV mutagenicity at 2.5uM, but not at 1uM. (2) Arsenic treatment does not alter the UV mutation spectra. (3) Arsenic at 1-5 uM enhances and prolongs RPAp34 phosphorylation induced by UV irradiation, suggesting the persistence of the DNA damage signal that induces the phosphorylation response. This effect is less prominent in NER-deficient XPA cells. (4) Arsenic does not increase the generation of thymine dimers induced by UV assayed by FACS with thymine dimer-specific antibody, but it inhibits the removal of thymine dimers. All of these results suggest that arsenic inhibits DNA repair, probably nucleotide excision repair. Further work will be required to identify the mechanism of this inhibition. This work is supported by NIESH Superfund Basic Research grant ES04908.

144 DISCOVERY AND FUNCTIONAL ANALYSIS OF XPA POLYMORPHISMS.

P. C. Porter[1], I. Mellon[2] and J. States[1]. [1]*Pharmacology & Toxicology, University of Louisville, Louisville, KY and* [2]*Pathology, University of Kentucky, Lexington, KY.*

Inter-individual differences in nucleotide excision repair (NER) alter cancer risk. NER gene polymorphisms provide a readily detectable source of genetic variation in NER, but little is known about their functional impact. XPA is essential for NER. We detected missense polymorphisms in XPA and investigated their effects by complementation of XPA cells. XP12RO-SV, an SV40-immortalized XPA mutant cell line, was complemented with wild type, R228Q or V234L XPA. Both polymorphisms provided wild type survival in response to both ultraviolet light (UV)(Mellon *et al. DNA Repair* (2002) *1*:531-46) and benzo[a]pyrene-diol-epoxide (BPDE) induced DNA damage despite low expression compared to wild type

XPA complemented cells . SV40-immortalization inactivates p53-dependent induction of global genomic repair, an NER sub-pathway. To provide a p53- and NER-proficient cell culture model, we investigated the use of telomerase-immortalized cells. Repair normal primary diploid human skin fibroblasts (GM00024) were immortalized with telomerase and named tGM24. tGM24 cells retain normal p53 induction and cell survival in response to UV and BPDE exposure. Primary XPA cells (GM02991) also were telomerase immortalized for complementation and named tGM2991. tGM2991 cells are hypersensitive to DNA damage compared to tGM24 cells. Similar survival decreases and p53 inductions were obtained in tGM2991 with 10-fold lower UV doses than in tGM24. Likewise, similar survival decreases were obtained with 5-fold lower BPDE doses. Our results support the hypothesis that telomerase immortalization does not alter the p53 DNA damage response. tGM2991 cells were stably transfected with wild type or one of three missense polymorphic XPA mini-genes (R228Q, V234L, L252V). Three cell lines expressing XPA protein at wild type levels are made: two contain the L252V XPA and one contains the R228Q XPA. (Support: University of Louisville Center for Envir. and Occup. Health Sciences and Center for Genetics and Mole. Med., KY EPSCoR, Markey Cancer Center, KY Research Challenge Trust Fund, and USPHS grant ES06460)

145 DIFFERENCES IN DNA REPAIR ACTIVITY AND INHIBITION OF REPAIR BY ALFLATOXIN B_1 CORRELATES WITH SUSCEPTIBILITY TO CARCINOGENESIS IN MOUSE.

L. Bedard[1], M. Alessi[2], S. K. Davey[3] and T. E. Massey[1]. *[1]Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada, [2]Chemistry, Queen's University, Kingston, ON, Canada and [3]Cancer Research Laboratories, Queen's University, Kingston, ON, Canada.*

Aflatoxin B_1 (AFB_1) is a mutagenic and carcinogenic metabolite produced by the fungus *Aspergillus*. To exert carcinogenicity, AFB_1 requires bioactivation to AFB_1-*exo*-epoxide, which binds to guanine (Gua) residues in DNA, forming AFB_1-N^7-Gua that can be hydrolyzed to AFB_1-formamidopyrimidine (AFB_1-FaPyr). Removal of AFB_1 DNA damage occurs primarily by nucleotide excision repair (NER). The objective of this study was to compare DNA repair synthesis activities of whole mouse lung and liver and rat liver protein extracts, and to determine whether *in vivo* treatment of mice with AFB_1 inhibits their ability to repair AFB_1 DNA damage. DNA repair synthesis activity of cell-free whole tissue protein extracts was assessed using an *in vitro* assay that reproduces the entire NER reaction against substrate plasmid DNA with AFB_1 damage. Mouse liver extracts repaired AFB_1-N^7-Gua and AFB_1-FaPyr ~ 5 fold and 30 fold more efficiently than did whole lung extracts respectively (N=4, $P<0.05$). Furthermore, mouse liver repair activities were ~ 6 fold and 4 fold higher than those of rat liver against AFB_1-N^7-Gua and AFB_1-FaPyr damage respectively. Lung extracts prepared from mice treated with a single tumourigenic dose of 50 mg/kg AFB_1 ip and sacrificed 2 h post-dosing demonstrated minimal repair synthesis activity against AFB_1-N^7-Gua damage (N=3, $P<0.05$). In contrast, liver extracts retained the ability to repair AFB_1-N^7-Gua (N=3, $P>0.05$). These results suggest that the lower DNA repair activity and sensitivity to inhibition by AFB_1 of mouse lung contributes to the susceptibility of mice to AFB_1-induced lung tumourigenesis relative to hepatocarcinogenesis. Furthermore, lower ability of rat liver to repair AFB_1-DNA damage may contribute to its relative susceptibility to hepatocarcinogenesis. In addition to AFB_1 DNA adducts being mutagenic, their genotoxicity may be enhanced by the ability of AFB_1 to inhibit their repair.

146 CHARACTERIZATION OF DNA REPAIR MECHANISMS FOLLOWING AFLATOXIN B_1 TREATMENT IN YEAST EXPRESSING HUMAN CYTOCHROME P450 1A2.

Y. Guo[1], H. Zarbl[3, 1], L. L. Breeden[3], B. D. Preston[2] and D. L. Eaton[1, 3]. *[1]Environ Occup Hlth Sciences, University Washington, Seattle, WA, [2]Pathology, University Washington, Seattle, WA and [3]Fred Hutchinson Cancer Research Cntr, Seattle, WA.*

Aflatoxin B_1 (AFB) is a human hepatotoxin and hepatocarcinogen produced by the mold *Aspergillus flavus*. In humans, AFB is bioactivated by hepatic CYP1A2 and 3A4 to a genotoxic epoxide that forms N^7-guanine DNA adducts. Little is known about how these bulky DNA adducts are repaired. A series of yeast haploid mutants defective in checkpoints and DNA repair were transformed with human CYP1A2. The stability of CYP1A2 expression in these mutants was characterized by western blots and Methoxyresorufin O-demethylase (MROD) enzymatic activity. Mutant clones were screened, and those with comparable CYP1A2 activity to wild-type strain were treated with various doses of AFB. Colony-forming ability and the *L*-Canavanine resistance forward mutation assay were employed to observe cell survival and mutation, respectively. Strains defective in nucleotide excision repair (NER) (*rad14*), postreplication repair (PRR) (*rad6* and *rad18*) and checkpoints (*mec1-1* and *rad9*) exhibited significantly decreased cell survival after AFB treatment, relative to their wild type, while strains defective in recombinational repair (RR) (*rad51* and *rad54*) had moderate sensitivity. *rad6*, *rad18*, *mec1-1*, *rad9*, *rad51* and *rad54* had significantly reduced AFB-induced mutation frequency. These results indicated the involvement of NER, PRR and RR in repair of AFB-induced DNA damage, and the requirement of PRR error-prone pathway in AFB-induced mutagenicity. Interestingly, while double mutant *rad51 rad14* showed greatly enhanced sensitivity and mutation frequency towards AFB treatment in comparison with either single mutant, *rad18 rad14* demonstrated neither lowered cell survival nor increased frequency of AFB-induced mutations comparing to either single mutant. These results demonstrate complex relationships among various DNA repair pathways and cell cycle control in AFB-induced cell cytotoxicity and mutagenesis in a yeast model. (Supported by grants R01ES05780, P30ES07033 and U19ES011387)

147 MUCOCHLORIC ACID INDUCES SINGLE STRAND BREAKS IN XRCC1 DEFICIENT CELLS.

E. Bodes, J. Nakamura, A. Molinelli, Y. Li, B. Pachkowski and J. A. Swenberg. *University of North Carolina, Chapel Hill, NC.*

Epidemiologic studies indicate that long-term consumption of chlorinated disinfected water is associated with increases in cancer. Mucochloric acid (MCA) is a by-product produced by the action of chlorine on humic acid in drinking water. While there is no direct link between MCA and cancer, we have shown that MCA is capable of damaging cells in culture. Our goal was to clarify whether the formation of single strand breaks (SSBs) was a mechanism by which MCA induces genotoxicity. We used the comet assay to detect SSB formation and a novel colorimetric assay that indirectly monitors PARP activation for SSB repair. While using NAD+ as substrate PARP binds to DNA nicks and recruits essential repair proteins to the damaged site. To understand the role of PARP activation in depleting intracellular NAD(P)H we applied several PARP inhibitors to our test systems. We used genetically matched CHO cells with and without functional XRCC1. XRCC1 is critical for genomic stability and it coordinates SSB repair. In both assays we detected a significant increase in SSBs across all doses after exposure to MCA. Transfecting deficient cells with hXRCC1 showed responses similar to proficient cells, supporting the role of XRCC1 in SSB repair after MCA exposure. In the colorimetric assay, pre-exposure with PARP inhibitors produced different results. 3-Aminobenzamide (AB) protected against a reduction in NAD(P)H at all doses whereas dihydroxyisoquinoline (DQ) was less effective at higher doses of MCA. Surprisingly, in the comet assay we detected dramatic differences in MCA-induced DNA damage upon pre-exposure to either AB or DQ, with AB forming significantly less SSBs. We hypothesized that opening of the lactone ring of MCA to an aldehyde allowed for the formation of a Schiff base conjugate with AB, but not DQ. We preincubated equal molar concentrations of MCA with the inhibitors and detected an MCA-AB conjugate by LC-MS/MS. These data explain the results obtained in both the comet and colorimetric assays. We concluded that covalent binding of AB with MCA prevents MCA from damaging DNA.

148 METHYL NITROSOUREA INDUCES LEUKEMOGENESIS WITH PRACTICAL THRESHOLD IN WILD TYPE MICE WHEREAS NONTHRESHOLD IN P53 DEFICIENT MICE.

Y. Hirabayashi[1], K. Yoshida[2], Y. Kodama[1], J. Kanno[1], Y. Kurokawa[3], I. Yoshimura[4] and T. Inoue[5]. *[1]Cellular & Molecular Toxicology. Division, NIHS, Tokyo, Japan, [2]Division of Biology and Oncology, NIRS, Chiba, Japan, [3]Sasaki Research Institute, Tokyo, Japan, [4]Faculty of Engineering, Tokyo University of Science, Tokyo, Japan and [5]Center for Biological Safety & Research, NIHS, Tokyo, Japan.*

A question, whether an absolute nonthreshold or a relative nonthreshold, i.e., a "practical" threshold, specifically at the low-dose level is present, may not be answered even with the use of a prohibitively large number of wild-type mice. Could the excessive incidence of tumorigenesis in p53-deficient mice contribute to our understanding of the threshold vs. nonthreshold issue in genotoxic carcinogenesis? This is considered because an exaggeration of tumorigenesis in p53-deficient mice is hypothesized to reduce or eliminate the range of threshold due to the p53-deficiency-mediated reduction of DNA repair and apoptosis. The present study of chemical leukemogenesis in p53-deficient mice by transplantation assay was designed to answer this question. Briefly, 218 C3H/He mice were lethally irradiated and repopulated with bone marrow cells from wild-type, heterozygous p53-deficient, and homozygous p53-deficient C3H/He mice. This was followed by treatment with a single and graded dose of methyl nitrosourea at 6.6, 14.8, 33.3, 50.0, and 75.0 mg/kg BW, with the vehicle-treated control groups treated with zero dose for each genotype. Whereas mice repopulated with p53-deficient bone marrow cells showed a marked reduction of the threshold for leukemogenicity, mice repopulated with wild-type bone marrow cells did not exhibit leukemia at a dose of 33.3 mg/kg body weight, and showed a curve with a high probability for the linear regression model with a positive dose intercept, predicting a threshold by the likelihood ratio test. Thus, the failure of wild-type mice to show an increase in incidence of leukemogenesis at low doses of genotoxic carcinogens may be due not to a statistical rarity, but to various p53-related functions possibly including DNA repair and apoptosis that may account for a threshold.

149 SEASONAL UV DOSE AND DNA REPAIR CAPACITY PREDICT NON-MELANOMA SKIN CANCER RISK.

J. L. Matta[1], A. Ruiz[1], R. A. Armstrong[2], Y. Detres[2] and J. M. Ramos[1]. [1]*Pharmacology and Toxicology, Ponce School of Medicine, Ponce, Puerto Rico and* [2]*Marine Sciences, University of Puerto Rico, Mayaguez, Puerto Rico.*

Non-melanoma skin cancer (NMSC) is the most common cancer worldwide. UV radiation is an important risk factor for NMSC. UVA (320-400 nm) and UVB (280-320 nm) can cause DNA damage and mutations. Cancer risk is partly determined by the capacity of the body to repair DNA damage. DNA repair capacity (DRC) has been reported as an independent risk factor for the development of NMSC. A 3-year clinical study was performed in Puerto Rico to develop a model of how environmental factors (e.g. UV exposure and sunblock) and genetics (e.g. DRC and skin type) influence the risk of NMSC. UVA and UVB measurements were obtained over a 5-year continuous period using a Biospherical Instruments GUV-511 radiometer. All participants (n=550) completed an Informed Consent and a questionnaire that elicited information on risk factors. DRC was measured in peripheral blood lymphocytes using a host cell reactivation assay with luciferase reporter gene. NMSC risk factors were determined by logistic regression analysis. Monthly means of daily UV radiation fluxes ranged from 569 to 1504 kJ m-2 d-1. UV fluxes were 24% higher from March-September when compared with the October-February period. Persons with NMSC had a statistical significant lower DRC (42%) compared with controls. Significant risk factors ($p<0.005$) included a minimal of 3-hour weekly cumulative UV dose (OR=2.0, 95% CI=1.2-3.3), skin type I and II (OR=8.5, 95% CI=5.2-13.9), and the use of sunblock (OR=0.16, 95% CI=0.10-0.26). A high DRC (highest 10%) reduced NMSC risk 7-fold and a low DRC (lowest 33%) increased risk by 3.4-fold. Overall, for every 1% decrease in DRC, the risk of developing NMSC increased 20%. The model presented illustrates how genes-environment-disease interact to influence NMSC risk. Genetic factors are more important than environmental factors for the development of NMSC in the population studied. These findings may be useful for NMSC prevention programs. Supported by NIH-NCRR grant 2G12RR03050-19.

150 ALTERATION OF CHEMOTHERAPEUTIC-INDUCED DNA DAMAGE BY A COMMON HEALTH FOOD SUPPLEMENT.

W. Trinachartvanit[1], B. M. Francis[2] and A. Rayburn[3]. [1]*Animal Biology, University of Illinois, Urbana, IL,* [2]*Entomology, University of Illinois, Urbana, IL and* [3]*Crop Sciences, University of Illinois, Urbana, IL.*

In the US, use of health food supplements has increased rapidly in the last decade, especially in cancer patients. However, recent concerns have been raised as to whether specific health food supplements taken during chemotherapy interact with chemotherapeutic agents. If interactions occur, they could enhance the action of chemotherapy or interfere with it by decreasing the conventional medication efficacies. Our aim was to investigate any potential interactions between an over-the-counter health food supplement, Fruit of LifeTM (which is advertised to consist of highly active antioxidants) and a chemotherapeutic agent, cytosine arabinoside (ara-C). Outbred CD1 mice were fed a semi-synthetic diet, supplemented with 0% (control), 0.2% (low dose) or 1.0% (high dose) of Fruit of LifeTM, for 4 weeks. Ara-C at 8 mg/kg was administered ip to half of the control and treated mice, 72 hours prior to the end of the feeding period. Prior studies had demonstrated that this dose of ara-C damages DNA in bone marrow without causing acute illness in the mice. Bone marrow was collected from all treatment groups and flow cytometry was used to determine DNA damage to the bone marrow cells. There were no significant differences in DNA damage between negative control mice and mice fed with 0.2% and 1.0% Fruit of LifeTM. Combined treatments of ara-C with either 0.2% or 1.0% Fruit of LifeTM significantly decreased the DNA damage level compared to ara-C treated mice on the unsupplemented diet. These results suggest that the health food supplement of interest (Fruit of LifeTM) interacts with the chemotherapeutic agent (ara-C) to decrease DNA damage caused by ara-C to normal bone marrow cells. Future studies are needed to determine how Fruit of LifeTM may alter the effectiveness of ara-C during chemotherapy. *This work was supported by a grant from the Office of the Attorney General of the State of Illinois.*

151 MODE OF ACTION FOR THE *IN VITRO* MUTAGENICITY OF BIOBAN CS-1246 AND IMPLICATIONS FOR ITS *IN VIVO* MUTAGENIC POTENTIAL.

B. B. Gollapudi[1], G. Charles[1], M. R. Schisler[1], M. Cifone[2], R. A. Budinsky[1] and P. J. Spencer[1]. [1]*The Dow Chemical Company, Midland, MI and* [2]*Covance Labs, Vienna, VA.*

The biocidal agent, 7-ethyl bicyclooxazolidine (Bioban CS-1246, CAS# 7747-35-5) was concluded to be non-mutagenic in a bacterial reverse mutation assay using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, and in an *in vitro* cytogenetics assay in Chinese hamster ovary cells both in the presence and absence of Aroclor-induced rat liver S-9. However, Bioban CS-1246 induced a mutagenic response in the mouse lymphoma (L5178Y TK+/-) forward mutation assay (MLA) both with and without S-9. Significantly, the mutagenicity was completely abrogated when the cultures were supplemented with formaldehyde (FA) dehydrogenase/NAD+ suggesting that the positive MLA response was attributable to the generation of FA in situ. The MLA findings are unlikely to be of relevance to an intact animal because of the efficient detoxification of low doses of FA *in vivo*. Evidence of this was obtained by the non-genotoxicity of Bioban CS-1246 in 2 *in vivo* assays. Firstly, in a mouse (male CD-1) bone marrow micronucleus test (MNT), in which there were no significant increases in micronucleated polychromatic erythrocytes following treatment with 0.5, 1 and 2 g/kg/day for 2 days and sacrificed 24 h later. Also, in an *in vivo*/*in vitro* unscheduled DNA synthesis (UDS) study, male F344 rats were given a single oral gavage at 0, 1 and 2 g/kg of CS-1246. Livers were perfused at 2 time points (2-4 and 14-16 h) and the extent of UDS was quantified in hepatocyte cultures after exposure to 3H-thymidine and autoradiography. CS-1246 did not elicit an UDS response indicating the lack of DNA reactivity *in vivo*. Based upon the weight of evidence from an extensive array of genotoxicity tests and the mode of action data, it is concluded that CS-1246 is not an *in vivo* mutagen. These results clearly demonstrate that *in vitro* genotoxicity of CS-1246 is associated with FA and unlikely to have relevance in animals or humans because of the occurrence of efficient detoxification mechanisms.

152 GENOTOXICITY EVALUATION OF THIODIGLYCOLND.

G. Reddy, M. A. Major and G. J. Leach. *Directorate of Toxicology, US Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD.*

Sulfur mustard (HD) undergoes hydrolysis to form thiodiglycol (TG) and small amounts of compounds in biological and environmental systems. The US Army has proposed to neutralize HD through a hydrolysis process with subsequent biodegradation of reaction products. TG has been detected in soil and water at certain Army installations. The toxicity data on TG are limited to only a few reports. Genotoxicity data on TG are not available to complete health and environmental risk assessments. Therefore, we developed genotoxicity data under extramural contract. These tests were conducted in accordance with Environmental Protection Agency Health Effects Testing Guide Lines in compliance with Good Laboratory Practice. The summary of test results of completed work will be reported here. Thiodiglycol did not produce mutagenic effects at any dose up to 5000 mg/plate in Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) and Escherichia coli (WP2uvrA) tester strains with and without a metabolic activation system. TG did not increase mutantion frequency at levels up to 5000 mg/ml in mouse lymphoma L5178ytk+/- cells with and without metabolic activation. The effects of TG on *in vitro* chromosomal aberrations in Chinese Hamster Ovary (CHO) cells showed chromosomal aberrations at 5 mg/ml (without metabolic activation) and 4 mg/ml with metabolic activation. Thiodiglycol (up to 2000 mg/kg) when tested *in vivo* in the mouse micronucleus assay was not mutagenic in mouse bone marrow. These studies revealed that TG is not mutagenic in three assays (Ames test, mouse lymphoma and mouse micronucleus assay) but it positive *in vitro* in CHO cells chromosomes. On the basis of these studies we believe that TG does not appear to pose a genetic hazard to humans (Abstract does not reflect US Army Policy)

153 DNA DAMAGE IN HUMAN LEUKOCYTES INDUCED *IN VITRO* BY 1- OR 2- BROMOPROPANE.

M. Toraason[1], N. P. Singh[2] and D. W. Lynch[1]. [1]*NIOSH, Cincinnati, OH and* [2]*University of Washington, Seattle, WA.*

1-Bromopropane (1-BP; n-propyl bromide) (CAS No. 106-94-5) is an alternative to ozone-depleting chlorofluorocarbons that has a variety of potential applications as a cleaning agent for metals and electronics, and as a solvent vehicle for spray adhesives. Its analogue, 2-brompropane (2-BP; isopropyl bromide) (CAS No. 75-26-3) impairs antioxidant cellular defenses, enhances lipid peroxidation, and causes DNA damage *in vitro*. In the present study, DNA damage was assessed in human leukocytes exposed *in vitro* to 1-BP or 2-BP at 0, 0.01, 0.1, or 1 mM for 8 hr; or at 1 mM for 1, 2, 4 or 8 hr. Exposures to 1-BP or 2-BP were performed in triplicate samples of fresh heparinized venous blood from an adult male volunteer. For estimation of DNA damage, comets were produced by alkaline microgel-electrophoresis. In each sample, DNA damage was estimated in a minimum of 100 leukocytes using VisComet image analysis software. Apoptosis was assessed in leukocytes exposed to 1-BP or 2-BP using the DNA diffusion assay. 1-BP or 2-BP induced a significant increase in comet tail moment at 1 mM but not at 0.01 or 0.1 mM. DNA strand break number was significantly increased at 0.1 and 1 mM of 1-BP or 2-BP. In temporal studies, a significant increase in DNA damage was evident 1, 2, 4, or 8 hrs after exposure to 2-BP, whereas increased DNA damage was observed only after 4 or 8 hrs of exposure to 1-BP. Significant increases in apoptosis were evident after

exposure to 0.1 or 1 mM 2-BP, but only after exposure to 1 mM 1-BP. Results demonstrate the potential for 1-BP and 2-BP to induce DNA damage *in vitro* in human leukocytes.

154 GENOTOXICITY EVALUATION OF THE CHLOROHYDRIN HYDROLYSIS PRODUCT OF BISPHENOL A DIGLYCIDYL ETHER (BADGE-2HCL).

J. M. Waechter, V. A. Linscombe, M. R. Schisler, K. M. Jackson and P. J. Spencer. *Toxicology & Environmental Research and Consulting, The Dow Chemical Co., Midland, MI.*

Bisphenol A diglycidyl ether is the monomer for epoxy resins used for the production of lacquer coatings for food and beverage cans. Therefore, human exposure to BADGE and/or its hydrolysis products *via* the migration of the residual monomer from cured can coatings into foodstuffs may occur. Previous work has shown that one of the hydrolysis products of BADGE in the presence of aqueous gastric fluid simulants is its *bis*-chlorohydrin (2, 2-*bis*[4-(3-chloro-2-hydroxypropoxy)phenyl]propane) (BADGE-2HCl) reported to increase micronuclei in cultured human lymphocytes treated *in vitro* (Suarez et al., Mutation Res. 470: 221-228, 2000). The genotoxicity potential of BADGE-2HCl was studied in three *in vitro* assays with or without the presence of S9 activation. In a Salmonella-Escherichia coli reverse mutation assay, six concentrations of the test article were tested ranging from 1 to 1000 micrograms/plate and 10 to 5000 micrograms/plate, respectively. In a chromosomal aberration assay, rat lymphocytes were treated with concentrations ranging from 6.25 to 37.5 micrograms BADGE-2HCl/ml of media for determining the incidence of chromosomal abberations. In a mouse lymphoma forward mutation assay, concentrations of BADGE-2HCl ranged from 1.25 to 80 micrograms/ml without S9 and from 1.25 to 100 micrograms/ml with S9. BADGE-2HCl did not induce a mutagenic or clastogenic response in these assays. The *in vivo* genotoxic potential of BADGE-2HCl was studied using the mouse bone marrow micronucleus (MN) test using oral doses of 0, 250, 500, or 1000 mg/kg/day administered on two consecutive days. Groups of six CD1 mice per dose were sacrificed at 24 hours after the second treatment for the evaluation of polychromatic erythrocytes for MN (2000 cell/animal) which were not significantly increased at any dose relative to the negative controls. In summary, these data indicate that BADGE-2HCl does not represent a genotoxic hazard. (Sponsored by the Epoxy Resins Committee of the Association of Plastics Manufacturers in Europe)

155 INDUCTION OF DNA DAMAGE (COMET ASSAY) BY BISPHENOL A IN CHINESE HAMSTER OVARY (CHO) CELLS.

K. Rao. *Toxicology, MicaGenix, Greenfield, IN.*

Induction of DNA Damage (Comet Assay) by Bisphenol A in Chinese Hamster Ovay (CHO) Cells. Yong Xu, Ellen Shaw, K.S. Rao, and J.W. Parton, MicaGenix Inc., Greenfield, IN Bisphenol A (4, 4-isopropylidene-2-diphenol, BPA) is a key industrial monomer used for the synthesis of polycarbonate plastics and epoxy resins. BPA produces a weak response in uterotropic assays and there has recently been renewed attention to estrogenic potential of BPA due to widespread contamination of laboratory experiments by BPA through leaching from autoclaved polycarbonate cages (Koehler et. Al. Lab. Animal 32:24, 2002). Epoxy resin-based sealers were reported to induce DNA damage with Comet assay (Huang et al. J Endodontics 27:744, 2001), which might be related to recent findings of genotoxic activity of BPA. This study investigated the potential activity of BPA on DNA damage using the Comet assay in CHO cells in the presence or absence of S9 activation. A trypan blue exclusion assay was performed to determine the cytotoxic activity of BPA. A 30-50% cytotoxicity was observed at concentration levels of 39.1 ug/ml with S9 and 78.1 ug/ml without S9. It was concluded that the microsomal fraction S9 enhanced cytotoxicity of BPA. A Comet assay was performed by treating CHO cells with BPA for 2 hours with and without S9. A dose-related increase of DNA damage, represented by Comet moment, Tail length and % tail DNA, was observed. The effects were again higher in the cells with S9 activation than without S9. The results indicate a DNA damage effect of BPA and suggest this effect may be related to the metabolism of BPA.

156 PHOTOMUTAGENICITY OF BERGAMOTTIN AND ISOPIMPINELLIN.

J. Cocchiara and A. Api. *Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ.*

The European Commission's Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) recently released an opinion to limit all "furocoumarin-like" substances in cosmetic products to 1 ppm based on potential photocarcinogenic and photomutagenic effects. Since it is not proven that all furocoumarins are photocarcinogens, the photomutagenicity of two furocoumarins found in citrus oils used in perfumery, bergamottin and isopimpinellin, was investigated in *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2. Treatments were performed in all strains at concentrations up to precipitating dose levels. Specifically the bacteria were plated with bergamottin at doses of 15.8-5000 μg/plate, and isopimpinellin at doses of 3.16-1000 μg/plate in both the presence and absence of UV light. Irradiations of each strain used appropriate UVA light exposures, the higher exposure being the mutagenicity limit for each strain, having previously been demonstrated to induce approximately a doubling of revertant numbers or a 50% decrease in cell survival, and the lower exposure being a non-mutagenic and non-toxic dose. For bergamottin, none of the treatments resulted in any increases in revertant numbers indicative of any photomutagenic activity, and this material was considered negative under the conditions of this study. For isopimpinellin, there were no notable increases in revertant numbers in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537. However, statistically significant increases in revertant numbers in strain TA102 were observed following irradiation (both with the low and high UV irradiation levels), and were dose-related up to the precipitating dose levels. A confirmatory test in this strain was conducted. The increases were reproducible and these data are therefore considered indicative of photomutagenic activity of isopimpinellin in strain TA102 in this assay system. Additional photomutagenicity testing on other furocoumarins identified in essential oils is planned.

157 BACTERIAL MUTAGENICITY OF CIGARETTE SMOKE GAS/VAPOR PHASE.

F. J. Tewes and T. J. Meisgen. *PHILIP MORRIS Research Laboratories GmbH, Cologne, Germany.* Sponsor: H. Haussmann.

The *in vitro* mutagenicity of cigarette smoke is generally determined from the particle phase. In this study the mutagenicity of the gas/vapor phase of cigarette smoke was investigated. Mainstream smoke from University of Kentucky standard reference cigarettes and a research cigarette, generated according to ISO standards, was passed through a glass fiber filter to trap the total particulate matter (TPM) and then bubbled through ice-cold phosphate-buffered saline to trap the ′watersoluble fraction of the gas/vapor phase′ (GVP). Mutagenicity was determined in the microsuspension modification of the Ames Salmonella mutation assay according to Kado (1983). Results revealed that GVP is mutagenic; however, in contrast to TPM, GVP contains significant amounts of direct-acting mutagens, as indicated by the response in the absence of a metabolic activation system, and causes mainly base-pair substitutions, as indicated by the higher response in strain TA100 than in strain TA98. Validation of the microsuspension assay using the solvent as negative control and methyl methanesulfonate as positive control showed that the intra-day and inter-day variability of the assay, with a coffecient of variation of less than 20%, was well within the usual range of other *in vitro* genotoxicity assays. Comparison of the reference cigarettes with the research cigarette showed that these cigarettes could be discriminated on the basis of GVP mutagenicity (e.g., response at equivalent TPM doses was approximately four-fold higher for the research cigarette compared to the Reference Cigarette 2R4F). Screening for mutagenic constituents in GVP showed that acrolein and formaldehyde were active in the microsuspension assay and that acrolein was responsible for up to 40% of the GVP activity. The results of the study demonstrate that the gas/vapor phase of cigarette smoke is mutagenic and that the microsuspension assay may be a useful tool for further investigations.

158 GENOTOXICITIES OF SAMPLES FROM NICKEL REFINERIES: PREDICTIONS OF CARCINOGENIC POTENTIALS.

R. Verma and J. Landolph. *Cancer Research Laboratory, Depts. of Mol. Microbiol. and Immunol., Path., and Mol. Pharmacology/Toxicol., USC/Norris Cancer Center, Keck School of Medicine/School of Pharmacy, University of Southern California, Los Angeles, CA.*

Epidemiological studies and animal carcinogenicity data indicate that different nickel compounds have different carcinogenic potentials. This poses the problem of how to predict carcinogenic potentials of those nickel samples for which there are no valid animal inhalation studies and no, or inconclusive, epidemiological data. We have used short-term *in vitro* assays to determine the relative genotoxicities of several nickel samples, finding good agreement between *in vitro* and *in vivo* results. We have now extended this work to include four samples of nickel refinery dust: two samples from a sulfidic nickel ore refinery (MD, RD) and two samples from a lateritic nickel ore refinery (NiCOM, GNiO [<15μm]). The sample of refinery matte dust (MD), was significantly cytotoxic to cultured C3H/10T1/2 (10T1/2) mouse embryo fibroblastic cells, with an LC_{50} concentration of (1.5 ± 1.2) μg/ml. This sample induced morphological transformation in 10T1/2 cells although it was

taken up by phagocytosis to approximately one fourth the extent that nickel subsulfide was. The roasting dust (RD) sample from the same refinery, was 20-fold less cytotoxic to 10T1/2 cells than the MD sample. The Nickel compact (NiCOM) sample from a lateritic ore refinery was phagocytosed and induced cytotoxicity, chromosomal aberrations (breaks and gaps), and morphological transformation in, 10T1/2 cells. The LC_{50} of the NiCOM sample was 18.5 µg/ml. The fourth sample corresponds to the less than 15 µm subfraction of green nickel oxide (GNiO [<15µm]). This sample was weakly cytotoxic compared to nickel subsulfide and other green (HT) nickel oxide samples previously tested. Our preliminary data suggest that the MD sample may have a carcinogenic potential similar to that of pure nickel subsulfide, in agreement with results from epidemiologic studies.

159 AMINO TERMINUS-DELETED CONSTITUTIVE ANDROSTANE RECEPTOR VARIANTS ARE EXPRESSED FROM DOWNSTREAM AUG AND CUG START CODONS.

M. A. Stoner[1], S. S. Auerbach[2] and C. J. Omiecinski[1]. [1]*Department of Veterinary Science, The Pennsylvania State University, University Park, PA and* [2]*Department of Pharmacology, University of Washington, Seattle, WA.*

The constitutive androstane receptor (CAR) (NR1I3) is a member of the nuclear receptor superfamily and regulates gene expression in response to xenobiotic signals, most notably phenobarbital-like compounds. CAR expression is highest in liver, where multiple mRNA splice variants have been characterized. Through use of a protein translation start site prediction program, we have identified at least six AUG and CUG putative start codons in the mRNA downstream of the usual start of CAR protein translation. Western blot analysis of human liver proteins reveals a complex banding pattern of CAR and CAR-like proteins, and *in vitro* translation of CAR using a wild-type DNA template yields the full-length product and a number of smaller proteins. One lower molecular weight band aligns with an amino terminus-deleted CAR variant that is missing the first 75 amino acids, apparently initiating translation at methionine 76. Truncated CAR variants were tested and did not efficiently drive transcription of a transiently transfected luciferase gene under control of a CAR/RXR binding site. However, co-expressed variant CAR proteins did appear to modulate the transcriptional activity of full-length CAR. Mutation of the full-length CAR translation start site results in increased expression of truncated CAR proteins, suggesting a cap-dependent model of protein translation, whereby the ribosome loads at one end of the transcript and scans for the next best putative Kozak sequence. Studies are ongoing to explain the involvement of amino terminus-deleted CAR variants in normal CAR signaling. (Supported by a Grant from the NIGMS, GM66411).

160 FUNCTIONAL ASSESSMENT OF A PUTATIVE PHOSPHORYLATION SITE IN A VARIANT ISOFORM OF HUMAN CAR.

S. S. Auerbach[2], M. A. Stoner[1] and C. J. Omiecinski[1]. [1]*The Pennsylvania State University, University Park, PA and* [2]*Department of Pharmacology, University of Washington, Seattle, WA.*

The constitutive androstane receptor (CAR, NR1I3) is one of 48 nuclear receptors encoded in the human genome. CAR mediates transcriptional activation of genes involved in phase1, 2 and 3 xenobiotic and steroid metabolism in response to inducing agents such as the barbiturate, phenobarbital. Multiple isoforms of the receptor have been identified recently that are derived from alternatively spliced transcripts. CAR2 contains an insertion of 4 amino acids (SPTV) that generates a putative phosphorylation site in the ligand-binding domain. Mutation of the serine in this motif to an alanine blocks phosphorylation and enhances the ability of the receptor to transactivate an (NR1)5-driven luciferase reporter. However, mutation of the serine residue to an aspartate, generates a mock phosphorylated form of the receptor that completely disrupts transactivation. Studies using specific protein kinase inhibitors together with phospho-specific immunoblot analyses are in progress to determine the potential functional role of protein phosphorylation on the interaction of CAR2 with DNA targets and with transcriptional coregulators. The existence of structurally variant CAR receptors may be important in expanding the repertoire of functional activities governed by this receptor in human tissues. (Supported by a grant from the NIGMS, GM66411)

161 ROLE OF CONSTITUTIVE ANDROSTANE RECEPTOR IN THE INDUCTION OF CYP2B AND XENOBIOTIC TRANSPORTERS BY OLTIPRAZ.

L. Bird[1], A. L. Slitt[2], W. Huang[3], D. D. Moore[3], C. D. Klaassen[2] and N. J. Cherrington[1]. [1]*University of Arizona, Tucson, AZ,* [2]*University of Kansas Medical Center, Kansas City, KS and* [3]*Baylor College of Medicine, Houston, TX.*

Oltipraz (OPZ) induces Phase I & II drug metabolizing enzymes such as Cyp2B and glutathione S-transferases. Coordinate regulation of Phase I & II enzymes with xenobiotic transporters has been shown after treatment with microsomal enzyme inducers. The purpose of this study was to determine whether OPZ also regulates xenobiotic transporter expression, and to determine the role of constitutive androstane receptor (CAR) in OPZ-mediated induction. To determine whether OPZ activates transporter gene expression *via* CAR, male and female Wistar-Kyoto rats were treated with OPZ and mRNA levels quantified by bDNA signal amplification. Wistar-Kyoto females express lower levels of CAR protein, such that they exhibit significantly lower induction of Cyp2B1 by phenobarbital as compared to Wistar-Kyoto males. OPZ induced UGT2B1 in males significantly higher than in females, suggesting activation through CAR. However, OPZ induced Mdr1b, Mrp3 and 4, mEH, NQO1, Cyp1A1, Cyp3A, and Cyp2B1/2 equally in both genders, indicating a CAR-independent mechanism of induction. Other transporters were unaffected by OPZ. To determine whether OPZ is capable of activating CAR-mediated transcription, $CAR^{-/-}$ mice were used to demonstrate the CAR-dependent induction of Cyp2B10 by OPZ. OPZ caused a robust induction of Cyp2B10 in wild type mice that was absent in $CAR^{-/-}$ mice. Finally, CAR-mediated induction of human CYP2B6 by OPZ was demonstrated using an *in vivo* transcription assay. Luciferase reporter constructs containing either a CYP2B6 promoter fragment containing the NR1 site or a multimeric NR1 site (NR1x5) alone were hydrodynamically transfected into mice. OPZ administration increased *in vivo* reporter activity of both the human CYP2B6 promoter and CAR specific response element constructs. In summary, OPZ coordinately increases hepatic xenobiotic transporter mRNA levels, along with Phase I & II enzymes *via* both CAR-dependent and CAR-independent mechanisms.

162 CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) INVOLVEMENT IN THE INDUCTION OF UDP-GLUCURONOSYLTRANSFERASE (UGT) IN RAT LIVER BY PHENOBARBITAL AND OTHER CYP2B INDUCERS.

M. K. Shelby[1], A. L. Slitt[1], N. J. Cherrington[2] and C. D. Klaassen[1]. [1]*University of Kansas Medical Center, Kansas City, KS and* [2]*University of Arizona, Tucson, AZ.*

UGTs are phase II metabolizing enzymes that catalyze the glucuronidation of numerous endobiotic and xenobiotic compounds. Phenobarbital (PB), a known Cyp2B inducer, increases UGT activity. Induction of Cyp2B by PB and other microsomal enzyme inducers is mediated by the nuclear receptor CAR. The purpose of this study was to determine the UGT isoforms in liver that are induced by the Cyp2B inducers PB, diallylsulfide (DAS), polychlorinated biphenyl 99 (PCB99), and trans-stilbene oxide (TSO), and whether CAR is involved in induction of those UGTs. In order to determine which UGTs are induced, UGT mRNA levels were determined in male Sprague-Dawley rats treated with the aforementioned Cyp2B inducers. UGT2B1 mRNA levels were increased by all four inducers, whereas UGT1A1 and 1A6 mRNA levels were induced by DAS, PCB99, and TSO. UGT1A5 mRNA levels were induced by DAS and TSO, and UGT2B12 mRNA levels were induced by PCB99 and TSO. The role of CAR in UGT induction was determined using Wistar Kyoto (WK) rats which posses a gender difference in liver expression of CAR where females express lower CAR levels than males. UGT mRNA levels were determined in male and female WK rats treated with PB, DAS, PCB99 and TSO. UGT1A1, 1A5, and 2B1 mRNA levels were induced in male WK rats by all four Cyp2B inducers. However, in female WK rats induction of UGT1A1, 1A5, and 2B1 mRNA levels was either abolished or diminished. UGT1A6 mRNA levels in WK rats were induced only by TSO. Additionally, there was no apparent difference in induction of UGT1A6 mRNA levels between male and female WK rats, suggesting induction of UGT1A6 by TSO is not CAR dependent. However, CAR appears to mediate, at least in part, induction of UGT1A1, 1A5, and 2B1 by PB, DAS, PCB99, and TSO. (Supported by NIH Grants ES-08156, ES-07079)

163 RESPONSE OF MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CLASS II PROMOTER REGION TO DEX AND RIFAMPICIN IN TRANSFECTED G3A CELLS AND PRIMARY RAT HEPATOCYTES WITH HUMAN PREGNANE X RECEPTOR (SXR).

E. Fuentes[1], B. D. Jimenez[1], L. C. Quattrichi[2], B. Joyce[2] and P. S. Guzelian[2]. [1]*Biochemistry, University of Puerto Rico, Medical Sciences Campus, San Juan, Puerto Rico,* [2]*Medical Toxicology, University of Colorado Health Sciences Center, Denver, CO and* [3]*Center for Environmental & Toxicological Research, University of Puerto Rico, Medical Sciences Campus, San Juan, Puerto Rico.*

Regulation of MHC class II gene expression is of considerable interest, the research presented here may provide new insight into novel pathways of immune regulation. Some evidence suggest that the expression of MHC class II proteins in non-APCs such as tumor cells could make the immune system respond better to tumors. Glucocorticoids such as Dexamethasone (DEX) and the antibacterial rifampicin (RIF) regulate gene expression by binding to the respective nuclear receptor and then interacting with specific promoter regions on the host cell DNA. To assess the

regulation of MHC II expression at the transcriptional level by DEX or rifampicin, we used the promoter region of HLA-DRa coupled to a luciferace reporter gene and transfected in G3A human fibrosarcoma cells line. Our results with transfected human fibrosarcoma cell line shows that the presence of SXR enhance MHCII gene expression significantly in these cells. This was confirmed with titration experiments using the SXR expression vector (pCDG-SXR). Our results also suggest that RIF treatment may mediate a non significant transcriptional activation of MHC II while DEX treatment inhibits it. Preliminary data in primary rat hepatocytes suggest that both Dexamethasone (DEX) and pregnenolone 16a-carbonitrile (PCN) induces the activity in these cells transfected with the human promoter of MHC II (HLA-DRa). These results are in agreement with those previously obtained in *in vivo* experiments conducted in our laboratory, which demonstrated induction of MHC II by these compound in non-APCs.

164 MECHANISTIC EXAMINATION OF GSK3 REGULATION BY PEROXISOME PROLIFERATORS AND ITS ROLE IN HEPATOCARCINOGENESIS.

K. A. Burns and J. P. Vanden Heuvel. *Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA.*

Peroxisome proliferators-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor family. PPARs can be activated by a large group of chemicals called peroxisome proliferators (PPs) that include industrial pollutants and hypolipidemic drugs. It has been shown in murine models that administration of PPs results in hepatomegaly, mitogenesis, and eventually hepatocellular carcinoma. PPs are thought to cause cancer by altering gene expression and ultimately cell cycle regulation. While it is known that PPARα mediates these effects, the mechanisms responsible for the responses observed are not known. To discern these responses, previous studies in our laboratory were done to comprehensively analyze gene expression in rat and human hepatoma cells exposed to PPs. These studies showed that glycogen synthase kinase 3 (GSK3) was being regulated in response to PP treatment. GSK3 is a serine/theronine-specific kinase with a number of important targets. Western blot analysis shows that both GSK3 isoforms (α and β) protein levels are decreased in the PPARα wild type hepatocyte model upon PP stimulation, but not in its PPARα -/- counter-part. Quantative RT-PCR analysis reveals no regulation at the transcriptional level; therefore, it is hypothesized that this regulation is occurring at the post-translational level. Experiments will be utilized to determine whether this regulation is the result of altered ubiquination and protein turnover. Since GSK3 is an important signaling molecule involved in diverse biological processes, regulation of this kinase may play a key role in PP induced cell cycle aberrant regulation.

165 PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORβ (PPARβ) ATTENUATES COLON CARCINOGENESIS.

H. E. Marin[1, 2], C. J. Nicol[3], F. S. Harman[2], F. J. Gonzales[3] and J. M. Peters[2]. *[1]Department of Veterinary Science and the Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA, [2]Biochemistry, Microbiology, and Molecular Biology, The Pennsylvania State University, University Park, PA and [3]Laboratory of Metabolism, National Cancer Institute, Bethesda, MD.*

Recent data has shown that PPARβ may be involved in colon cancer. PPARβ expression is reported to be upregulated in human colon tumors containing an inactivated APC gene, as well as in human and azoxymethane-induced rodent tumors. In these studies, the functional role of PPARβ in colon carcinogenesis was investigated using azoxymethane (AOM)-treated PPARβ(-/-) mice as well APC(+/-) x PPARβ(-/-) mutant mice. In the first study, PPARβ(-/-) mice were treated with AOM and colon polyp formation was examined. AOM treatment caused a dose-dependent increase in colon polyp formation in wild-type mice, and this effect was exacerbated in PPARβ(-/-) mice. In the second study, APC(+/-) mutant mice were crossed with PPARβ(-/-) mice, generating double mutants, APC (+/-) x PPARβ (-/-). Similar to AOM-treated PPARβ(-/-) mice, double mutant mice also exhibited increased polyp formation compared to control APC(+/-) mice. In addition, the double mutant mice showed increased mortality rate as compared with APC(+/-) controls. Interestingly, PPARγ mRNA expression was decreased in colons of AOM-treated wild-type and PPARβ(-/-) mice, and more so in colon polyps. Decreased expression of PPARγ mRNA was also found in colon polyps of both APC(+/-) and double mutant mice. Given the difference in phenotype observed between genotypes, this demonstrates that the increased incidence of polyp formation is not due to differences in PPARγ expression. Further, in contrast to recent reports, results from these studies provide evidence that PPARβ attenuates colon carcinogenesis in both a chemically-induced and genetic model. Supported by National Cancer Institute (CA 97999)

166 THE ACTIVATION OF MOUSE AND HUMAN PPARα, PPARβ AND PPARγ BY PHTHALATE MONOESTERS.

M. T. Bility[1], J. M. Peters[2], R. H. McKee[3], R. M. David[4] and J. H. Butala[5]. *[1]The Center for Molecular Toxicology, The Pennsylvania State University, State College, PA, [2]Veterinary Sciences, The Pennsylvania State University, State College, PA, [3]ExxonMobil Biomedical Sciences Inc., ExxonMobil, Annandale, NJ, [4]Health and Environment Laboratories, Eastman Kodak, Rochester, NY and [5]Toxicology, Toxicolog Consultants Inc., Gibsonia, PA.*

Phthalate diesters are industrial plasticizers with ubiquitous human exposures due to their ability to readily leach from commercial products. Phthalate diesters are hydrolyzed into phthalate monoesters by esterases in the gut, liver and blood. Phthalates are known to activate peroxisome proliferator-activated receptors (PPARs), and the monoesters appear to be the active metabolite that binds PPARs. In this study the activation of both human and mouse PPARα, PPARβ, and PPARγ by phthalate monoesters were investigated by trans-activation assays using stably-transfected 3T3-L1 cell lines. MI7P, MINP, MEHP, MIDP, MnOP, MBenP and MI6P activated both mouse and human PPARα. The mouse PPARα was generally more sensitive than the human PPARα to activation by phthalate monoesters. MIDP, MBenP, MEHA, MButP and MI6P activated mouse PPARβ, but human PPARβ was not significantly activated by any of the phthalate monoesters. MI7P, MINP, MEHP, MIDP, MnOP, MBenP, MEHA and MI6P activated both mouse and human PPARγ. Further, both mouse and human PPARγ had similar sensitivity to activation by phthalate monoesters. The hierarchy of potency for the activation of PPARα among the phthalate monoesters was determined to be MnOP> (MIDP≈MINP≈MI7P)> (MEHP≈MI6P)> MBenP>MEHA> (MButP≈MEP). The hierarchy of potency for the activation of PPARγ among the phthalate monoesters was determined to be MnOP>MINP> (MIDP≈MI7P)> (MEHP≈MBenP)> (MI6P≈MEHA)> (MButP≈MEP). These results indicate that the potency of phthalate monoesters for the activation of PPARα and PPARγ increases with increasing alkyl side chain length. This research was supported by The Phthalate Esters Panel of The American Chemistry Council and The Huck Institute for Life Sciences, The Pennsylvania State University.

167 CITED2 IS A COACTIVATOR OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-α AND -γ TRANSCRIPTIONAL ACTIVITY.

E. Tien and J. P. Vanden Heuvel. *Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University, University Park, PA.*

The peroxisome proliferator-activated receptors (PPARs) are a class of nuclear receptors which respond to chemicals known as peroxisome proliferators (PPs). The PPARs can be further classified into three types (α, β or δ and γ). These three subtypes of PPAR have been implicated in many biological functions ranging from adipocyte differentiation to hepatocarcinogenesis. The ultimate physiological response of the PPARs to their respective ligands is dependent in large part to the recruitment and function of other proteins known as coregulators. The coregulator class of proteins can be divided into two main categories: coactivators and corepressors. It is becoming apparent that the composition of the transcriptional complex formed around the nuclear receptor hetero- or homo- dimer can vary depending on the ligand which activated the nuclear receptor. These recruitment differences can greatly alter the ability of a transcriptional complex to regulate aspects of cellular function in response to a chemical insult. In these studies, a novel PPAR coactivator (CITED2) was characterized and has been shown to be a basal and ligand induced coactivator for PPARα and PPARγ but not PPARβ. Also, CITED2 acts as a coactivator for PPARα in the presence of a wide range of agonists including fibrate hypolipidemic drugs and fatty acids. Expression of CITED2 is ubiquitous throughout the mouse which suggests that CITED2 may act as a general coactivator for PPARα-dependent effects. In addition, CITED2 is involved in the regulation of several PPARα-dependent target genes as well as PP-induced cell proliferation, suggesting a larger role for CITED2 in the overall maintenance of cellular homeostasis.

168 MODULATION OF PKCα/MAPK SIGNALING PATHWAY BY PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORβ (PPARβ).

D. J. Kim[1, 2] and J. M. Peters[1, 2]. *[1]Department of Veterinary Science and The Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA and [2]Graduate Program in Molecular Toxicology, The Huck Institute for Life Sciences, The Pennsylvania State University, University Park, PA.*

Previous work has shown an enhanced epidermal hyperplastic response induced by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA), and increased incidence of skin tumor formation in a two stage bioassay in PPARβ-null mice, suggesting a critical role of PPARβ in attenuating epidermal cell proliferation. Protein kinase C (PKC) is the major cellular receptor that is activated by TPA. In

these studies, the role of PPARβ in the regulation of PKCα was examined. Intracellular phosphorylation analysis showed phosphorylated PKCα and mitogen-activated protein kinase kinase (MEK) were increased in PPARβ-null mouse skin treated with TPA as compared to wild-type control. Similarly, western blot analysis showed that the level of phosphorylated PKCα was higher in TPA-treated PPARβ-null skin and keratinocytes compared to wild-type controls. Additionally, phosphorylated Raf, MEK and p42 MAPK (ERK2) were higher in TPA-treated PPARβ-null skin compared to wild-type control. Two downstream effector molecules of the MAPK pathway, RSK1 and COX-2, were also higher in TPA-treated PPARβ-null skin compared to wild-type control indicating enhanced PKCα/MAPK signaling pathway in the absence of PPARβ expression. Inhibition of PKCα by selective inhibitors in cultured keratinocytes revealed that inhibition of PKCα effectively retarded cell proliferation in PPARβ-null keratinocytes in a dose-dependent manner, whereas it only slightly influenced cell proliferation in wild-type keratinocytes. Combined, these studies provide evidence that PPARβ attenuates cell proliferation by modulating PKCα/MAPK signaling pathway. (Supported by NIH, CA89607)

169 FORKHEAD BOX O1A (FOXO1A) AND PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR ALPHA (PPARα) EXPRESSION LEADS TO MUTUAL REPRESSION OF THEIR TRANSCRIPTIONAL ACTIVITY.

T. Murosky and G. Perdew. *Pennsylvania State University, State College, PA.*

The FOXO Subclass of the Forkhead Box (FOX) proteins has been implicated in cell cycle regulation, metabolism, and apoptosis. The FOXO proteins are modulated by direct AKT (PKB) phosphorylation. AKT phosphorylation of FOXO1a blocks nuclear transport causing a repression in its transcriptional activity. Evidence exists showing FOXO proteins complexing with certain members of the Nuclear Hormone Receptor superfamily (e.g. Androgen Receptor, Estrogen Receptor) when the receptors are activated. Peroxisome Proliferator Activating Receptors (PPARs) are also implicated in cell cycle regulation, metabolism, and apoptosis. A continued high level activation of PPARα can lead to hepatocarcinogenesis and its tumor promotional properties in murine models. Activated PPARα can suppress apoptosis in many different model systems. To contrast, FOXO1a has been shown to induce apoptosis. The hypothesis that PPARα can antagonize FOXO1a activity was tested. In transient transfections in HepG2 cells, PPARα was co-transfected with wtFOXO1a or FOXO1a-AAA (constitutively active) and the PPRE Aco-Luc reporter vector. The wtFOXO1a was not able to repress PPARα, but the constitutively active form mediated a 2-fold repression of PPARα activity. In a reverse experiment, the presence of PPARα repressed the wtFOXO1a and FOXO1a-AAA activity on a FOXO-driven reporter about 3-fold and 2-fold respectively. The conclusions of these experiments point to a functionally significant interaction between PPARα and FOXO1a, which may at least in part mechanistically explain the anti-apoptotic activity of PPARα. Interestingly, the repressive activity of FOXO1a was also observed with PPARβ and PPARγ in transient cell transfection assays. Additional studies will be required to determine the actual mechanism of the mutual repression observed. (Supported by NIEHS grants ES04869 and ES07799)

170 CONJUGATED LINOLEIC ACID BINDS TO PPAR AND CAUSES DIFFERENTIATION OF 3T3-L1 CELLS.

B. J. Belda, J. T. Thompson and J. P. Vanden Heuvel. *Penn State University, University Park, PA.*

Conjugated linoleic acid (CLA) is a class of long chain, polyunsaturated fatty acids that are positional and geometrical isomers of linoleic acid (LA). CLA has many beneficial effects in laboratory animals including reduction of cancer and amelioration of symptoms of type 2 diabetes. To understand these effects we looked at the role CLA plays in differentiation of 3T3-L1 preadipocytes. CLA treatment increased Oil Red-O staining 1.5 to 2 fold over control treated cells, indicating that the CLA causes these cells to undergo differentiation. To elucidate the mechanisms involved in differentiation we conducted microarray analysis of CLA treated cells. In total 537 genes were significantly regulated in at least one of seven time points. Expression of genes indicative of adipocyte differentiation (ADFP, CD36 and the PPAR responsive UCP2) are increased with CLA treatment. However, expression of pro-apoptotic genes (BCL2, Bak, Trp53, and TNF) is reduced while anti-apoptotic gene (Birc4 and DAD1) expression is reduced. Because PPAR has been reported to play a key role in 3T3-L1 cell differentiation into adipocytes we also investigated the ability of CLA to activate a PPAR luciferase reporter construct. CLA along with two known PPAR activators, rosiglitazone and PGJ2 are all able to activate this reporter construct. These data support the claim that CLA may prevent diabetes by activating PPAR and causing 3T3-L1 cells to differentiate. This may also suggest a mechanism by which CLA inhibits cancer as PPAR ligands inhibit breast and prostate tumor cell growth. Research into the expression of PPAR regulated genes in this system is ongoing.

171 VAN/NAF1 IS A PPAR COREGULATOR.

A. M. Flores, R. Wilson, L. E. Vasina and B. J. Aneskievich. *Pharmaceutical Sciences, University of Connecticut, Storrs, CT.*

Peroxisome proliferators increase differentiation and cell-specific gene expression in keratinocytes, the major cell type in the epidermis or upper layer of skin. This effect is mediated through peroxisome proliferator activated receptors (PPARs), members of the nuclear receptor (NR) superfamily of ligand activated transcription factors. In addition to ligand, NR function is governed by coregulator proteins, which contribute to either activation or repression of PPAR target gene transcription. To better understand PPAR function in keratinocytes, we sought to identify NR coregulator proteins present in these cells, define the basis for NR-coregulator interaction, and examine their activating or repressing effect on NR function. We screened a human keratinocyte cDNA library using the carboxyl portion of human PPARα as bait in a yeast two-hybrid assay. In addition to previously identified PPAR-interacting proteins such as TRAP220/PBP and RXR, one candidate coregulator was represented nine times as the same two overlapping cDNA fragments of a previously identified protein known as Naf1 (Nef-associated factor 1) or VAN (Virion-associated nuclear shuttling protein). Interaction of the Naf1/VAN clones varied with the different PPARs. Each of the two overlapping clones contained the same LXXLL domain (NR box) which was demonstrated by site directed mutagenesis to be responsible for interaction with the receptors. In a similar manner, the PPAR AF-2 was also found to be required for interaction with the Naf1/VAN clones. Studies with the full-length Naf1/VAN protein have also detected strong interaction with PPAR, again in an isoform-specific fashion. Both full-length and the more-carboxyl clone regulate PPAR transcriptional activity at defined PP response elements. Immunostaining for Naf1/VAN protein in intact skin revealed a differentiation-associated expression pattern. Given this and its interaction with PPARs, we expect Naf1/VAN may play a significant role in regulating epidermal keratinocyte response to peroxisome proliferators.

172 CELL CONTEXT-DEPENDENT DIFFERENCES IN HORMONAL REGULATION OF E2F-1 IN HUMAN BREAST CANCER CELLS.

S. Ngwenya[1] and S. Safe[2]. *[1]Biochemistry & Biophysics, Texas A&M University, College Station, TX and [2]Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.*

E2F-1 is a member of the E2F transcription factor family that plays an important role in cell cycle progression and cell proliferation. 17β-Estradiol (E2) induced E2F-1 gene expression in MCF-7 human breast cancer cells, and deletion analysis of the E2F-1 gene promoter showed that the -146 to -54 region was the minimal estrogen-responsive sequence. Subsequent analysis of this promoter in MCF-7 cells indicated that E2-induced transactivation was dependent on both upstream GC-rich and downstream CCAAT motifs that bound estrogen receptor α (ERα)/Sp1 and NF-YA, respectively. The cooperative interaction of the resulting ERα/Sp1/NF-YA complex was required for enhanced gene expression. E2 also induced E2F-1 gene expression in ZR-75 human breast cancer cells, and deletion analysis showed that the -146 to -54 region was the minimal E2-responsive sequence. Mutation and deletion analysis of the E2F-1 promoter showed that both the upstream GC-rich and downstream CCAAT sites were independently E2-responsive, and this was in direct contrast to results obtained in MCF-7 cells where cooperative interaction of the ERα, Sp1 and NF-YA was required for transactivation. Analysis of the promoter constructs showed that the GC-rich sites, which bind Sp1, were activated by genomic ERα/Sp1, whereas the activation of the CCAAT sites, where NF-YA binds, was associated with activation of cAMP/PKA which in turn activated NF-YA in ZR-75 cells. Both indirect and direct antiestrogens ICI 182, 780 and TCDD inhibited E2-induced activity in cells transfected with pE2F-1h (-169/-111) and pE2F-1j (-146/-54). In ZR-75 cells, TCDD was more effective than ICI 182, 780 as an inhibitor of E2 induced activity in constructs containing GC- rich or CCAAT sites. These results demonstrate the importance of cell context importance in molecular mechanisms of E2F-1 activation and inhibition. (Supported by NIEHS E209106 and ES04176)

173 GENISTEIN REGULATES THE STEROID COACTIVATOR GRIP-1 IN THE RAT MAMMARY GLAND.

T. G. Whitsett and C. A. Lamartiniere. *University of Alabama at Birmingham, Birmingham, AL.*

Genistein is the predominant isoflavone found in soy and has been shown to suppress the development of chemically-induced mammary cancer in rats. One mechanism through which genistein may exert this chemoprotective effect is *via* the estrogen receptors alpha and/or beta. Nuclear receptors are known to interact with nuclear receptor coactivators, especially the SRC family (SRC-1, GRIP-1, and AIB1), to increase the efficiency of transcriptional regulation. The current study investigates the relationship between dietary genistein consumption and GRIP-1.

Female Sprague-Dawley CD rats were fed 250 mg genistein/kg AIN-76A diet or AIN-76A alone from conception until either day 21 or day 50 postpartum. Western blot analysis was performed on the mammary glands of these female rats. There was a 2 fold increase in the level of GRIP-1 protein in the 21 day old mammary glands of animals that were treated with dietary genistein compared to the 21 day old females consuming only AIN 76A. Conversely, the animals treated with dietary genistein until 50 days showed a 2.8 fold decrease in the levels of GRIP-1, when compared to the animals on the AIN-76A diet. We hypothesize that prepubertal exposure to genistein increases cell differentiation and maturation of the mammary gland resulting in protection against mammary cancer. An up-regulation of GRIP-1 due to genistein early in life could interact with nuclear receptors, especially ER, enhancing activation and increasing transcriptional rate at target genes, thus furthering an ER-based mechanism of action for genistein's protective effect against breast cancer. Subsequently, the adult rat which is characterized as having a more differentiated mammary gland has lower GRIP-1 levels and reduced susceptibility for carcinogenesis. Understanding the mechanism of action of genistein during critical periods of life should allow us to development novel approaches to cancer prevention.

174 EFFECTS OF DOPAMINE D1 FULL AGONISTS ON RECEPTOR CYCLING.

J. P. Ryman-Rasmussen[1] and R. B. Mailman[1, 2, 3]. *[1]Curriculum in Toxicology, UNC Chapel Hill, Chapel Hill, NC, [2]Psychiatry, UNC Chapel Hill, Chapel Hill, NC and [3]DarPharma, Inc., Chapel Hill, NC.*

Dopamine D1 receptor full agonists show promise in treating a number of neurological and psychiatric disorders including Parkinson's disease. Occupation of GPCRs by agonists is known to cause a variety of compensatory events (e.g., desensitization, internalization, etc.) that can affect drug response, yet there is minimal information on how different D1 full agonists compare to dopamine in affecting the processes of receptor internalization and intracellular trafficking that are important in the regulation of receptor function. We have used an HEK cell line stably transfected with an epitope-tagged, human D1 receptor to address this issue. We have found that internalization of cell surface receptors following agonist exposure in this model system occurs at a similar rate for dopamine and the novel agonists A77636 and dinapsoline, and reaches as steady-state by 30 min. Only with dopamine itself, however, is there a rapid up-regulation (within 1 h) following the removal of agonist, although a slower (over 24 h) up-regulation occurs with receptors exposed to dinapsoline. This up-regulation does not occur for receptors treated with A77636. These data raise the hypothesis that occupancy by dopamine targets the D1 receptor to a rapid recycling pathway, whereas A77636 targets D1 receptors for degradation. This hypothesis was supported by preliminary confocal microscopic studies of cells that had been treated with these three drugs. The regulation of the receptor by dinapsoline appears to involve both novel recycling and biosynthetic pathways that remain uncharacterized. [This work was supported by NIH grants NS39036 and MH 40537, and a gift from DarPharma, Inc. RBM has a COI related to equity in DarPharma, Inc.]

175 EVALUATION OF HEPATIC RECEPTOR CONCENTRATION BY QUANTITATIVE OPTICAL IMAGING.

L. M. McIntosh[3], D. R. Vera[1], R. F. Mattrey[1], S. Fournier[2], S. Authier[2], P. Gallant[3] and F. Lesage[3]. *[1]University of California, San Diego, San Diego, CA, [2]LAB Pre-Clinical Research International, Inc., Laval, QC, Canada and [3]ART Advanced Research Technologies Inc., Saint-Laurent, QC, Canada.*

Kinetic modeling is critical to isolate variables, such as blood flow, binding, dissociation constants and non-specific uptake, involved in targeting of receptors. Optical imaging has high sensitivity to fluorophores and can be utilized to non-invasively measure uptake. However, the method has not yet been used as a quantitative tool. In this study, a novel time-domain small animal optical imaging system was used to quantify receptor concentration of an optically labeled receptor binding diagnostic agent. The optical fluorophore Cy5.5 was covalently coupled to DPTA-galactosyl-dextran or DPTA-dextran. The Cy5.5 density for both agents was approximately 8 moles of Cy5.5 per mole of dextran T70. Male BALB-c mice were anesthetized and the Cy5.5 labeled agents were administered (24 nmole/kg dextran) intervenously. Immediately post-injection the animal was placed supine in the ART SAMI system for optical imaging. Regions of interest over the heart and liver were imaged over 30 minutes. Time-intensity curves were generated over these regions which represented fluorescent intensity in the heart and liver as a function of time. Images of the area were also produced to recover object shape, location and concentration. The time-activity curve and volumetric images for Cy5.5-DTPA-dextran did not show hepatic uptake. The agent resembled a blood pool tracer without any tissue specificity. However, the receptor-binding agent, Cy5.5-DPTA-galactosyl-dextran, displayed hepatic accumulation, peaking within 20 minutes. The study demonstrates the role for time-domain optical imaging in the evaluation of receptor diagnostic agents. Optical imaging has higher sensitivity (75-fold increase) than nuclear imaging, which allows quantification of receptor density, improved kinetic modeling and tomographic representations.

176 DURING CHOLESTASIS LOSS OF RXRα IS HEPATOPROTECTIVE AND INCREASES EXPRESSION OF GENES FOR METABOLISM AND TRANSPORT IN LIVER.

A. L. Slitt[1], N. J. Cherrington[2], C. Chen[1], J. M. Maher[1], Y. Wan[1] and C. D. Klaassen[1]. *[1]University of Kansas Medical Center, Kansas City, KS and [2]University of Arizona, Tucson, AZ.*

Retinoid-X-receptor alpha (RXRα) is an important heterodimeric partner for nuclear hormone receptors (NHRs) such as Pregnane-X-receptor (PXR) and Farnesoid-X-receptor (FXR). PXR and FXR are activated by bile acids, and are responsible for the regulation of genes involved in bile acid metabolism and transport. Because RXRα functions as a dimeric partner, it was hypothesized that ablation of RXRα in liver would decrease the expression of a variety of genes that are induced *via* NHRs during cholestasis. Therefore, liver-specific RXRα-null mice (KO) and corresponding wild-type mice (WT) underwent either sham or common bile-duct ligation (BDL) surgery. Three days after BDL, serum bile acid concentrations were elevated 5.7-fold and 6.2-fold above control concentrations in WT and KO mice, respectively. Unexpectedly, the amount and severity of BDL-induced focal necrosis was greater in WT mice than that in KO mice. BDL decreased the mRNA levels of the bile acid uptake transporters Ntcp, Oatp1, Oatp3, and Oatp4 by at least 40% in both WT and KO mice. Oatp2 and Oatp5 mRNA was increased after BDL, with a larger induction occurring in KO mice. The mRNA levels of the phase I enzymes Cyp3a11, Cyp7a, NQO1, and the liver excretory transporters Mrp1, Mrp3, Mrp4, Mrp5, and Bsep were increased in livers from WT and KO mice, with the magnitude of induction being higher in livers from KO mice. The decreased hepatotoxicity in KO mice is likely due to increased liver gene expression of transporters that export BA from hepatocytes and not due to decreased expression of BA uptake transporters, because there was no difference in Oatp1 or 4 mRNA expression after BDL between WT and KO mice. The absence of RXRα may allow other NHRs to activate gene transcription. Alternatively, other RXR isoforms, such as RXRβ and RXRγ may heterodimerize with PXR and FXR to activate gene transcription. (Supported by grants ES-09716, ES-09649, ES-07079, CA053596, AA014147)

177 HUMAN DIETARY ALKALOIDS INHIBIT SONIC HEDGEHOG SIGNALING.

R. Lipinski and W. Bushman. *Molecular and Environmental Toxicology Center, University of Wisconsin-Madison, Madison, WI.* Sponsor: R. Peterson.

The Sonic hedgehog (Shh) signaling pathway plays an integral role in the patterning and development of diverse structures in the vertebrate embryo. Genetic abrogation of Shh signaling induces a spectrum of developmental field defects such as cyclopia, cleft lip, and cleft palate (collectively termed holoprosencephaly). Shh is a secreted peptide which binds to the transmembrane receptor Patched (Ptc), releasing its inhibition of Smoothened and inducing a signaling cascade that results in the upregulation of target genes including Gli1 and Ptc1. The plant steroidal alkaloid cyclopamine, which is an inhibitor of the Shh signaling pathway, induces holoprosencephaly when administered to a variety of vertebrate species. Although cyclopamine is not present in the human diet, there are several other dietary alkaloids of similar chemical structure, including tomatidine (derived from α-tomatine in tomatoes), solanidine (derived from α-chaconine and α-solanine in potatoes), solasodine (derived from solasonine in eggplants), and diosgenin (from wild yams). To investigate the possible effects of these compounds on Shh signaling, we used NIH3T3 cells transfected with a Shh-responsive Gli1 luciferase construct. Addition of Shh peptide (ShhNp) increased luciferase activity six-fold. This ShhNp-mediated increase in luciferase activity was reduced to control levels by addition of cyclopamine (10 μM) or tomatidine (10 μM), reduced by 25% with solanidine (10 μM), and reduced by 50% with solasodine (10 μM). Diosgenin had no effect. Control experiments confirmed the specificity of inhibition and the lack of cytotoxicity. In a Ptc1-null primary fibroblast cell line with constitutive Shh pathway activation, cyclopamine, tomatidine, solanidine and solasodine all inhibited Shh signaling — suggesting that they act through a common mechanism downstream of Ptc1. Shh signaling is important in a variety of developmental processes. Its inhibition by dietary alkaloids may have important implications on human health, particularly during fetal development.

178 EVALUATION OF THE DEVELOPMENTAL TOXICITY OF 1-(1, 2, 3, 4, 5, 6, 7, 8-OCTAHYDRO-2, 3, 8, 8-TETRAMETHYL-2-NAPHTHALENYL)ETHANONE.

C. Letizia, J. Cocchiara, D. A. Isola and A. Api. *Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ.*

The developmental toxicity of 1-(1, 2, 3, 4, 5, 6, 7, 8-Octahydro-2, 3, 8, 8-tetramethyl-2-naphthalenyl)ethanone (OTNE), a widely used fragrance ingredient, was evaluated in rats. OTNE, or the control article, were administered orally (gavage) to one-hundred pregnant female rats (25 rats/group) on days 7 through 17 of gestation at doses of 0.0 (control), 96, 240 and 480 mg/kg/day. Control animals were treated with reverse osmosis membrane processed deionized water. The rats were observed for *via*bility twice daily. Clinical observations and observations for abortions, premature deliveries and deaths were conducted daily. On day 21, all rats were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed, the uteri were removed and examined for pregnancy, number and distribution of implantations, fetal mortality and resorptions. Each fetus was examined macroscopically for the presence, shape and size of all organs. Approximately one half of each litter was examined for soft tissue alterations. No deaths or premature deliveries were caused by OTNE. The 480 mg/kg/day dose group produced persistent clinical observations and reductions in body weights and feed consumption. This dose also produced a minimal, but not statistically significant reduction in fetal body weights, but no other effects on development. No fetal gross external, soft tissue or skeletal malformations or variations were produced by OTNE. The number of ossification sites per fetus per litter was unaffected at dosages as high as 480 mg/kg/day. Dosages of 96 and 240 mg/kg/day caused excess salivation and transient reductions in body weight gains and feed consumption generally only during the first days of the dosage period. On the basis of this data, both the maternal and the developmental no-observable-adverse-effect-levels (NOAELs) of OTNE are 240 mg/kg/day.

179 CLONING OF RAT 5α-REDUCTASE TYPE2 GENE PROMOTER REGION AND AN EVIDENCE OF NO RELATIONSHIP BETWEEN ITS TRANSACTIVATION REGULATION AND ARYLHYDROCARBON RECEPTOR.

S. Ohsako, K. Kubota and C. Tohyama. *Environmental Health Sciences Division, National Institute of Environmental Studies, Tsukuba, Ibaraki, Japan.*

It has been established that development of ventral prostate of male laboratory animals was suppressed when exposed to a relatively low dose of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) during pregnancy. This typical endocrine disrupting phenomenon was demonstrated to be completely dependent on arylhydrocarbon receptor (AhR) gene. Several studies showed that 5α-reductase type 2 (5RD) expression was increased during postnatal period in the rat ventral prostate exposed to TCDD *in utero*. We here cloned 5′-franking region of 5RD gene and examined if its transcriptional activity was affected by AhR. Rat 5RD-promoter region (5RDp, 1713 bp) cloned by genome walking had an androgen responsive element at −659 to −679 and the same transcription start site as mouse 5RDp. Despite the absence of TATA-box, the cloned 5RDp-luciferase reporter construct showed clear transcription activation in COS-1 cells and suppression in TM4 cells in an androgen receptor (AR) dependent manner. However, co-transfection of rat AhR and ARNT expression plasmid could not change the luciferase expression level regardless of in the presence or absence of testosterone. This level was also not changed by an increased amount of 3-methyl-cholanthrene. Therefore, it could be concluded that at least the 1713-bp rat 5RDp region was not affected by liganded-AhR complex, suggesting that the increase of 5RD expression during postnatal days is not caused by direct interaction TCDD-AhR with 5RD promoter, but that an indirect effect occurred in the ventral prostate of offspring exposed to dioxin *in utero*.

180 MATERNAL UNDERNUTRITION DURING PREGNANCY POTENTIATES INTRAUTERINE GROWTH EFFECTS OF ETHANOL.

K. Shankar[1], M. Hidestrand[1], L. D. Humphrey[3], M. J. Ronis[1, 3, 2] and T. M. Badger[3, 2, 1]. *[1]Pharmacology/Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR, [2]Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR and [3]Arkansas Childrens Nutrition Center, Little Rock, AR.*

The incidence of fetal alcohol syndrome surprisingly represents a small proportion of all the children exposed to alcohol *in utero*. Since ethanol (EtOH) metabolism is significantly increased in pregnant rats infused EtOH *via* total enteral nutrition (TEN), this may serve as a protective mechanism. However, undernutrition during pregnancy abolished the increased metabolism of ethanol. To dissect the role of maternal undernutrition in alcohol-induced fetal toxicity, time-impregnated rats were fed either optimal (220 kcal/ $kg^{3/4}$/d) or undernourished (154 kcal/ $kg^{3/4}$/d) diets containing either 12 g/kg/d EtOH or isocaloric amount of carbohydrates from gestation d 6-15. Undernourished dams had 1.6 fold higher urine EtOH concentrations (UECs) compared to optimally fed rats ($p = 0.007$) given the same dose of EtOH. Undernourished EtOH fed rats also showed marked increase in fetal toxicity parameters including decreased fetal weight and litter size ($p < 0.05$). 55% litter resorptions were seen in the undernourished EtOH-fed rats compared to only 2% in the optimally fed EtOH treated animals. Undernutrition alone in the absence of EtOH did not increase any parameters of fetal toxicity, indicating that EtOH toxicity is potentiated by undernutrition. To further investigate the mechanisms of intrauterine growth retardation we assessed maternal IGF1 status, thought to be an important regulator of fetal growth. Serum levels of IGF1 were measured by radioimmunoassay and hepatic IGF1 mRNA levels were quantitated using real-time PCR. Consistent with the fetal growth data, optimally fed EtOH treated animals did not show a decrease in IGF1. In contrast undernourished EtOH fed animals showed 2-fold lower levels of IGF1 mRNA ($p = 0.0002$). The present data suggest that undernutrition potentiates the fetal toxicity of EtOH in part by disrupting IGF1 signaling. Supported in part by R01 AA12819 (MJJR).

181 DEVELOPMENTAL TOXICITY OF ACETYL CEDRENE IN RATS.

A. Lapczynski, J. Cocchiara, D. Isola and A. Api. *RIFM, Woodcliff Lake, NJ.*

Acetyl cedrene, a widely used fragrance material was studied for its potential to produce developmental toxicity in rats. The test article was administered orally to 100 (25 rats/group) female rats on days 7 through 17 of presumed gestation at dosages of 0 (control), 25, 50 and 100 mg/kg/day. Control group was treated with corn oil. All rats were observed for *via*bility at least twice each day. They were also examined daily for clinical observations of effects of the test article, abortions, premature deliveries and death before and after dosage administration. Body weights were recorded on day 0, daily during the dosage and postdosage periods and at sacrifice. Feed consumption values were recorded on day 0, 7, 10, 12, 15, 18 and 21. All rats were sacrificed on day 21 and gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites was recorded. Tissues with gross lesions were retained. Each fetus was weighed and examined for sex and gross external alteration. Approximately 50% of the fetuses in each litter were examined for soft tissue alteration. The remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red S and examined for skeletal alterations. The results showed that the 100 mg/kg/day dosages caused significant reduction in body weight gain and reduced the absolute and relative feed consumption values. Rats in both 50 and 100 mg/kg/day dosage group exhibited a dose dependent incidence of salvation, but the clinical observation was attributed more to the gavage dosing and was not considered to be a direct toxic effect of the test article. Litter parameters were not affected by dosages of the test article. No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by dosages of Acetyl Cedrene as high as 100 mg/kg/day. On the basis of these data, the maternal no observable adverse effect level (NOAEL) of Acetyl Cedrene is 50 mg/kg/day and the developmental NOAEL is greater than 100 mg/kg/day.

182 FOLIC ACID PROTECTS AGAINST VALPROIC ACID-INDUCED NEURAL TUBE DEFECTS IN CD-1 MICE.

J. E. Dawson[1] and L. M. Winn[1, 2]. *[1]Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada and [2]School of Environmental Studies, Queen's University, Kingston, ON, Canada.*

Neural tube defects (NTDs) are the most common type of human congenital malformations, with an incidence frequency of 1-2 per 1000 live births. The use of antiepileptic drugs, such as valproic acid (VPA), during pregnancy has been associated with a 5-20 fold increase in the rates of NTDs. Rodent *in utero* exposure to VPA has been shown to induce NTDs in a dose and strain dependent manner. The mechanism of how VPA induces NTDs, and why certain strains are more susceptible than others, remains unclear. Folic acid (FA) supplementation during pregnancy has been shown to protect human and some rodent embryo's from teratogenic insult, however FA's ability to protect embryos from VPA induced NTDs is controversial. Human epidemiological studies provide evidence leaning toward a protective effect, in the mouse model this protection appears to be strain dependent. Recently, it has been demonstrated that FA prevents VPA induced NTDs in the TO mouse model. The objective of our study was to determine if FA prevents NTDs in a different mouse strain, the CD-1 mouse, after administration of a previously demonstrated teratogenic dose of VPA (400 mg/kg) during the period of neural tube closure (Gestational Day (GD) 9). Briefly, pregnant (plug = GD 1) CD-1 mice were administered 4 mg/kg FA three times daily on gestational days 5 to 10. These animals were also exposed to a teratogenic dose of VPA (400 mg/kg) on GD 9 prior to neural tube closure. VPA treatment alone caused a 24 percent incidence of NTDs in CD-1 mice which was reduced to 3 percent by the prolonged

folic acid treatment. These results suggest that the CD-1 mouse model can be used to investigate the molecular mechanism mediating folic acid protection against valproic acid induced neural tube defects. We are currently investigating the role of the p53 tumor suppressor gene in neural tube closure and VPA induced teratogenesis. Support: JP Bickell Foundation

183 EXPRESSION AND TERATOLOGICAL RELEVANCE OF INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) AND CYCLOOXYGENASE-2 (COX-2) IN EMBRYONIC AND PLACENTAL TISSUES OF WILD-TYPE AND INOS KNOCKOUT MICE.

G. McCallum[1] and P. G. Wells[1, 2]. [1]*Pharmacy, University of Toronto, Toronto, ON, Canada and* [2]*Pharmacology, University of Toronto, Toronto, ON, Canada.*

Previous studies from this laboratory have shown that wild-type embryos in culture from B6129PF2/J mice were more susceptible to the reactive oxygen species (ROS)-initiating teratogens phenytoin and benzo[a]pyrene (B[a]P) than embryos from iNOS (B6;129P-*Nos2*^{tm1Lau}) knockout animals (Toxicologist 54: 71 [No. 335], 2000). This suggested that iNOS is constitutively expressed during organogenesis, and can contribute to ROS-initiated embryopathies. In the current studies however, immunoprecipitation experiments with gestational day (GD) 13 wild-type B6129PF2/J mice detected iNOS-specific immunoreactivity in placenta but not in embryonic tissue. Since COX-2 can catalyze the bioactivation of both phenytoin and B[a]P to ROS-initiating free radical intermediates, and nitric oxide has been implicated in the modulation of COX expression, the embryonic expression of COX-2 was also examined. Although embryonic COX-2 was constitutively expressed, protein levels in iNOS knockout and wild-type mice were identical. These results suggest that placental transfer of nitric oxide and related reactive nitrogen species can influence embryonic susceptibility to ROS-initiating teratogens *via* a mechanism unrelated to constitutive embryonic expression of iNOS or alterations in the level of embryonic COX-2 protein. (Support: Canadian Institutes of Health Research)

184 A COMPARISON OF EFFECTS ON REPRODUCTION AND NEONATAL DEVELOPMENT IN CYNOMOLGUS MONKEYS GIVEN HUMAN SOLUBLE IL-4R AND MICE GIVEN MURINE SOLUBLE IL-4R.

L. L. Carlock[1], L. A. Cowan[1], S. Oneda[2], A. M. Hoberman[3] and J. L. Bussiere[1]. [1]*Toxicology, Amgen Inc., Thousand Oaks, CA,* [2]*SNBL USA Ltd.,, Everett, WA and* [3]*Argus Research Laboratories, Horsham, PA.*

Inhibition of interleukin-4 (IL-4) is being evaluated to treat asthma. Amgen Inc. (formerly Immunex) evaluated effects of human soluble IL-4 receptor (sIL-4R) on monkey reproduction and murine sIL-4R on mouse reproduction. When pregnant cynomolgus monkeys received human sIL4R intravenously (IV) 2/week during organogenesis [gestation days (GD) 20-50] at 0, 0.2 or 2.0 mg/kg, there were no effects in clinical signs, body weight, food consumption, clinical pathology, or immunological evaluations. There was an increase in abortion/embryo-fetal death in the 0.2 (9/21 or 42.9%) and 2.0 (5/19 or 26.3%) mg/kg groups (controls: 3/17 or 17.6%). At cesarean, all fetuses were alive, no treatment effects noted. Three neonates were stillborn (2.0 mg/kg group). No neonates died after birth, no test article-related abnormalities noted. When pregnant Crl:CD-1®(ICR)BR mice (10 mice/group) received murine sIL-4R IV 1/day during organogenesis (GD 6-15) at 0, 25, 75, 250, or 625 µg/mouse, there were no effects in clinical signs, body weight, number of pregnancies (9-10/group), gestation, litter size, liveborn, sex ratio or pup weights. Mean trough serum concentrations after first doses were slightly higher in high dose monkeys than in high dose mice (cyno: 11.175+/-2.9 µg/mL; mouse: 3.47 +/-0.8 µg/mL). Mean serum conc. after last dose (within 1 h) had similar ranges for both species (cyno: 5.38-54.51 µg/mL; mouse: 5.3-237 µg/mL). Antibody development did not influence toxicity in monkeys. However at last dose, 2/6 (25 µg), 5/6 (75 µg), or 6/6 (250 & 625 µg) mice had antibodies to drug (mean serum conc. lower after last dose than after first dose; decrease more pronounced at 250 & 625 µg doses). It is unclear whether the lack of effects noted in mice and the increase in abortions and stillbirths observed in cynomolgus monkeys are due to species-specific responses to IL-4 inhibition, or whether the antibody response in the mice altered the activity of the drug.

185 DEVELOPMENTAL TOXICITY OF BETA-THUJAPLICIN (TP) IN RATS.

M. EMA, A. HARAZONO, S. FUJII and K. KAWASHIMA. *Risk Assessment, National Institute of Health Sciences, Tokyo, Japan.*

beta-Thujaplicin (TP) is a phenolic component of essential oils extracted from cypress trees. TP has been found to act as an antibacterial agent and an antitumor agent. In addition, it possesses phytogrowth-inhibitory effects. TP is used as a natural food preservative in Japan. In this study, the developmental toxicity of TP was determined. Pregnant rats were given TP by gavage at 15, 45, or 135 mg/kg on days 6-15 of pregnancy. The maternal body weight gain during administration at 45 and 135 mg/kg and after administration at 136 mg/kg and adjusted weight gain at 45 and 135 mg/kg were significantly reduced. A significant decrease in food consumption during and after administration was found at 45 and 135 mg/kg. A significant increase in the incidence of postimplantation loss was found at 135 mg/kg. A significantly lower weight was found in female fetuses at 45 and 135 mg/kg and in male fetuses at 135 mg/kg. Although a significantly increased incidence of fetuses with skeletal variations and decreased ossification were found at 135 mg/kg, no significant increase in external, skeletal and internal malformations was detected after administration of TP. The data demonstrated that TP had adverse effects on embryonic/fetal survival and growth only at maternal toxic doses. No adverse effects on morphological development were found in rats fetuses. Based on the significant decreases in maternal body weight gain and weight of female fetuses at 45 mg/kg and higher, it is concluded that the NOAELs of TP for both dams and fetuses are considered to be 15 mg/kg in rats.

186 INHALATION DEVELOPMENTAL TOXICITY STUDIES IN RATS WITH ANTIMONY TRIOXIDE (SB2O3).

P. E. Newton[1], R. E. Schroeder[1], L. Zwick[1] and T. Serex[2]. [1]*MPI Research, Inc., Mattawan, MI and* [2]*Great Lakes Chemical Company, West Lafayette, IN.*

In a range-find (RF) study, 5 groups of CD rats (5/group) were exposed to Sb2O3 *via* nose-only inhalation, 6 hrs/day, on Gestation Days (GD) 0 through 19. The mean exposure levels were 0, 0.39, 0.73, 1.48, and 6.07 mg/M3. The mass median aerodynamic diameters (MMAD) and geometric standard de*via*tions (GSD) ranged from 1.59 to 1.96µM and 1.60 to 1.68, respectively. The NOEL for maternal toxicity in the RF was 6.07 mg/M3, the highest exposure level evaluated. The NOEL for developmental toxicity in the RF was 1.48 mg/M3 based on the suggestion of lower (8%) fetal body weights and smaller (4%) fetal size (crown-rump distance [CRD]) at 6.07 mg/M3. In the definitive Sb2O3 study, 4 groups of CD rats (26/group) were exposed to 0, 2.6, 4.4, and 6.3 mg/M3, respectively, from GD 0-19. The MMAD and GSD ranged from 1.59 to 1.82 µM and 1.713 to 1.744, respectively. Animals were observed daily for mortality/clinical findings and gestation body weight and food consumption were measured at regular intervals. Females were euthanized on GD 20 and uterine implantation data recorded and fetuses taken for evaluation. Packed RBC components were collected from 10 females/group at GD 20 to determine bound Sb2O3 levels. A statistically significant dose-related increase in lung weights was seen in the treated groups. Absolute lung weights were 24.4, 31.1, and 38.6% heavier than control in the 2.6, 4.4, and 6.3 mg/M3 groups, respectively. Microscopically this was characterized by pigmented alveolar macrophages and inflammation. RBC levels of Sb2O3 were also statistically increased in the treated groups with values ranging from 3.1 to 5.6 µg/g compared to 0.1 µg/g in controls. The NOEL for developmental toxicity was 6.3 mg/M3, as no effects were seen in uterine implantation data, fetal body weights, CRD, sex ratios or from external, visceral, or skeletal examinations. In summary, under the conditions of this study, Sb2O3 was not developmentally toxic. (Supported by the International Antimony Oxide Industry Association)

187 EFFECTS ON RAT EMBRYONIC DEVELOPMENT *IN VITRO* OF DI-(2-ETHYLHEXYL) PHTHALATE (DEHP) AND ITS METABOLITES.

J. REGNIER[1], C. Bowden[2] and J. Lhuguenot[3]. [1]*Toxicology and Environment, ATOFINA, Paris-la-defense, France,* [2]*Huntingdon Life Science, Eye, United Kingdom and* [3]*ENSBANA, Dijon, France.*

The embryotoxicity of DEHP and its main metabolites (mono-2-ethylhexyl phthalate (MEHP), 2-ethylhexanol (2-EH), 2-ethylhexanoic acid (2-EHA) and MEHP-oxidized metabolites I, V, VI, and IX was evaluated on CD rat embryos *in vitro*. First, day 9 embryos were cultured in serum from pregnant CD rats collected approximately 1.5 hours after the final administration of 1000 mg/kg DEHP on GD 6-11. Afterwards, DEHP and its metabolites were evaluated individually. Embryos were cultured in roller bottles for approximately 48 hours at 38.0 +/- 1.0degC. Embryotoxic potential was assessed from effects on the survival, overall growth and development, and teratogenic potential from effects on the morphology of embryos. Serum from the DEHP-treated rats and culture media were analyzed by liquid scintillation and/or GC-MS to determine the achieved concentrations of DEHP and DEHP-metabolites. Embryos cultured in the serum from DEHP-treated rats exhibited reductions in crown-rump and head lengths, somite number and morphological score, when compare to controls. An increase in the number of malformations was also observed, which principally included malformations of the yolk sac circulation, flexion, forebrain, otic system, branchial bars and fore limb buds and unfused caudal neural tubes. The type of malformation observed with

DEHP-metabolites was similar to those observed with serum from DEHP-treated rats. A comparison of the lowest effect concentration at which statistically significant effects were observed on morphology parameters suggests the following order of developmental toxicity potency of the DEHP-metabolites: Metabolite IX > I >> VI = V > DEHP = MEHP = 2-EH >> 2-EHA. In comparison with the concentrations detected in the serum from the DEHP-treated rats, MEHP-oxidized metabolites, especially no. IX and I seem to be the ultimate developmental toxicants.

188 TERATOGENIC RESPONSES ARE MODULATED IN MICE LACKING EXPRESSION OF EPIDERMAL GROWTH FACTOR (EGF) AND TRANSFORMING GROWTH FACTOR-ALPHA (TGF).

B. D. Abbott, D. S. Best and M. G. Narotsky. *Repro Toxicology Division, USEPA, Research Triangle Park, NC.*

EGF and TGF regulate proliferation and differentiation in the embryo. EGF appears to mediate teratogenic responses, as the incidence of cleft palate (CP) is reduced and the severity and incidence of hydronephrosis are increased in EGF knockout (KO) mice after exposure to TCDD on gestation day (GD)12. Similarly, EGF KO mice have a lower incidence of CP after GD-12 exposure to retinioc acid (RA), whereas TGF KO mice show an increased incidence of CP after GD10 exposure. In the present study, pregnant C57BL/6J and TGF KO mice were exposed to a single oral dose of RA (100 mg/kg, 10 ml/kg) or vehicle on GD10, and wild type (WT) and EGF KO mice were exposed on GD12. GD18 fetuses were examined for external and visceral alterations. TGF KO mice and EGF KO mice had increased incidences of dilated renal pelvis compared to mice of background genotype; however, treatment of KO mice with RA significantly reduced the incidences compared to vehicle-treated KO mice. RA exposure produced effects on limb and heart development, however the incidence of specific limb defects was influenced by the developmental stage as well as the expression of EGF or TGF. After GD10 RA exposure, the incidence of forelimb digital and long-bone defects was significantly higher in TGF KO compared to C57BL/6J fetuses. Also, micrognathia, hindlimb digital deficits, and oligodactyly (fore and hind limbs) were only observed in the RA-treated TGF KO. RA exposure on GD12 of WT and EGF KO produced forelimb brachy-/oligodactyly and long-bone reduction of fore and hind limbs. However, the EGF KO was significantly more susceptible to digital deficits of the hind limb. In summary, in the developing limbs, the teratogenic effects of RA were influenced by developmental stage and responses were exacerbated in the absence of EGF and TGF expression. This study provides further evidence that the EGF pathway mediates responses to teratogens and that the influence depends on developmental stage and the target tissue. This abstract does not necessarily reflect EPA policy.

189 COMPARATIVE STUDY OF ALCOHOL TERATOGENIC EFFECT IN C57BL/6 AND DBA/2 MOUSE EMBRYOS USING EMBRYO CULTURE.

M. Kuwagata[1], T. Ogawa[2, 1] and F. C. Zhou[1]. *[1]Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN and [2]Anatomy, Showa University School of Medicine, Tokyo, Japan.*

Children born to chronic alcoholic women have a high incidence of fetal alcohol related spectrum disorder featuring psychosocial disorder, and craniaofacial malformations. Animal models also demonstrated that fetal alcohol exposure impaired development. In the rodent studies, inbred strains of different genetic background were often applied to investigate the role of genotype in their susceptibility to alcohol-induced teratogenecity. However, studies reported so far have generated inconsistent results which may, in part, be due to differential progressing in the stage of embryos. In the present study, we used two inbred strain mice, C57BL/6 (B6) and DBA/2 (D2), which were often used in alcohol studies. First, we investigated the embryonic stage with 2-hour breeding paradigm in the two strains of mice. When they were sacrificed on gestation day 8.25 at the same time, D2 embryos were all at presomite stage, while almost all B6 were at 3-6 somites stage. This result suggests D2 embryos are usually younger than B6 when treatments are applied in the study *in vivo*. Therefore, we applied whole embryo culture technique to compare sensitivity to alcohol between the two strains. Embryos with 3-6 somites were collected in both strains and cultured for 44 hours in the medium containing 400 mg/dL of ethanol and scored for morphological features. The alcohol treatment affected the growth of embryos in both strains and induced abnormalities including hypoplasia of optic vesicle in both strains with a different severity between B6 and D2 embryos. In conclusion, we found that in the current paradigm, differential progress of development exists between B6 and D2, which may contribute to inconsistent results in the literature. (Supported by AA07611)

190 COMPARATIVE ASSESSMENT OF TWO EMBRYO CULTURE METHODS IN EVALUATING EMBRYOTOXICITY.

H. Huuskonen and H. Komulainen. *Department of Environmental Health, National Public Health Institute, Kuopio, Finland.* Sponsor: M. Viluksela.

Rat whole embryo culture (WEC) has been widely used in evaluating developmental toxicity of chemicals *in vitro*. In the ECVAM validation study, its predictivity and precision for strongly embryotoxic compounds was 100%. However, the precision for non-embryotoxic and weakly embryotoxic compounds was lower. In the present study, the embryotoxicities of five model compounds in mouse preimplantation embryo culture and rat whole embryo culture assays were compared. Preimplantation mouse embryos were collected from the superovulated and mated females at morula stage and cultured in microspots of medium at 37 ○C in 5% CO_2 atmosphere for two days up to the blastocyst stage. The morphology and developmental rate were assessed at 24 and 48 hours using an inverted microscope with relief optics. In WEC, rat embryos were collected from pregnant females at 1-5 somite stage. They were dissected from the desidua and Reichert's membrane and cultured in bottles containing medium and a gas phase. The gas phase was changed twice a day for two days. The growth and morphology were assessed after the culture period of 46 hours under a stereomicroscope. The model chemicals, 5-fluorouracil (5-FU), methotrexate (MET), salicylic acid (SAL), dimethadione (DMD) and saccharin (SAC) were dissolved in the medium in both culture system. In both assays, 5-FU and MET were strongly embryotoxic, SAL and DMD weakly embryotoxic and SAC non-embryotoxic. The no-effect concentrations (NOECs) were 0.01 μg/ml for 5-FU and MET, 100 μg/ml for SAL, 500 μg/ml for DMD and 1000 μg/ml for SAC in preimplantation embryo culture method. In WEC, the NOEC-values were 0.1 μg/ml for 5-FU, 0.05 μg/ml for MET, 100 μg/ml for SAL, 500 μg/ml for DMD and 1000 μg/ml for SAC. The results showed a good correlation of the embryotoxic potential of these chemicals in both assays. The preimplantation embryo culture method may provide a simpler mammalian model to study embryotoxic potential of chemicals. (Supported by TEKES, the National Technology Agency, Finland, Project 70035/02)

191 TWO ZEBRAFISH ALCOHOL DEHYDROGENASES SHARING COMMON ANCESTRY AND FUNCTIONAL CHARACTERISTICS WITH MAMMALIAN CLASS I AND III GENES.

M. Reimers[1, 3], M. E. Hahn[2] and R. L. Tanguay[3]. *[1]Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO, [2]Biology Department, Woods Hole Oceanographic Institute, Woods Hole, MA and [3]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Ethanol is teratogenic to many vertebrates and we are using zebrafish as a model system to determine if there is an association between alcohol dehydrogenase (ADH) activity and developmental toxicity. To test this hypothesis, it is necessary to determine if zebrafish express ADH enzymes capable of ethanol metabolism during development. With the duplication of many genes in zebrafish, it is critical to ascertain the functional characterization of all identified enzymes. Here we report the isolation and characterization of two cDNAs encoding zebrafish ADH. Phylogenetic analysis of these zebrafish ADHs reveals that they are most closely related to the mammalian class 1 ADH enzymes and we have named them adh8a and adh8b. Both adh8a and adh8b were genetically mapped to chromosome 13 approximately 3.6 megabases from each other. At the predicted amino acid level, adh8a and adh8b are 77% identical to each other, and are 73% and 68% identical to the human class 1 ADH, respectively. To characterize the functional properties of ADH8A and ADH8B, the enzymes were recombinantly expressed and purified from SF-9 insect cells. Kinetic studies with a variety of substrates revealed differential substrate preferences. Consistent with mammalian class I enzymes, ADH8A metabolizes ethanol, with a 0.679 mM Km. Conversely, ADH8B does not oxidize ethanol, but does efficiently metabolize longer chain primary alcohols greater than 5 carbons and S-hydroxymethlyglutathione similar to class III enzymes. ADH8A does not efficiently metabolize these substrates. Finally, mRNA expression studies indicate that both ADH8A and ADH8B mRNA are expressed during early development. Together these results indicate that class I ADH enzymes are conserved in zebrafish, albeit with mixed functional properties. Supported by NIH grants AA12783, ES03850, and ES00210.

192 DEVELOPMENTAL TOXICITY OF METAM SODIUM IN ZEBRAFISH.

M. A. Haendel[1, 3], F. Tilton[2, 3], R. L. Tanguay[2, 3] and G. S. Bailey[1, 2, 3]. *[1]Linus Pauling Institute, Oregon State University, Corvallis, OR, [2]Environmental and Molecular Toxicology, OSU, Cor., OR and [3]EHSC and MFBSC, OSU, Cor., OR.*

Sodium metam (NaM), a dithiocarbamate, is a potent agricultural biocide applied prior to planting for the elimination of nematodes, soil pathogens and weeds. There is a remarkable paucity of information about the risk that this class of chemicals

may pose to developing vertebrates and their mechanism of action. Zebrafish have been utilized as a model system because early developmental events and gene function are conserved with humans. We have characterized the toxicity during early life stage exposure to NaM in zebrafish. Zebrafish embryos were exposed to varying concentrations of NaM between 0 and 4.05 μM for a 20-hour period beginning at 4 hours post fertilization (hpf). The LC50 curves are steep, and are estimated to be 2.3 μM (300 ppb) at 24 hpf and 0.4 μM (50 ppb) at 13 days. The most notable malformation, observed in 100% of the exposed animals at 0.8 μM, was an enlarged and severely twisted notochord; the NOAEL was 0.05 μM. Surprisingly, this notochord defect is not immediately lethal and the animals continue to grow; however, they exhibit delays in hatching, apparent paralysis, and are unable to feed. To evaluate the effects on the notochord and nervous system, we have characterized changes in gene expression. Expression of α-collagen II mRNA is localized to the notochord sheath cells in controls, whereas in NaM-exposed embryos, it is misexpressed in the notochord cells. MyoD and histological staining indicate muscle abnormalities; the myotomes of the NaM-exposed embryos are less defined, compacted and block-shaped compared to controls. There are more peripheral axonal branches in NaM-exposed embryos than in controls, as shown by ZN-12 antibody staining. Our preliminary results indicate that developing zebrafish are exquisitely sensitive to NaM sodium and we believe that this model is ideal to elucidate the molecular mechanism(s) and etiology of NaM toxicity in vertebrates. Supported by NINDS #NS11170, NIEHS #ES00210, and #ES03850.

193 THE TERATOGENIC EFFECTS OF ETHANOL EXPOSURE IN ZEBRAFISH.

E. J. Loucks[1, 2], B. A. Wimpee[1, 2] and M. J. Carvan[1, 2]. *[1]University of Wisconsin-Milwaukee, Milwaukee, WI and [2]Great Lakes WATER Institute, Milwaukee, WI.*

Developmental ethanol exposure from maternal consumption of alcoholic beverages and many consumer products has been linked to developmental abnormalities in humans and laboratory animals. The long-range goal of this project is to elucidate the molecular mechanism by which ethanol perturbs embryonic and fetal development, and to identify genes that plays a role in the sensitivity to ethanol-induced teratogenesis. Fetal exposure to ethanol is primarily dictated by voluntary maternal behavior and societal influences. Consequences of fetal exposure can range from embryotoxicity, gross malformations and a large variety of more subtle morphological, biochemical and functional abnormalities depending on the duration and time of exposure. This can have a tremendous toll on the affected individuals, their families, and society as a whole. The sensitivity of an indivdual to ethanol-induced perturbation of developmental processes can also involve genetic factors. Twin studies in humans demonstrated that identical twins are equally susceptible to FAS, whereas fraternal twins have shown a marked discordance in the severity of FAS. Genetically distinct strains of zebrafish (Danio rerio) are differentially sensitive to alcohol and provide the best model system for genetic analysis of the developmental effects of ethanol to embryos. We have determined that zebrafish are very sensitive to the effects of ethanol on embryo/larval mortality, neurocranial and craniofacial skeletal development, and CNS apoptosis. We have also determined that the magnitude of these effects is strain-dependent, and that no single strain is the most sensitive in each assay. This data will be discussed in relation to our molecular characterization of genes that influence sensitivity to ethanol-induced teratogenesis.

194 DEVELOPMENTAL TOXICITY OF CARBARYL IN ZEBRAFISH.

H. Cheng, A. Lin and E. Chan. *Biology and Chemsitry, City University of Hong Kong, Hong Kong, N/A, Hong Kong.*

Carbaryl (1-naphthyl-N-methycarbamate; commercial name, Sevin) is one of the most commonly used broad-spectrum pesticides in urban and agricultural settings. It was used widely in turf management of golf courses, in controlling burrowing shrimp in oyster fields, in exterminating animal pests in forests, and even in treating head lice in school children. In the environment, field concentrations of carbaryl up to 4.8 mg/L have been found immediately following application. In streams draining agricultural and urban areas, carbaryl up to 1.5 and 2.5 ug/L have been reported respectively. Fertilsed zebrafish eggs were exposed to nominal concentrations of carbaryl from 1 to 100ug/ml from 4 hours post fertilisation (hpf) to 28 hpf. Percentages of mortality and abnormality were scored. All embryos died at carbaryl concentrations of 50 ug/ml or higher. Growth arrest was observed at 30 ug/ml while pericardial edema was observed at 10 ug/ml. No gross abnormality was observed at concentrations below 10 ug/ml. Zebrafish is an appealing experimental model for pesticide induced developmental toxicity. We will establish the chains of events following the anticholinestarase activity of carbaryl to the resulting target organ toxicity. developing vasculature. This project will contribute to the development of bioindicators for developmental toxicity and provide mechanism basis of pesticide risk assessment.μμμ

195 ETHANOL PERTURBS CARDIOVASCULAR DEVELOPMENT IN JAPANESE MEDAKA, *ORYZIAS LATIPES.*

A. K. Dasmahapatra[1] and M. L. Haasch[1, 2]. *[1]Environmental Toxicology Research Program, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS and [2]Department of Pharmacology, University of Mississippi, University, MS.*

Although the cardiovascular defects in developmental ethanol exposure have been identified in both human and animal models, the molecular mechanism(s) for these abnormalities has not yet been fully characterized. We used the Japanese medaka(*Oryzias latipes*) developmental model to evaluate cardiovascular defects produced by acute ethanol exposure. Viable medaka eggs within 1h of fertilization were exposed to ethanol (0-400mM)in hatching solution. After 48h, ethanol was omitted from the embryo culture medium. Cardiovascular development was observed from 1-7 dpf. The embryos exposed to low ethanol concentrations (0-100mM) started circulation at 2 dpf but active circulation was significantly delayed at higher concentrations (200-400mM). Moreover, these higher concentrations (200-400mM) developed tube heart with blood clots in blood island and in circulating vessels. We hypothesized that the cardiovascular abnormalities seen in the developing Japanese medaka was due to one or more metabolites of ethanol metabolism. To examine this possibility, we have begun to characterize the genes for alcohol metabolizing enzymes in medaka. By applying-PCR based technologies, we have partially cloned two subtypes of alcohol dehydrogenase mRNA (ADHa and ADHb)in adult medaka liver. ADHa mRNA was expressed in liver, gill, eye, muscle, heart and GI of adult fish but not in brain and gonad. ADHb was expressed in all tissues examined. The expression patterns of ADHa and b mRNA were also different from each other during embryonic development. ADHa was expressed from the 2-cell stage to neurula (1dpf) but ADHb was first expressed 2dpf. Our results indicate that ADHa is the major enzyme expressed during the early part of the embryonic development and likely plays a significant role in the generation of ethanol metabolities and the subsequent development of cardiovascular defects. [Supported by ETRP and ORSP of the University of Mississippi]

196 EXENATIDE (SYNTHETIC EXENDIN-4) DEVELOPMENTAL TOXICOLOGY IN RABBITS: COMPARISON TO PAIR-FED CONTROLS.

R. Hiles[1], T. Carpenter[1], A. Hoberman[2] and R. Byrd[3]. *[1]Amylin Pharmaceuticals, Inc., San Diego, CA, [2]Argus Research, Horsham, PA and [3]Eli Lilly & Co., Greenfield, IN.*

SC administration of exenatide (EX) to pregnant rabbits on gestation days (GD) 6-18 caused a dose-related reduction in food consumption (FC) and reduced body weight compared to pre-dose value (ΔBW) (1). Fetal anomalies included umbilical hernias (UH) in 1.6, 5.6, 11.8% of fetuses at 11, 78, 130 μg/kg EX, respectively. The current study's purpose was to evaluate the effect of reduced FC on UH. Groups of 20 pregnant dams were dosed SC with EX-diluent (ED), 1, 11, or 130 μg/kg EX BID on GD 6-18, while pair-fed (PF) control groups were dosed with ED and fed to match EX groups. EX caused a dose-related reduction in both FC (GD 6-9: 62, 32, 12% of ED group for 1, 11, 130 μg/kg EX, respectively) and WC (GD 6-9: 82, 52, 19% of ED group for 1, 11, 130 μg/kg EX, respectively). ΔBW was +1.2, -1.7, -5.9, -10% for ED group, 1, 11, 130 μg/kg EX, respectively. Reduced WC was not observed among the PF groups. PF groups to match EX groups for 1, 11, 130 μg/kg EX exhibited ΔBW of -1.6, -3.5, -5.4%, respectively. Dose-related increases in serum beta-hydroxybutyric acid (BHBA), a starvation biomarker, were more elevated compared to ED group in EX groups than in PF groups (0.91, 1.14, 2.67, 2.82 mg/dL for ED group, 1, 11, 130 μg/kg EX, respectively and 0.77, 0.85, 2.33 mg/dL for PF groups for 1, 11, 130 μg/kg EX, respectively). UH (GD 29 Caesarean sections) were observed in 11 and 130 μg/kg EX doses [5.9% (1 fetus in 1 litter) and 29% (8 fetuses in 5 litters of litters, respectively] (historical = 0 - 6.2%); no litters from 1 μg/kg EX or PF controls had UH. Based on ΔBW and BHBA, the PF group intended to match the 130 μg/kg EX groups was more similar to 1 and 11 μg/kg EX group and not the 130 μg/kg EX group. Mean UH fetal BW was less than non-UH fetal BW ($p<0.05$). UH occurred concomitantly with fetal growth retardation related to severe maternal weight loss. EX does not appear to be a selective developmental toxicant. (1) Hiles, R.A., Hoberman A.M., & Christian, M.S. (2001). Toxicological Sciences 60 (Ab 1182), 248

197 COMPARISON OF GESTATIONAL DOSE (MG/DAY) IN GAVAGE VS. CONTINUOUS EXPOSURE STUDIES IN RATS.

S. P. Parker[1], C. B. Myers[1], R. W. Tyl[1], J. P. Van Miller[2] and R. L. Joiner[3]. *[1]Life Sciences and Toxicology, RTI International, Research Triangle Pk, NC, [2]TRS, Charlottesville, VA and [3]GE, Pittsfield, MA.*

In developmental toxicity studies, maternal animals are dosed during much or all of gestation. If administration is by gavage, the dose in mg/kg/day is held constant by adjusting the volume (ml/kg), based on the most recent body weight (BW) to correct for maternal BW gain during pregnancy. This convention has continued for other reproductive screening and definitive studies (e.g., 2-generation and OECD 421/422). The maternal rat may gain >150 g during gestation, but with the BW gain in the last trimester (>100 g) almost entirely from uterine contents. Test chemical (TC) intake (mg/day) in dosed feed/water studies is not increased from gestational day (gd) 14 to 20. In gavage studies, TC intake (mg/day) during the same time period is increased as much as 30% because of the adjustment for maternal BW. Thus, the dose (mg/kg/day), based on the actual maternal BW (i.e., minus uterine contents), is similarly increased (30%). This can result in overdosing the dam and conceptuses and is the likely cause of excessive maternal and perinatal toxicity observed in some studies. The risk of increased toxicity from bolus gavage dosing is also increased by the following: 1) the maternal liver is enlarged in late pregnancy, presumably in response to pregnancy and increased TC load, but it is not enlarged commensurate with increased TC dose; 2) TC may not be evenly distributed into the fetal compartment, and the relative maternal burden may, therefore, be even higher; 3) GI tract motility is reduced with potentially increased absorption of TC during late pregnancy. Such effects confound the interpretation of reproductive toxicity. Therefore, we recommend adjusting the dose from gd 0 to 14 of pregnancy and maintaining the dose (in mg/day by maintaining the gd 14 dosing volume) from gd 14 to pnd 0. This approach provides a dose appropriate to maternal BW (minus the uterine contents) in the latter part of gestation. This work is supported by the General Electric Company, Pittsfield, MA.

198 MATERNAL FUMONISIN EXPOSURE AND NEURAL TUBE DEFECTS: MECHANISMS IN AN *IN VIVO* MOUSE MODEL.

J. B. Gelineau-van Waes, L. Starr, J. Wilberding, F. Aleman and J. Maddox. *Department of Genetics, Cell Biology & Anatomy, University of Nebraska Medical Center, Omaha, NE.* Sponsor: R. Riley.

Fumonisin B1 (FB1) is a mycotoxin produced by F. verticillioides, a common contaminant of corn worldwide. An association between maternal FB1 exposure and increased risk for neural tube defects (NTDs) has recently been observed in human populations relying on corn as a dietary staple. FB1 disrupts sphingolipid biosynthesis by inhibiting ceramide synthase, resulting in elevated sphinganine levels, and depletion of downstream glycosphingolipids. We have previously demonstrated that early gestational exposure to FB1 results in NTDs in 80% of exposed inbred LMBc mouse fetuses. FB1 depletion of ganglioside GM1 altered plasma membrane microdomains, compromising folate uptake *via* the GPI-anchored folate receptor. Daily maternal folate supplementation reduced the incidence of NTDs, while replacement therapy with ganglioside GM1 restored folate uptake and rescued the phenotype. Since previous reports indicate that FB1 toxicity may be mediated by TNFa, the role of TNFa is evaluated in our model. Atypical expression of TNFa is seen in the developing neural tube following FB1 exposure. TNFa expression corresponds to positive TUNEL staining in the ventricular zone, but co-localizes with NF-κB in the marginal zone. TEM sections show a loss of adherens junctions following FB1 exposure, and phalloidin staining indicates disruption of the apical actin belt. Further studies show altered expression and/or post-translational modification of cytoskeletal proteins. FB1 exposure resulted initially in an increase in apoptosis, loss of cell adhesion and cell polarity, disruption of cytoskeletal integrity, disorganized cell migration, and failure of the neural folds to elevate. This was followed by eversion of the neural folds, and increased proliferation, as demonstrated by increased PCNA staining. Preliminary microarray data also indicate an alteration in cell cycle checkpoint genes, and an increase in tumor antigens/proto-oncogenes, growth factors, and cell survival (bcl-2) pathways.

199 ZEBRAFISH ASSAYS FOR ASSESSING DEVELOPMENTAL TOXICITY.

C. Zhang, C. Parng, C. Willett, C. Ma and P. McGrath. *Phylonix Pharmaceuticals, Inc., Cambridge, MA.* Sponsor: P. Mayeux.

Developmental toxicity is a major problem with numerous pharmaceutical agents and screening for potential adverse effects is critical for assessing drug safety. Transparent zebrafish embryos offer unique advantages for assessing the effects of drugs on various developmental events. The processes involved in embryogenesis as well as the function of numerous genes have been well studied in zebrafish and are highly conserved compared to mammals. In this study, we developed a reproducible, multi-endpoint scoring system to determine developmental toxicity using zebrafish embryos. We have established 26 parameters to visually assess drug induced effects on morphology and organogenesis. After drug treatment, we also established dose-response curves for mortality (LC50) and malformation (ED50), and the teratogenic index (TI ratio= LC50/ED50). As strong support for use of zebrafish as a model for assessing drug toxicity, we recently examined the effects of 100 known compounds and noted that LC50 and teratogenicity were remarkably similar to results in mammals, suggesting that zebrafish has a high predictive value for toxicity testing in humans.

200 CELL CYCLE INHIBITION AND CLEFT PALATE INDUCTION BY SECALONIC ACID D.

V. C. Dhulipala, W. Welshons and C. S. Reddy. *Vet. Biomedical Sciences, University of Missouri, Columbia, MO.*

Cleft palate (CP) is one of the most common birth defects affecting one in seven hundred newborns annually. Exposure in mice to secalonic acid -D (SAD), a mycotoxin produced by Pencillium oxalicum, is known to induce CP in association with smaller embryonic palatal shelves. Since the reduction in shelf size can result from a reduction in either palatal cell number or in the amount of extracellular matrix (ECM), the objective of this study was to delineate which of these mechanisms contribute to the teratogenic effect of SAD using the human embryonic palatal mesenchymal (HEPM) cells. The effect of increasing concentrations of SAD on HEPM cell numbers, survival, proliferation and ECM synthesis was assessed using cell counting, uptake of trypan blue, 3H-thymidine uptake and a combination of 3H-glucosamine incorporation and western analysis for the various ECM components (hyaluronic acid, sulfated glucosaminoglycans, fibronectin and tenascin), respectively. Exposure to SAD resulted in a dose-dependent reduction in HEPM cell number, levels of proliferating cell nuclear antigen (PCNA) in the nucleus and 3H-thymidine uptake with a no-effect concentration of 0.025 micro gm SAD/ml and an IC50 of 12.3 micro gm SAD/ml. Significant cytotoxicity and reduction in the ECM transport, but not synthesis, occurred only at the highest concentrations tested. Further studies using flow-cytometry and cyclin-dependent kinase (CDK) assay suggested that SAD blocked HEPM cell-cycle at the transition of G1/S phases, a conclusion supported by inhibition of CDK2 (active in G1/S phase) but not CDK1 (active in G2/M phase)by SAD. The results of this study strongly suggest that SAD-induced reduction in palatal shelf size and CP may be a result of its inhibition of cell-cycle and warrant further studies on the mechanism of its cell-cycle block.(Supported by Alternatives Research and Development Foundation).

201 IMMUNE PROTECTION AGAINST MNU-INDUCED DIGITAL DEFECTS.

M. R. Prater[2, 1], S. D. Holladay[1] and E. D. Strahl[1]. *[1]Veterinary Medicine, Virginia Tech, Blacksburg, VA and [2]Biomedical Sciences, Edward Via Virginia College of Osteopathic Medicine, Blacksburg, VA.*

The present study investigated the role of the maternal immune system in protection against methylnitrosourea (MNU)-induced limb and digital birth defects in CD-1 mice. MNU's teratogenic effects are dose-specific and dependent upon the time of exposure in relation to gestational age. Maternal exposure to MNU at gestation day (GD) 9 resulted in reductions in limb length, formation of digital defects including syndactyly, oligodactyly and polydactyly, and distal limb hypovascularization in fetuses at GD 12 and 14. Limb lengths of GD 12 and 14 mice in the MNU group were 71% and 68% of control, respectively. Maternal intraperitoneal injection with interferon-gamma (IFN-gamma) two days prior to the GD 9 MNU treatment resulted in significantly increased limb lengths that were 88% and 84% of controls, respectively. These data suggest that nonspecific maternal immune stimulation with IFN-gamma, prior to MNU exposure, partially normalizes teratogen-induced fetal limb developmental lesions. This normalization may in turn relate to reduced limb defects seen in these animals at end-gestation. Further, histologic examination of placenta in MNU treated animals revealed intracellular and extracellular alterations in the spongiotrophoblast layer. This placental pathology was again lessened in pregnant mice that received IFN-gamma two days prior to MNU. It is not yet known if reduced day 12 and 14 distal limb dysmorphogenesis in MNU-exposed fetuses may be the result, in part, of improved placental integrity caused by IFN-gamma in MNU-exposed dams.

202 ORGANIC ANION TRANSPORTING POLYPEPTIDES (OATP) 9 AND 12 MNRA EXPRESSION: TISSUE DISTRIBUTION DURING PREGNANCY COMPARED WITH MALE AND NON-PREGNANT FEMALE SPRAGUE DAWLEY RATS.

T. M. Leazer and C. D. Klaassen. *University of Kansas Medical Center, Kansas City, KS.*

Pregnancy can influence the disposition and handling of chemicals as the maternal system changes to accommodate fetal and placental development. Knowledge of transporters is vital for the prediction of placental/fetal chemical accumulation and

susceptibility to toxicity. Hence, the purpose of this research was to characterize the tissue distribution of Oatp9 (Slc21a9) and 12 (Slc21a12) mRNA expression in male, non-pregnant female and pregnant rats. Oatp9 and 12 mRNA expression was determined with branched DNA signal amplification technology. Oatp9 mRNA was expressed highest in large intestine, stomach and term placenta; moderately expressed in liver, kidney, cerebellum, cerebral cortex and ileum; and was lowest in lung, duodenum and jejunum. Interestingly, for all sections of intestine, Oatp9 mRNA levels were higher in pregnant rats than in male and female rats. Oatp9 mRNA levels in placenta increased with the progression of pregnancy. Oatp12 mRNA levels were by far the highest in term placenta. In fact, Oatp 12 mRNA in placenta was 30-fold higher at term than any other tissue. Moreover, Oatp12 mRNA increased in placenta from gestation day 15 to 21. The next highest concentration of Oatp12 was in small intestine and stomach; expression was moderate in kidney, lung and large intestine; and lowest in brain. In ileum, Oatp12 mRNA transcripts were higher in pregnant female than non-pregnant or male rats. In summary, Oatp9 mRNA was most highly expressed in intestine, whereas Oatp12 was exceptionally highly expressed in placenta. The expression of both Oatp9 and 12 increased in the intestine during pregnancy, suggesting that these transporters are important in the absorption of chemicals needed during pregnancy. In addition, the extremely high level of Oatp12 in placenta and the increase in expression of Oatp9 and 12 in placenta during pregnancy suggest that they may be important in the transport of nutrients to the fetus. (Supported by NIH grants ES-09649 and ES-07079)

203 DEVELOPMENTAL EFFECTS IN RABBITS ASSOCIATED WITH ELEVATED PLASMA TYROSINE.

G. J. Moffat, M. E. Moxon, J. W. Botham and R. W. Lewis. *Syngenta CTL, Alderley Park, Cheshire, United Kingdom.* Sponsor: I. Kimber.

Selective inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) by the herbicide mesotrione leads to perturbation of the tyrosine catabolism pathway. Subsequent tyrosinemia has been associated with a range of biological changes in rats, but to a much lesser extent in mice, where the plasma tyrosine elevation is less pronounced. Indeed, the incidence and severity of these changes in rodents correlated directly with plasma tyrosine concentration but not with concentrations of mesotrione. Importantly, the magnitude of tyrosinemia in humans following HPPD inhibition resembles that seen in mice. In a rabbit developmental toxicity study with mesotrione, the only treatment-related findings were minor changes in foetal ossification. To assess the human relevance of this finding, it was important to examine whether this skeletal effect was also attributable to elevated plasma tyrosine. Groups of 20 time-mated New Zealand White rabbits received 1% tyrosine in the diet (1%T) or 500 mg/kg/day mesotrione (MST) or a combination of 1% dietary tyrosine plus 500 mg/kg /day mesotrione (1%T+MST) during gestation. Assessment of maternal plasma tyrosine, revealed the expected range of tyrosinemia: 1%T<MST<1%T+MST. Similar to the previous study, there was no effect on foetal number, growth or survival and no evidence of teratogenicity or any effect on the incidence of external or visceral defects or variants. Indeed, even at plasma tyrosine levels exceeding those achieved with mesotrione alone, the only observed changes were in the patterns of foetal ossification. Furthermore, a clear relationship between the incidence of specific skeletal findings and plasma tyrosine was established ie the highest plasma tyrosine levels (1%T+MST) produced the largest difference from controls while the lowest increase in plasma tyrosine (1%T) had little effect on the pattern of ossification. This strong relationship between incidence of specific changes in foetal ossification and plasma tyrosine indicates a causal relationship with tyrosine rather than a direct effect of mesotrione.

204 THE SENSITIZING POTENTIAL OF NATIVE AND REDUCED/ALKYLATED BRAZIL NUT 2S ALBUMIN (BER E 1) IN AN ORAL BROWN NORWAY RAT FOOD ALLERGY MODEL.

S. J. Koppelman, L. M. Knippels, P. F. Nieuwenhuizen, E. I. Klein Koerkamp, H. H. de Jongh and A. H. Penninks. *Experimental Immunology, TNO Nutrition and Food Research, Zeist, Netherlands.* Sponsor: V. Feron.

2S albumin, a storage protein from Brazil nut, is an important allergen and classified as Ber e1. In this study the disulphide bounds of 2S albumin were reduced and alkylated (RA-2S albumin) resulting in loss of protein structure and an increased digestibility *in vitro*. The purpose of this study is to relate specific structural aspects of Brazil nut 2S albumin to the sensitizing capacity *in vivo* using an oral Brown Norway rat food allergy model. In addition, we wanted to demonstrate that the oral Brown Norway rat food allergy model is able to distinguish between the different potential allergenicities of native and RA-2 S albumin. Young Brown Norway rats (n=10) were orally exposed to 0.1 or 1 mg native- or RA-2S albumin by daily gavage dosing for 42 days without adjuvants. Serum samples were obtained at days 0, 28 and 42. As positive controls several animals (n=5) were injected i.p. with native or RA-2S albumin at days 0, 2, 4, 7, 9 and 11. At day 0, alum was added as an adjuvant. These animals were bled at day 28. Serum samples were tested for antigen specific IgE, IgG1 and IgG2a using a passive cutaneous anaphylaxis assay and enzyme-linked immunosorbent assay (ELISA). Oral exposure to 0.1 or 1 mg native 2S albumin resulted in both native-2S albumin-specific IgG1 and IgG2a responses in 50-70% of the animals. In 50% of the animals of both groups native 2S albumin-specific IgE (day 42)was developed. In contrast, oral exposure to 1 mg RA-2S albumin only resulted in the development of RA-specific IgG1 and IgG2a in 60% of the animals whereas no specific IgE could be detected. Exposure to 0.1 mg RA-2S albumin did not result at all in a detectable immune response in any of the tested animals. The results of this study demonstrate the importance of the stability of protein structure in inducing food allergy. In addition, we show that the oral Brown Norway rat food allergy model is able to distinguish the different potential allergenicity of both proteins.

205 INCREASED ANTIBODY RESPONSE BY BDF1 MICE CO-ADMINISTERED OVALBUMIN AND LIPOPOLYSACCRIDE (LPS).

M. R. Woolhiser, J. M. Rase and T. D. Landry. *Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI.*

There is an urgent need for an animal model that predicts the allergenicity potential of novel proteins associated with biotechnology. Many protein test materials will be purified from bacterial sources and thus have the potential to contain appreciable amounts of LPS, a potential confounder in the interpretation of antibody responses. A recent study suggested that concurrent allergen and LPS administration would reduce an IgE response towards specific, allergenic protein(s). This study evaluated the antibody response in mice following concurrent, i.p. administration of ovalbumin (OVA) and LPS. Mice were injected on days 1 and 8 with OVA prepared in PBS at 0.1mg/ml [0.08 endotoxin units (EU)] or in combination with LPS (1-400 EU). Serum samples were prepared on day 14 for ELISA (OVA-specific IgG) and to measure OVA-specific IgE *via* passive cutaneous anaphylaxis (PCA). IgG sera titers from naive mice were undetectable at dilutions of 1:10, while mice treated with OVA alone had elevations in specific IgG (titer = 286) and specific IgG1 (titer = 149). Co-exposure to OVA and LPS (≥ 1.6 EU) more than doubled these IgG and IgG1 titers. LPS had no impact on the incidence of positive PCA responses as all mice injected with OVA (+/- LPS) developed specific IgE; undiluted sera from naive mice lacked OVA specific IgE. LPS and OVA administration enhanced the magnitude of IgE responses as determined *via* maximum PCA titer and PCA reaction area using undiluted sera. Maximal PCA titers using pooled sera samples indicated a two-fold increase in specific IgE titers when OVA was co-injected with 40-80 EU. IgE titer increased to greater than 100 in mice treated with OVA and 400 EU. These results confirm that LPS can alter a murine antibody response when co-administered with an allergenic protein. The nature of the antibody response (i.e., enhanced or diminished) may depend upon factors such as exposure route, interval or duration. This suggests LPS might serve as a confounder when predicting a novel protein's allergenic potential *via* IgE and IgG production.

206 MIXED ANTIBODY AND T-CELL RESPONSES TO PEANUT AND THE PEANUT ALLERGENS ARA H1, ARA H2, ARA H3 AND ARA H6 IN A MURINE ORAL SENSITIZATION MODEL.

F. van Wijk[1, 2], S. Koppelman[3], R. Pieters[1] and L. Knippels[2]. *[1]Immunotoxicology, IRAS, Utrecht, Utrecht, Netherlands, [2]Experimental Immunology, TNO Nutrition and Food Research, Zeist, Netherlands and [3]Protein Technology, TNO Nutrition and Food Research, Zeist, Netherlands.*

Peanut allergy is known for its severity and persistence through life. Several peanut proteins have been identified as allergenic and are indicated as Ara h1-7. Very little is known about the mechanisms that underlie sensitization to peanut proteins. The purpose of the present study was to reveal the immune responses that are induced against peanut and the peanut allergens Ara h1, Ara h2, Ara h3 and Ara h6 during sensitization. Humoral and T-cell responses against peanut and the peanut allergens were examined in an early and later stage of sensitization in an established murine model of peanut anaphylaxis. Oral sensitization to peanut was characterized by a mixed cytokine response (IL-4, IL-5, IL-10 and IFN-γ) and polyisotypic humoral responses (IgE, IgG1 and IgG2a). Furthermore we demonstrated that these TH1/TH2 cytokine and antibody responses are also directed specifically against the major peanut allergens Ara h1, Ara h2, Ara h3, and Ara h6. The major peanut allergen Ara h1 induced the highest antibody responses followed by Ara h3, Ara h6 and Ara h2. This study implicates that both TH1 and TH2 phenomena are involved in the development of peanut allergy. Furthermore, we show that the present oral model is suitable to examine immune responses to food allergens during sensitization.

207 THE LOCAL LYMPH NODE ASSAY: CURRENT REGULATORY STATUS.

D. A. Basketter[1], R. J. Dearman[2], C. A. Ryan[3], F. G. Gerberick[3], R. J. Fielder[4] and I. Kimber[2]. *[1]SEAC, Unilever, Sharnbrook, United Kingdom, [2]Syngenta CTL, Macclesfield, United Kingdom, [3]Procter & Gamble, Cincinnati, OH and [4]Department of Health, London, United Kingdom.*

The local lymph node assay (LLNA) is an alternative to guinea pig tests for identification of skin sensitising chemicals. In the LLNA protocol, CBA strain mice receive 3 consecutive daily topical applications of test substance (at 3 dose levels). Five days after the initiation of exposure, proliferative responses in lymph nodes draining the application site are measured by incorporation of radiolabelled thymidine. Chemicals that induce proliferative responses >3x those observed in concurrent vehicle treated control are considered to be skin sensitisers. The assay has been approved as a standalone method for the determination of skin sensitisation hazard by the FDA, the EPA, CPSC and others following approval by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The LLNA has also received formal approval from the European Centre for the Validation of Alternative Methods (ECVAM). The LLNA became OECD Guideline 429 in 2002, and in the EU awaits endorsement as Test Method B42 in the 29th Adaptation to Technical Progress. The UK Health and Safety Executive led the way as a European Competent Authority by announcing that for both scientific and animal welfare reasons the LLNA was the preferred method. Currently, regulatory authorities in the Far East increasingly are considering the method. However, challenges remain insofar as validation does not mean automatic acceptance, nor is the LLNA necessarily the most appropriate method in all circumstances. Experience with the assay for the assessment of complex mixtures is no better validated than are such evaluations using guinea pig methods, although at least for the LLNA considerable data are available on the impact of vehicles on the expressed potency of allergens.

208 VALIDATION OF A MINIMAL TRANSCRIPT BIOMARKER SET TO DIFFERENTIATE BETWEEN SENSITIZERS AND IRRITANTS IN THE LOCAL LYMPH NODE ASSAY.

W. R. Foster[2], G. S. Ladics[1] and C. M. Glatt[1]. *[1]Haskell Laboratory, DuPont, Newark, DE and [2]Bristol-Myers Squibb, Wilmington, DE.*

Previous studies utilized the LLNA in conjunction with DNA microarrays to evaluate expression profiling as a method to distinguish between sensitizers and irritants and to identify potential transcript biomarkers. Using a defined training set and analysis criteria, a set of potential biomarker genes that included proteases and protease inhibitors, and lymphocyte, cell cycle, and inflammatory related response transcripts were defined and had expression patterns in the array experiments suggesting they may discriminate between sensitizers and irritants. The objective was to validate the biomarker candidates using real-time PCR. Analyses were performed on auricular lymph node total RNA isolated from 8-week-old female CBA/JHsd mice (n=7-15/group) dosed with either 4:1 acetone/olive oil (vehicle), one of several different sensitizers, or one of several different irritants according to the standard LLNA protocol. Real-time PCR experiments were performed using the TaqMan® technology and primer/probe sets of each specific biomarker. Expression patterns for transcripts such as mLag3 (lymphocyte activation gene), TYMS (thymidylate synthase gene), and mBub1A (a mitotic checkpoint protein kinase) were consistent with the array results. These transcripts were generally upregulated in sensitizer-treated groups and unchanged in irritant-treated groups. The validated clusters of potential biomarkers effectively confirm proliferation-based scoring of sensitizers in the LLNA. Expression profiling performed on higher doses of the irritant, benzalkonium chloride, that typically score positive in the LLNA, revealed a similar induction of many cell cycle and inflammatory-response transcripts, providing a molecular basis for high-dose irritant scoring in the LLNA. These results illustrate the utility of expression profile databases that can be queried against for compound classification, and reveal several candidate biomarkers that may serve as sensitive endpoints for discriminating irritants from sensitizers using the LLNA.

209 EXPERIENCE IN THE ROUTINE USE OF THE LOCAL LYMPH NODE ASSAY (LLNA).

J. Le Bigot, B. Griffon, X. Manciaux, S. de Jouffrey and R. Forster. *CIT, Evreux, France.*

The Local Lymph Node Assay (LLNA) is an *in vivo* alternative to traditional Guinea pig testing methods for assessment of the potential of chemicals to cause skin sensitization. In this assay, sensitizing activity is measured using parameters of the induction phase, rather than of the elicitation phase, by quantification of the proliferative responses in draining lymph nodes after topical application of the test item. Testing procedures have been formalized in a recently approved OECD guideline (No. 429). In this guideline, skin sensitizers are defined as test items which, at one or more tested concentrations, elicit a 3-fold or greater increase in the incorporation of radioactivity (a measure of cellular proliferation) compared with vehicle controls, together with consideration of a dose-response relationship. As the method is described in the OECD guideline, the LLNA and its interpretation seem easy. However, based on our experience of over 150 individual studies performed in the last 12 months, it appears that interpretation may sometimes be problematic. We have previously shown that definitive classification of a test item as a skin sensitizer cannot be based only on the criterion of a stimulation index (SI). Integration of a quantitative measure of skin irritation (for example, by measurement of ear thickness) in the study protocol is necessary to permit unequivocal interpretation of the results since it is well established that an irritant action can give rise to "false positive" increases in cellular proliferation. More recently, in routine studies, we have been confronted by various data sets for which interpretation was problematic. For example, an SI greater than 4 at the highest tested concentration but accompanied by slight irritation? An SI exceeding 2 at the highest feasible dose-level, without evidence of dose-relationship? In our experience the LLNA is an informative and robust test but routine use requires careful attention to the interpretation of results.

210 COMPARISON OF TWO PROTOCOLS OF A MODIFIED LYMPH NODE ASSAY.

K. Riecke and P. Kurth. *Experimental Toxicology, Schering AG, Berlin, Germany.* Sponsor: R. Stahlmann.

The Local Lymph Node Assay (LLNA) is a method to study sensitization using tritiated thymidine for assessment of cell proliferation. We tested the usefulness of auricular lymph node weight, lymph node cellularity and weight of an ear punch as alternative end points using two different study protocols. Female BALB/c mice (4 or 6 per group) were treated on three consecutive days on the back of both ears with the sensitizing reference compounds dinitrochlorbenzene (DNCB) or hexylcinnamic aldehyde (HCA) or mercaptobenzothiazole (MBT). Three concentrations of DNCB, HCA or MBT were selected which included the respective threshold concentrations (EC3) in the LLNA. The end points were assessed one ("short protocol", DNCB and HCA) or three days ("LLNA protocol", DNCB, MBT and HCA) after the last treatment. Lymph node cells from DNCB-treated animals were also studied by three color flow-cytometry analyzing CD4 or CD8 and CD44 and CD62L to differentiate between naive T-cells and cells having had antigen contact. Using the short protocol, HCA was positive at all concentrations exceeding the EC3 but DNCB was positive only at the 4-fold EC3. Using the LLNA protocol, all three reference substances were positive from their respective EC3 upwards. At the EC3, lymph node weights were approximately 1.5-times higher than in controls using the LLNA protocol. Flow cytometric analysis did not show clear cut effects in DNCB-treated mice up to a concentration of 0.25 mg/ml. Compared to the short protocol, the LLNA-protocol led to more pronounced increases of lymph node weight and lymph node cellularity. The LLNA protocol was able to classify all concentrations at or beyond the EC3 as positive with three reference substances which are considered as weak to moderate or strong sensitizers. The increase of lymph node weight (increase approx. 1.5 fold at EC3) seemed to be more sensitive than the increase of lymph node cellularity (increase 1.1 to 1.7 fold at EC3). Inclusion of flow-cytometric end points did not improve sensitivity of this assay.

211 ROUND II OF AN INTER-LABORATORY VALIDATION OF ALTERNATIVE ENDPOINTS OF THE MURINE LLNA.

J. Huesler[9], P. Ulrich[1], H. Vohr[2], G. Ehling[3], M. Hecht[4], A. Heusener[10], A. Gamer[5], H. van Loveren[6], L. Ullmann[8], T. Maurer[9] and K. Riecke[11]. *[1]Preclinical Safety, Novartis Pharmacology AG, Muttenz, Switzerland, [2]Bayer AG, Wuppertal, Germany, [3]Aventis Pharmacology AG, Frankfurt, Germany, [4]ITA Fraunhofer Institute, Hannover, Germany, [5]BASF AG, Ludwigshafen, Germany, [6]RIVM, Bilthoven, Netherlands, [7]University of Bern, Bern, Switzerland, [8]RCC, Itingen, Switzerland, [9]Swiss Agency for Therapeutic Products, Bern, Switzerland, [10]Merck, Darmstadt, Germany and [11]Schering AG, Berlin, Germany.*

The original Local Lymph Node Assay (LLNA) is based on the use of radioactive labelling to measure cell proliferation. However, other endpoints for assessment of proliferation are also authorized by the OECD guideline 429 provided there is appropriate scientific support, including full citations and description of the methodology. Here we describe the outcome of the 2nd round of an inter-laboratory validation of alternative endpoints in the LLNA conducted in nine laboratories in Europe. The validation study was managed and supervised by the Swiss drug agency. Statistical analysis was performed at the university of Bern, Department of Statistics. Ear-draining lymph node (LN) weight and cell counts were used to assess proliferation instead of radioactive labelling of lymph node cells. In addition, the acute inflammatory skin reaction was measured by ear swelling and weight of circular biopsies of the ears to identify skin irritating properties of the test items. Eleven

blinded test items were applied to female NMRI and/or BALB/c mice. The assessment of skin irritation potential supported the differentiation of pure irritative from contact allergenic potential. In contrast to the radioactive method irritants have been picked up by all the labs. An assessment scheme developed by the group turned out to be of extreme value in evaluation of the data from the different parameter. Similar to the EC3 values defined for the radioactive method, threshold values were calculated for the endpoints measured in this modification of the LLNA. It was concluded that all parameters measured have to be taken into consideration for the categorisation of compounds due to their relative sensitising potencies.

212 USE OF LOCAL LYMPH NODE ASSAY POTENCY DETERMINATIONS IN EXPOSURE-BASED RISK ASSESSMENT FOR SKIN SENSITIZATION.

C. Ryan[1], P. McNamee[2] and F. Gerberick[1]. *[1]Procter & Gamble Company, Cincinnati, OH and [2]Procter & Gamble Company, Egham, Surry, United Kingdom.*

The fact that a chemical is determined to be a contact allergen does not mean it cannot be formulated into a consumer product at safe levels, provided that one has a thorough understanding of ingredient exposure, allergenic potency and dose response. The local lymph node assay (LLNA), a validated method for the prediction of skin sensitization hazard, has been shown to be useful for the determination of relative sensitization potency. Relative potency is based upon calculation, from the LLNA dose response curve, of an EC3 value - the estimated concentration of chemical required to induce a threshold positive response (stimulation index = 3). To demonstrate both the application and value of LLNA-derived potencies to the risk assessment process, the example of two theoretical preservatives is used. For illustration purposes, we assume that in calculations from the resulting LLNA dose response curves Preservative A has an EC3 value of 12.9%, with a relative sensitization potency of weak and Preservative B has an EC3 value of 0.3%, giving it a relative potency of strong. Using an exposure-based risk assessment approach in which a default No Observed Effect Level (NOEL) is assigned based on the potency classification; the anticipated consumer exposure to the ingredient is compared to a sensitization reference dose calculated from the default NOEL and appropriate uncertainty factors to determine a margin of safety (MOS). An acceptable exposure is generally represented by an MOS≥1. Based on this risk assessment methodology, both preservatives were found to be acceptable for use in a 'rinse-off' product (e.g. shampoo) at a level of 50 ppm, while only Preservative A, the weak allergen, was appropriate for use in a 'leave-on' product (e.g. moisturizer) with the same level of preservative. These examples demonstrate the utility and importance of LLNA-derived potency classifications in the conduct of scientifically sound risk assessments for skin sensitizing chemicals.

213 CATEGORISATION OF HUMAN SENSITISATION POTENCY USING LOCAL LYMPH NODE ASSAY EC3 VALUES.

N. J. Gilmour[1], D. A. Basketter[1], G. Y. Patlewicz[1], P. S. Kern[2], C. A. Ryan[2], F. G. Gerberick[2], R. J. Dearman[3] and I. Kimber[3]. *[1]SEAC, Unilever, Sharnbrook, United Kingdom, [2]Procter & Gamble, Cincinnati, OH and [3]Syngenta CTL, Macclesfield, United Kingdom.*

Effective evaluation of skin sensitisation demands that potential contact allergens are identified and the risk of sensitisation amongst exposed populations assessed. Sensitisation hazard is not an all or nothing phenomenon; dose response relationships can be discerned and thresholds identified for induction and elicitation of contact allergy. These parameters, under the heading of potency, are vital for the risk assessment process. The murine local lymph node assay (LLNA) is a validated and accepted method for the identification of sensitisation hazards (a sensitiser is a chemical that induces a stimulation index 3 or more times that of the concurrent vehicle control). The LLNA has also been used to determine the potency of sensitisers, by derivation of an EC3 value, the estimated concentration of chemical required to induce a stimulation index of 3. At present, various agencies, including in EU and OECD, together with industry bodies, are considering whether and how to adapt these data into regulations which would place sensitisers into one of several potency categories. It is our view, based on an evaluation of over 250 chemicals, that five categories, extreme, strong, moderate, weak and negative, represent the optimal approach and is consistent with what is understood regarding the potency of skin sensitisers in humans. To illustrate this approach, an example dataset of 100 selected organic chemicals is shown, where we judge the accuracy of prediction of human potency to be in the region of 90%. This categorisation has utility as a simple guide for risk management strategies as well as representing an important first step in risk assessment.

214 OBSERVATIONS ON THE UTILITY OF THE LLNA FOR DERMATOLOGIC DRUG PRODUCTS.

A. Jacobs, D. Allen, P. C. Brown, B. A. Hill, N. See and J. Wilkin. *USFDA, Rockville, MD.*

The local lymph node assay (LLNA) may be useful in the evaluation of contact hypersensitivity for some chemical substances. In Feb 1999, the Interagency Coordinating Committee on the Validation of Alternative methods (ICCVAM) published a validation study for the use of the LLNA as an alternative to traditional hypersensitivity methods. The LLNA was validated for neat liquids and for 50%, 25% and 10% solutions in acetone olive/oil. However, the LLNA was not validated for mixtures, clinical formulations, or with many substances with pharmacologic activity. Topical drug formulations usually have concentrations of active components at <1%. Because results using these formulations may be vehicle dependent, inclusion of a positive control in the clinical vehicle is desirable. Several issues related to drug substance and or drug products tested with the LLNA have arisen that have confounded interpretation of the LLNA These issues include: irritant vehicle, failure of DNCB in the clinical vehicle, vehicles that appear positive, pharmacologic activity (e.g., cytokine induction), false positives relative to both guine pigs and humans, and potency relative to DNCB not correlating with the guinea pig assay. Furthermore, intra-laboratory reproducibility of LLNA results has been inconsistent. It is not clear for which human population the LLNA was validated. Persons with or without atopic dermatitis may respond differently, as would persons in a challenge test or persons exposed without occlusion. Therefore, the LLNA should be used with caution; it may not be useful for many dermal drug products. In some cases, absence of effects in repeat-dose studies in animals, followed by repeat-dose studies in humans, may ob*via*te the need for nonclinical evaluation of dermal hypersensitivity.

215 APPLICATION OF A MODIFIED LLNA TO PETROLEUM-BASED PRODUCTS: DERMAL SENSITIZATION POTENTIAL OF CALCIUM LONG-CHAIN ALKYLBENZENE SULFONATES.

S. A. Signs[1] and G. L. DeGeorge[2]. *[1]The Lubrizol Corporation, Wickliffe, OH and [2]MB Research Laboratories, Spinnerstown, PA.*

Calcium long-chain (C15-30) alkylbenzene sulfonates are oil-based detergents used in a variety of mechanical lubrication applications. Low overbased calcium sulfonates (LOB) are generally regarded as dermal irritants whereas high overbased calcium sulfonates (HOB) are non-irritants. Existing data reveals a strong correlation between dermal irritant properties of calcium sulfonates and dermal sensitization potential observed in guinea pigs and humans. A flow cytometry-based murine local lymph node assay (LLNA) was used to further elucidate the influence of dermal irritancy on assessments of dermal sensitization potential. Female CBA/J mice (five per group) were treated with 3 concentrations of either LOB, HOB, 50% HCA (positive control) or ultra-pure petrolatum (vehicle control) by daily topical application to the dorsum of each ear for three consecutive days. Five days following the initial dose, the mice were injected with 5-bromo-2-deoxy-uridine (BrdU). This thymidine analog is incorporated into the DNA of proliferating cells. Five hours later, the auricular lymph nodes were isolated and single-cell suspensions of lymph node cells (LNC) were generated for each individual animal. An aliquot of each LNC suspension was analyzed by flow cytometry for immunotype expression (B220+, CD3+) and the total number of proliferating (BrdU+) cells was determined. Treatment with HOB resulted in no significant dermal irritation as measured by ear swelling and yielded a cellular SI <3 (not classified a dermal sensitizer). LOB treatment induced a dose-dependent increase in cellular proliferation (30% LOB; SI=4.54) which was accompanied by significant increases in ear thickness indicating a dermal irritation response. Additionally, the lack of increases in B220+ lymph node cells or in the B:T cell ratio following LOB treatment further confirms that changes in LNC proliferation are due to irritation rather than induction of dermal sensitization.

216 EFFECTS OF LIPOPHILICITY AND VISCOSITY OF SOLVENTS ON DPM/LN BACKGROUND LEVEL IN MURINE LOCAL LYMPH NODE ASSAY (LLNA).

L. G. Ullmann, W. Wang-Fan, G. Arcelin, S. Corney and K. Blumbach. *Toxicology, RCC Ltd., Itingen, Switzerland.*

In 253 LLNA studies with 1012 CBA/CaOlaHsd mice performed at RCC, 11 different solvent systems, most of them are recommended by OECD Guideline 429, have been used. As a standard level in the calculation of the stimulation index (S.I.), disintegrations per minute per lymph node (DPM/LN) of the control group plays a key roll in getting reliable and correct test results. However, the level of DPM/LN can be varied or influenced by many factors. The variety of vehicles used in LLNA is one of the most important factors which can directly effect the background level

of DPM/LN. Although some systematic de*via*tions in a LLNA study can be eliminated by using a vehicle control group, further investigations on the background level of DPM/LN are still necessary in different solvent systems. This poster presents the results of our investigations into the following aspects of the LLNA: - The effect of different solvents on DPM/LN control level; - The possible range of variation of DPM/LN control level with different solvent systems; - How to interpret the solvents effects on DPM/LN background level. The results have helped to optimize the experimental methods and to increase the accuracy of the tests.

217 BISPHENOL A IS NOT SKIN SENSITIZING OR PHOTOALLERGENIC AS MEASURED BY A MODIFIED LOCAL LYMPH NODE ASSAY IN MICE.

H. Vohr, H. Ahr and G. D. Stropp. *Toxicology, Bayer AG, Wuppertal, NRW, Germany.* Sponsor: R. Shiotsuka.

A major chemical intermediate for the production of polycarbonates and epoxy resins is Bisphenol A (BPA). Experimental studies for dermal sensitisation potential of BPA have led to generally negative or equivocal results. Photosensitization studies were based on non-validated test systems or the results were not definitive. Case-reports of dermal sensitisation are rare and generally involve presumed exposure to compounds produced from BPA and not the BPA monomer. Thus, the purpose of this study was to assess BPA for a skin sensitising or photoallergenic potential using a validated test system. A modified Local Lymph Node Assay (LLNA), the Integrated Model for the Differentiation of Skin reactions (IMDS; Homey, B, et.al, Toxicol. Appl. Pharmacology 153, 83-94, 1998; Vohr, H.-W., et al, Arch. Toxicol., 73, 501-509, 2000) was used to distinguish skin sensitization from acute irritation. The UV-IMDS assay was used to test for photo-reactivity. Female outbred NMRI mice (6/group) received dermal applications of either vehicle (DAE433), 3%, 10% or 30% (solubility limit) BPA on three consecutive days. For the investigation of photo-reactivity, mice were irradiated with 20J UV-A/cm2 directly after each application. Results showed no skin reaction in the BPA groups. In contrast to positive controls (HCA, Chlorpromazine, 8-MOP), neither a skin sensitizing or photoallergenic nor an irritating or photoirritating potential was observed. Therefore, BPA is not considered to be a skin sensitizer or photosensitizer in this validated test system.

218 EVALUATION OF THE SENSITIZATION POTENTIAL OF *PFIESTERIA* TOXIN IN BALB/C MICE.

R. M. Patterson[1], E. Noga[2] and D. Germolec[1]. *[1]NIEHS, Research Triangle Park, NC and [2]N.C. State University, Raleigh, NC.*

Members of the estuarine dinoflagellate genus, *Pfiesteria*, are reported to have been responsible for massive fish kills in the southeastern United States. The classic signs of *Pfiesteria* poisoning in fish are skin ulceration and swimming disruption. Some studies suggest that people exposed to waters having *Pfiesteria* blooms might exhibit toxin-induced skin and respiratory irritation and inflammation. High levels of exposure related to occupational setting have suggested disruptions in cognitive function. Although the putative toxin has not been isolated, available data suggest both hydrophilic and hydrophobic toxic components. The dermonecrotic properties have been proposed to be associated with the hydrophobic fraction. The C-18 bound fraction of the crude toxin extract from dinoflagellate cultures, confirmed to be ichthyotoxic, was examined in the current studies to evaluate its potential to produce inflammation and dermal sensitization. This fraction was cytotoxic to mouse Neuro 2A cells at 1 x 10^3 µg/ml, validating potency as described previously (McClellan-Green, 1997). In primary human epidermal keratinocytes, proliferation and *via*bility were also inhibited at 1 x 10^3 µg/ml of the C-18 fraction, as determined using the 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt (MTS) and Neutral Red cytotoxicity assays. Exposure of Balb/C mice to 0.25-30 x 10^3 µg/ml of the C-18 bound fraction induced mild, localized inflammation, as evidenced by a 5-10‰ increase in ear swelling relative to control mice. There was no increase in lymph node cell proliferation as measured using the ICCVAM-validated (Dean, *et al.* 2001) local lymph node assay. These studies suggest that the C-18 bound fraction of the toxin is cytotoxic to keratinocytes in culture at high concentrations; that it induces mild, localized irritation, but not sensitization in mouse skin; and that the degree of dermal irritation and inflammation reported in human exposure studies may result from the combination of *Pfiesteria* contact and other confounding factors.

219 EVALUATION OF THE CONTACT HYPERSENSITIVITY-INDUCING POTENTIAL OF A COMMERCIAL WEAPON CLEANING AND MAINTENANCE COMPOUND.

S. Azadi[1], D. P. Arfsten[2] and B. J. Meade[1]. *[1]NIOSH, Morgantown, WV and [2]Naval Health Research Center Toxicology Detachment, Wright-Patterson AFB, OH.*

Commercial cleaning, lubricating, and preserving compounds are used in both the military and civilian sectors for maintenance of small and large caliber weapons. The cleaning of weapons is a very labor intensive, hands-on operation and frequent, repeated dermal contact with materials used in weapons maintenance is highly probable. Cleaning compounds are usually complex mixtures containing many constituents including polyalphaolefin oil, synthetic oils, esters, isoparaffinic hydrocarbons, and dibasic ester, some of which have the potential for being dermal sensitizers. These studies assessed the irritant and sensitizing potential of a weapon cleaning compound using a modified local lymph node assay (LLNA) and phenotypic analysis (PA) of draining lymph node cells. Female BALB/c mice (n=5) were topically exposed on the dorsal ear pinna for 3 (LLNA) or 4 (PA) consecutive days. In the LLNA, mice were injected i.v. with 3H-thymidine on day 6 and draining lymph nodes were radioassayed. Ear thickness was measured using a micrometer prior to sacrifice to evaluate irritancy. For PA, on day 10 post initial exposure, draining lymph node cells were stained with FITC labeled rat anti-mouse IgE or PE labeled rat anti-mouse CD45 B220. Blood was collected at the time of sacrifice for analysis of total serum IgE. The compound induced a dose-responsive increase in lymph node cell proliferation with the neat material resulting in a stimulation index of 13.4. PA demonstrated an elevation in B220+ cells. No increase in total serum IgE was detected. Animals exhibited thickened skin and hair loss at the site of exposure and a 42.5% increase in ear swelling was observed in animals exposed to the neat material as compared to controls 3 days following the final exposure. These studies indicate the potential for the compound tested to induce contact sensitization with no evidence of an IgE inducing potential. These studies were supported in part by NIEHS intra-agency agreement # Y1-ES-0001-03.

220 EFFECT OF CHLORINATED ORGANIC SOLVENTS IN DRINKING WATER ON TYPE I ALLERGIC REACTION.

M. Seo, T. Yamagiwa, T. Ikemoto, M. Satoh and H. Nagase. *Department Of Hygienics, Gifu Pharmaceutical University, Gifu, Japan.*

The incidence of allergic diseases has been increasing. Some epidemiological studies suggest that this is due to exposure to environmental toxic substances. Chlorinated organic solvents are incombustible and low molecular chemicals, and have been used widely in a variety of industrial processes as solvents, softeners, paint thinners, dry-cleaning fluids at present. But chlorinated organic solvents have become major environmental pollutants. Previously we observed that some chlorinated organic solvents, such as tetrachloroethylene (PCE), trichloroethylene (TCE), and 1, 1, 1-trichloroethane, increased histamine release from mast cells *in vitro*. In the present study, we investigated the enhancing effect of low concentration of PCE, TCE and 1, 1, 1-trichloroethane dissolved in drinking water on Wistar rat (male, 8weeks old). Each chlorinated organic solvent was dissolved in distilled water at a concentration of Japanese guideline for drinking-water quality, or its 100-fold concentration. After 2 or 4 weeks, we executed PCA reaction. The chlorinated organic solvents enhanced PCA reaction in a dose-dependent and time-dependent manner. In pathological studies, the skin lesions elicited in rat drinking PCE and TCE for 2 weeks showed a cellular infiltrate in the dermis that contained lymphocytes and mast cells. To further investigate the effects of chlorinated organic solvents, we measured IL-4 production from mast cells, rat basophilic leukemia cells (RBL-2H3), treated with chlorinated organic solvents for 6 hours. IL-4 production from the cells treated with 1mg/L PCE markedly enhanced. The mechanism of enhancing PCA reaction is assumed that chlorinated organic solvents increase IL-4 production, and it causes a Th1-Th2 imbalance and increases histamine release from mast cells accumulated excessively. The results suggest that the intake of chlorinated organic solvents as drinking water, even in a low concentration, would lead to the initiation of allergic diseases.

221 INVOLVEMENT OF PERTUSSIS TOXIN SENSITIVE G PROTEIN ACTIVATION IN HISTAMINE RELEASE INDUCED BY FLUOROQUINOLONE ANTIBACTERIAL AGENTS (FLUOROQUINOLONES) AND THEIR STRUCTURE-HISTAMINE RELEASE RELATIONSHIP.

M. Kazuhiko, M. Chikako and K. Furuhama. *Drug Safety Reseach Laboratory, Daiichi Pharmaceutical Co., Ltd.,, Tokyo, Japan.*

The present study was designed to clarify the involvement of pertussis toxin sensitive G protein activation in histamine release induced by fluoroquinolones and then to elucidate the relationship between their structures and histamine release using rat peritoneal mast cells. Levofloxacin (LVFX) at 1000 µM or more dose-dependently induced a non-cytotoxic histamine secretion. This action was rapidly completed within 30 s and was dependent on temperature, energy, pH and intracellular Ca2+. Histamine secretion due to LVFX was prevented by pretreatment with pertussis toxin or benzalkonium chloride which was a selective inhibitor of G proteins of Gi subtypes, or neuraminidase which induced hydrolysis of sialic acid residues on the cell surface. Moreover, LVFX increased GTPase activity in the supernatant of mast cell membrane homogenate. Next, histamine releasing activity of the 10 fluoroquinolones such as LVFX, ciprofloxacin (CPFX), lomefloxacin (LMFX), sparfloxacin (SPFX), gatifloxacin (GTFX), grepafloxacin (GPFX),

sitafloxacin (STFX), HSR-903, moxifloxacin (MXFX) and T-3762 was compared under the same experimental conditions. All of the fluoroquinolones tested except for T-3762 induced a non-cytotoxic histamine secretion in a concentration-dependent fashion. The order of its potency (10% histamine releasing concentration, μM) was GPFX (70) = HSR-903 (70)≥GTFX (110)≥STFX (120)≥CPFX (130) ≥SPFX (140)≥MXFX (200)≥LMFX (250)>LVFX (1870)>T-3762 (>3000). The activation caused by these fluoroquinolones was evidently blocked by pretreatment with pertussis toxin. These results demonstrate that fluoroquinolones interact with sialic acid residues, followed by the activation of pertussis toxin sensitive G proteins leading to histamine release. Furthermore, the order of the potency of histamine release would be, at least in part, related to the positive charge of substituents at the 7 position of fluoroquinolone core structure.

222 DEVELOPMENT OF AN ORAL EXPOSURE ANIMAL MODEL WITH REPORTER ANTIGENS TO ASSESS IMMUNE-MEDIATED DRUG-HYPERSENSITIVITY REACTIONS.

S. Nierkens, M. Aalbers, M. Bol and R. Pieters. *IRAS-Immunotoxicology, Utrecht University, Utrecht, Netherlands.*

Adverse immune responses to drugs are of major concern to pharmaceutical companies, physicians and susceptible patients. Due to its idiosyncratic nature and complex etiology adverse immune effects are usually missed in routine toxicity studies. Previous reports suggest that the murine or rat popliteal lymph node assay (PLNA) may be a possible screen for hypersensitivity reactions. However, the PLNA involves administering the test compound *via* footpad injections rather than the relevant route of exposure. In addition, drug-induced immune responses may vary from chemical- to neo-antigen-specific, thus hampering straightforward detection of immunosensitizing potential in animal models. By using well-defined reporter antigens (RA) this problem has been successfully circumvented in the PLNA. The aim of this study was to investigate whether the RA-approach can be used in combination with oral exposures to assess the immunogenicity of drugs. Accordingly, female C3H/HeJ mice were injected i.p. with the RA TNP-OVA on day 0 and received 50 or 150 mg/kg D-penicillamine (D-Pen) by gavage or i.p. on day 0-7. On day 10, all experimental groups showed increased TNP-specific serum antibody responses and only the 150 mg/kg-dosed groups had increased DTH responses on day 15 and TNP-specific antibodies on day 20. In a separate study we found that a single oral dose of D-Pen (50 mg/kg) sensitized the immune system so that a paw-challenge with a sub-sensitizing dose of D-Pen in combination with the RA TNP-Ficoll (T cell independent antigen susceptible for neo-antigen-specific help) induced TNP-specific DTH and antibody secreting B cells in the draining lymph node. The response was drug-specific, as diclofenac did not induce TNP-specific responses in D-Pen-sensitized animals. In conclusion, we present two promising variations of an oral exposure model using RA to assess the immunosensitizing capacity of drugs. Other drugs are currently investigated using these models. This project is financially supported by ILI-HESI Immunotoxicology Technical Committee (ITC)

223 TEST STRATEGIES FOR IMMUNE SENSITIZATION BY PHARMACEUTICALS: A COMPARISON OF THE SUBCUTANEOUS LOCAL LYMPH NODE ASSAY AND POPLITEAL LYMPH NODE ASSAY USING REPORTER ANTIGENS.

R. Pieters, S. Nierkens, L. Nieuwenhuijsen and M. Thomas. *IRAS-Immunotoxicology, Utrecht University, Utrecht, Netherlands.*

Immune-mediated idiosyncratic drug reactions are a major problem for susceptible patients, physicians and pharmaceutical industries. Currently, validated screening tools to assess the immunosensitizing capacity of pharmaceuticals are not available. Recently, the Immunotoxicology Technical Committee of ILSI-HESI initiated a collaborative effort to evaluate the popliteal lymph node assay (PLNA) in comparison with a modified version of the local (auricular) lymph node assay (LLNA). Both assays are evaluated with regard to their potency to recognize drug-induced sensitization. We modified the LLNA to enable s.c. exposure, i.e. similar to the PLNA, and injected the compounds of interest between the layers of the ear. Compounds were injected together with the T cell independent antigen TNP-Ficoll, which only elicits switched isotypes when antibody producing B cells receive additional neo-antigen specific help. Using this approach, we positively identified D-penicillamine, streptozotocin (STZ), ofloxacin, and diphenylhydantoin as drugs with immunosensitizing capacity. Sulphamethoxazole and procainamide (known to require metabolism) and phenobarbital and metformin were tested negative. Thus, the number of TNP-specific IgG antibody secreting cells is a very useful and sensitive parameter to identify drug-induced hypersensitivity in both PLNA and modified LLNA. However, the type-1 associated parameters (CD8 cells, macrophages, IFN-γ, TNF-α and IL-1β) that are induced in the PLN by STZ were less pronounced in the auricular lymph node. Together, the PLNA and modified LLNA can both be used to distinguish sensitizing compounds from non-sensitizers with the use of reporter antigens. However, the modified LLNA is practically less convenient than the PLNA and the PLNA seems to provide more relevant immune information on the mechanisms of chemical-induced hypersensitivity reactions.

224 CYTOKINE RELEASE AS AN ENDPOINT TO IMPROVE THE SENSITIVITY AND SPECIFICITY OF THE POPLITEAL LYMPH NODE ASSAY (PLNA).

J. Descotes[2], G. Ravel[1, 2], M. Christ[1], N. Eltschinger[1] and J. Guichard[1]. *[1]MDS Pharmacology Services, L'Arbresle, France and [2]Poison Center & INSERM U503, Lyon, France.*

The PLNA has been proposed for the preclinical prediction of the autoimmunogenic and sensitizing potential of drugs. The evaluation of over 130 compounds in this assay has resulted in several false positive (e.g. ethanol, imipramine) or false negative (e.g. ofloxacin, sulfamethoxazole) responses placing in question the sensitivity and specificity of the test. In this study, cytokine production in the popliteal lymph nodes of female Balb/c mice was evaluated as a possible endpoint to improve the sensitivity and specificity of the assay. Diclofenac, imipramine, hydralazine, glafenin and minocycline were tested in groups of 6 Balb/c mice using the classical primary PLNA procedure. Cellularity measurements were made 7 days after injection of the drug. In addition, Th1 (IL-4 and IL-5), Th2 (IL-2, IL-12 and IFN-γ and pro-inflammatory (IL-6, TNF-α and Monocyte Chemoattractant Protein-1) cytokines were measured in suspensions of popliteal lymph node cells by flow cytometry. Cellularity indexes above 5 (defined as positive responses) were obtained only in mice treated with diclofenac and imipramine. Diclofenac induced a significant increase in the Th1 and pro-inflammatory cytokines. All other drugs had no effects on the production of any cytokine. The measured cytokine levels did not help rule out the false positive and false negative responses obtained with imipramine and glafenin, respectively. These findings suggest that measuring cytokine release is unlikely to improve the sensitivity and specificity of the PLNA. In addition, minocycline, a tetracycline antibiotic not previously tested in the PLNA, but recognized as a cause of many autoimmune and hypersensitivity reactions in human patients, produced a negative PLNA response.

225 INTER-ANIMAL VARIATION IN CYTOKINE FINGERPRINTING OF CHEMICAL ALLERGENS.

H. Caddick, R. J. Dearman and I. Kimber. *Syngenta CTL, Macclesfield, United Kingdom.*

Repeated topical exposure of BALB/c strain mice to chemical contact and respiratory allergens stimulates cytokine secretion phenotypes consistent with the selective activation of type 1 and type 2 T cell subpopulations, respectively. Lymph node cells (LNC) isolated after treatment with the contact allergen 2, 4-dinitrochlorobenzene (DNCB) display a type 1 cytokine secretion pattern with relatively high levels of interferon γ (IFN-γ) and interleukin (IL) 12, but little of the type 2 cell products IL-4, IL-5, IL-10 and IL-13. Exposure to the respiratory allergen trimellitic anhydride (TMA) results in the converse type 2 cytokine expression profile. In previous experiments, in order to provide for sufficient supernatant to analyze the complete panel of cytokines by enzyme-linked immunosorbant assay, lymph nodes have been pooled on an experimental group basis. In these studies, DNCB-stimulated LNC invariably expressed higher levels of type 1 cytokines whereas TMA-activated LNC produced higher levels of type 2 cytokines. It is now possible to measure multiple cytokines in small volumes, using cytokine arrays with a fluorescent end point, allowing cytokine secretion patterns to be measured for individual animals. Very marked type 1 and type 2 cytokine secretion profiles, respectively, were observed for LNC isolated from DNCB- and TMA-exposed mice (n=5). LNC derived from DNCB-treated animals produced significantly more IFN-γ ($p<0.05$) and IL-12 ($p<0.01$) than did those isolated from TMA-treated mice, whereas exposure to TMA stimulated significantly higher levels of IL-4, IL-5 and IL-10 ($p<0.01$). These data suggest that there is little inter-animal variation in the ability of DNCB and TMA to elicit distinct type 1 and type 2 cytokine secretion profiles and demonstrate that similar cytokine phenotypes are detected whether lymph nodes are pooled on a group basis or individual animals are assessed.

226 SENSITIZATION WITH DINITROTHIOCYANOBENZENE (DNTB) : COMPARISONS WITH DINITROCHLOROBENZENE (DNCB).

P. S. Friedmann[1], C. Pickard[1], M. Cumberbatch[2], R. J. Dearman[2] and I. Kimber[2]. *[1]Southampton University, Southampton, United Kingdom and [2]Syngenta CTL, Macclesfield, United Kingdom.*

DNCB is a potent contact sensitizer in man and rodents whereas it has been reported that prior exposure to the chemically related hapten DNTB can tolerize mice to subsequent sensitization with DNCB. We have therefore examined the

ability of DNTB to modify responses to DNCB and to induce de novo contact sensitization in humans. Ten volunteers were sensitized by application of 4 doses of DNCB (6.25, 8.8, 12.5, 18.8μg) to the forearm. Four weeks later, the other arm was challenged with the same doses of DNCB and elicitation reactions quantified at 48h as skinfold thickness using calipers. Marked responses were induced in all subjects (AUC sum of responses at each challenge dose, mean+/-SE; 5.9+/-1.02). Using the same treatment and challenge regimen, responses to DNTB were uniformly negative (n=7). Prior exposure (4 weeks earlier) to the same concentrations of DNTB was without significant effect on the subsequent response to sensitization and elicitation with DNCB (AUC mean+/-SE; 4.3+/-1.6). However, using the same exposure schedule, initial priming with high dose (35μg) DNTB did augment the elicitation reactions to DNCB (AUC mean+/-SE; 13.4+/-6.02, n=7). The differential activity of DNTB was not a result of poor penetration as volunteers that had been sensitized and challenged with DNCB exhibited equivalent skin reactions when challenged with the same doses of DNTB. Furthermore, priming with a very high dose DNTB (75μg) followed by challenges with DNTB 4 and 8 weeks later resulted in sensitization of 2 out of 10 volunteers. These data demonstrate that DNTB is not a tolerogen in man, but is a relatively weak sensitizer compared with DNCB. DNTB is equally effective as DNCB at eliciting challenge reactions, suggesting that it is less efficient at stimulating dendritic cells (DC) to activate naive T cells, but can drive DC to activate memory T cells.

227 MOLECULAR SCREENING FOR SKIN SENSITISATION HAZARD *IN VITRO* USING PROTEOMICS TECHNIQUES.

M. Divkovic[2], D. A. Basketter[1], C. K. Pease[1], A. Dell[2] and H. R. Morris[2]. [1]*SEAC, Unilever, Sharnbrook, United Kingdom and* [2]*Biological Sciences, Imperial College, London, United Kingdom.*

Covalent binding of hapten to a skin protein is a key process required for sensitisation. It has long been postulated that only sensitising chemicals will modify skin protein(s), whereas non-sensitising compounds and irritants do not. To pinpoint the amino acid residues involved in such modifications, we have employed mass spectrometry (MS) based proteomics techniques. Known sensitisers, non-sensitisers and irritants were incubated with human serum albumin (HSA). Modified HSA samples were digested by trypsin and matrix assisted laser desorption/ionisation (MALDI-MS) analyses were conducted on tryptic digests. Nano-electrospray tandem mass spectrometry (ES-MS/MS) analyses were conducted on modified peptides purified by high performance liquid chromatography (HPLC). The HSA sequence was mapped and modified peptides were identified. In HSA samples incubated with non-sensitisers and irritants there was no change compared to HSA incubated without chemical. Modified peptides were purified by HPLC and sequenced using ES-MS/MS. Lysine, histidine and cysteine were found to be involved in covalent modifications by the sensitising chemicals used. A complex adduct of one sensitiser (methylchloroisothiazolinone, courtesy of Prof. J.P.Lepoittevin, University Louis Pasteur, Strasbourg, France) on His 338 of the HSA molecule was also identified. Many more sensitisers need to be investigated in this way to establish similarities between covalent protein modifications of chemically related compounds. These results can be further employed to develop a simple, medium- throughput assay for covalent protein binding ability of chemicals. This could form part of a battery of tests needed to completely replace animal testing of new chemical entities for skin sensitisation potential.

228 AN INTER-LABORATORY STUDY FOR THE DEVELOPMENT OF AN *IN VITRO* SKIN SENSITIZATION TEST USING HUMAN CELL LINES.

Y. Yoshida[1], T. Ashikaga[2], H. Sakaguchi[1], M. Miyazawa[1], M. Hirota[2], M. Ogo[2], H. Itagaki[2] and H. Suzuki[1]. [1]*Kao Corporation, Haga, Tochigi, Japan and* [2]*Shiseido Corporation, Yokohama, Kanagawa, Japan.* Sponsor: J. Avalos.

In regard to *in vitro* skin sensitization tests, dendritic cells (DCs) derived from human peripheral blood have been considered in the development of new tests. Meanwhile, some methods using human cell lines have been reported recently. In our previous study, we suggested that measuring CD86 and/or CD54 expression on THP-1 cells (monocytic leukemia cell line) could be used as an *in vitro* skin sensitization method. An inter-laboratory study among Kao and Shiseido was undertaken in Japan in order to further develop our *in vitro* skin sensitization model. In the present study, we used two human cell lines: THP-1 and University-937 (histiocytic lymphoma cell line). We measured the expression of CD86 and CD54 on the above cells using flow cytometry after a 24-hour and 48-hour exposure to known allergens (e.g., DNCB, PPD, $NiSO_4$) and non-allergens (e.g., SLS, Triton X-100). Both facilities used same test protocol, treatment concentration and clone of anti-CD86 and CD54 mAb for the study. For the sample test concentration, 4 doses (2x, 1x, 0.5x and 0.1x of the 50% inhibitory concentration (IC50)) were evaluated. IC50 was calculated using MTT assay. We found that allergens/non-allergens were better predicted using THP-1 cells compared to University-937 cells following a 24-hour exposure, but for a 48-hour exposure, there was no significant difference. We also found that the 48-hour treatment time had a better accuracy than the 24-hour treatment time for both cell lines. Expression of CD86 and CD54 were good predictive markers for THP-1 cells, but for University-937 cells, expression of CD86 was a better predictor than CD54, especially at the 48-hour treatment time. The accuracy also improved when both markers (CD86 and CD54) were used as compared with a single marker, especially with THP-1 cells. These results suggest that our method, using human cell lines THP-1 and University-937, but especially THP-1 cells, may be a useful *in vitro* skin sensitization model to predict various contact sensitizers.

229 DEVELOPMENT OF AN *IN VITRO* SKIN SENSITIZATION TEST USING HUMAN CELL LINES.

T. Ashikaga[1], Y. Yoshida[2], M. Hirota[1], M. Ogo[1], H. Sakaguchi[2], M. Miyazawa[2], H. Suzuki[2] and H. Itagaki[1]. [1]*Shiseido Corporation, Yokohama, Kanagawa, Japan and* [2]*Kao Corporation, Haga, Tochigi, Japan.* Sponsor: J. Avalos.

Several studies have shown that allergens enhance CD86 expression on surface of dendritic cells (DCs) and it has been thought that this phenotypic change could be applied to the development of an *in vitro* skin sensitization test. In our previous study, we found that CD86 and/or CD54 expression on THP-1 cells (monocytic leukemia cell line) was enhanced after a 24-h exposure to allergens but not non-allergens. We suggest that THP-1 cells could be used for an *in vitro* skin sensitization test as a replacement of DCs. However, much work remains to be done in order to make the cell substitution suitable for practical use. In this report, we examined various test conditions to optimize a protocol to be used in an inter-laboratory study between Kao and Shiseido. At first, effect of subculturing time on the CD86 expression was studied. THP-1 cells were incubated for 24, 48 and 72 hours, respectively. After the incubation, the cells were transferred to fresh medium, and then the cells were exposed to DNCB for 24 hours. The expression of CD86 on DNCB-treated THP-1 cells tended to be high in the 48-hour and 72-hour subculturing time. The augmentation of CD86 expression has been often observed when cells were treated with a subtoxic dose of allergen. To determine dose setting, the cytotoxicity of the test sample was performed (MTT assay) using THP-1 cells and University-937 cells (histiocytic lymphoma cell line) and 50% inhibitory concentration (IC50) of each test samples was calculated. Furthermore, antibodies were diluted to several concentrations and cells treated with allergens/non-allergens (e.g., DNCB, NiSO4 / SLS) were stained in order to identify the appropriate level of antibody to use. The staining studies confirmed that the working dilutions of both CD86 and CD54 antibodies were appropriate for use in our method. Based on our findings, the experimental conditions to be used in a Kao-Shiseido inter-laboratory study were identified and the protocol for that study was optimized.

230 MECHANISMS OF CCR7 UP-REGULATION BY NISO4 ON HUMAN DENDRITIC CELLS.

F. Boisleve and M. Pallardy. *Faculty of Pharmacy, INSERM U461, Chatenay-Malabry, France.*

Despite progress in elucidating mechanisms of contact hypersensitivity induced by chemical haptens, the first stages including activation of the dendritic cells (DC) are still unknown. Haptens, like Nickel, penetrate into the organism and are taken up by immature DC and then presented to naive T-lymphocytes (LT) thus eliciting hypersensitivity reactions. Activation of DC by haptens induces the maturation of DC. During this process, DC migrates to lymph nodes to activate LT. Migration of DC involves many factors including the expression of the chemokine receptor CCR7. In this study, we tested the effects of nickel sulfate (NiSO4) on CCR7 expression on immature DC and investigated the signalling pathways involved in its expression. Immature DC were differentiated from cord blood CD34+ cells. Treatment of DC by NiSO4 (500 μM) during 24 hrs induced the maturation of these cells as assessed by the expression of CD83 and CD86. NiSO4 also elicited the migration of these cells in response to CCL19 through induction of CCR7 on their surface. To establish the mechanisms involved in the expression of CCR7, we examined the activation of the Mitogen-activated protein kinases (MAPKs), p38MAPK and c-jun N-terminal Kinase (JNK), and the activation of the Nuclear Factor (NF)-κB in NiSO4 stimulated-DC. In our model, stimulation of immature DC with NiSO4 induced phosphorylation of p38MAPK and JNK and also the activation of NF-κB. To evaluate the role of these transduction pathways in the expression of CCR7, we used specific inhibitors for each pathways. Cells pretreated with SB203580, a specific inhibitor of p38MAPK or parthenolide, an inhibitor of NF-κB activation, showed a down-regulation of CD83 and CCR7 expression induced by NiSO4. For the first time, the role of JNK in the maturation of DC was also demonstrated since CD83 and CCR7 expression were inhibited by SP600125, a specific inhibitor of JNK. These results suggested the predominant roles of p38MAPK, JNK and NF-κB pathways in the expression of CCR7 induced by the contact sensitizers.

231 STUDIES ON THE RESPIRATORY IMMUNE RESPONSE TO A PROTEASE AND IMPLICATIONS FOR THE SAFETY ASSESSMENT OF ENZYME-CONTAINING PERSONAL CARE PRODUCTS.

E. S. Finn[1], S. P. Chapoval[3], A. Xue[2], V. Chowdhary[2], L. C. Limardi[1], A. C. Pursifull[1], E. V. Marietta[2], T. A. Gaffey[2], B. Kirchner[1], K. Sarlo[1], C. S. David[2] and D. N. Rubingh[1]. *[1]Procter & Gamble Co., Cincinnati, OH, [2]Mayo Clinic, Rochester, MN and [3]Yale University, New Haven, CT.*

Microbial enzymes have been safely used in detergents for over 30 years. Their use is expanding in new applications such as personal care products resulting in the potential for development of IgE-mediated allergy. Understanding the immune response to enzymes is a key step in the safety assessment of new enzyme-containing applications. Changing the enzyme so that it is less likely to induce an allergic response is one approach to address safety of these new applications. Humans and HLA Class II transgenic (tg) mice were evaluated for Ab and T cell responses and genetic susceptibility to the protease Y217L BPN. MHC Class II alleles were evaluated in occupationally exposed workers using SSP-PCR. A number of alleles were found among workers with Ag-specific IgE: DQ8 was statistically associated with development of IgE (susceptible) and DQ6/DR15 was associated with absence of IgE (protection). T cell epitope mapping studies in humans identified several epitopes with patterns of epitopes varying among individuals. Immunization of HLA-DQ6, DQ8, DR3 and DR4 tg mice showed that DQ8 mice were susceptible to development of Y217L BPN hypersensitivity based on development of Ag-specific IgG1/IgE and lung eosinophilia. DQ6 and DR3 mice developed an Ab response but required additional immunizations over a longer period of time. T cell epitope mapping studies in DQ8 and DQ6 mice identified several T cell epitopes many of which were similar between the two strains. Some of these epitopes were similar to epitopes recognized by humans. In summary, studies in humans and tg mice show the allergic response to the protease Y217L BPN is complex and diverse. This increases the complexity of safety programs needed to assure that native and modified enzymes are safe for use in personal care products. These safety programs must include exposure assessment and robust clinical testing.

232 ASSESSMENT OF IMMUNE RESPONSES TO *PENICILLIUM CHRYSOGENUM* AND CHARACTERIZATION OF ITS ALLERGENS.

Y. Chung[1], M. E. Viana[2], L. B. Copeland[3], M. K. Selgrade[3] and M. D. Ward[3]. *[1]ESE, UNC/USEPA, Chapel Hill, NC, [2]CVM, NCSU, Raleigh, NC and [3]USEPA, Research Triangle Park, NC.*

Molds have been associated with allergic disease but have not been studied extensively despite their widespread presence in the indoor environment. *P. chrysogenum* has been associated with both water damaged buildings and sick building syndrome. Our objectives in this study were to assess immune responses to *P. chrysogenum* and to identify allergenic (IgE inducing) proteins of the fungus. BALB/c mice were exposed four times over a four-week period by involuntary aspiration (IA) to 1, 10 or 100 μg of *P. chrysogenum* extract (PCE) or Hank's balanced salt solution as vehicle control. Positive control mice were exposed to 10 μg of *Metarhizium anisopliae* extract (MACA). Serum and bronchoalveolar lavage fluid (BALF) were collected before (D0), at 1 (D1) and 3 (D3) days following the final IA exposure. Only mice exposed to 100 μg of PCE showed responses different from vehicle control, but some of these mice were moribund. The mice exposed to PCE (100 μg) demonstrated significant increases in BALF levels of total protein, lactate dehydrogenase (LDH), total cell, macrophages, neutrophils, lymphocytes, and eosinophils at D0, D1 and D3, compared to vehicle control. Mice exposed once to 100 μg of PCE (inflammatory control) showed significantly increased levels of total protein, LDH and neutrophils in BALF at D1 and D3. Four exposures to PCE (100 μg) also resulted in significantly increased IgE in both serum and BALF compared to vehicle and inflammatory control. Eosinophils in BALF of mice exposed to 100 μg of PCE was lower than that of mice exposed to 10 μg of MACA but higher than the vehicle or inflammatory control. Western blot analysis showed 8 IgE inducing proteins in PCE, ranging from approximately 28 to 65.5 kD. Current data indicate that although PCE may induce allergic responses with less potency than MACA, it does contain IgE-inducing proteins. (Supported by UNC/EPA Cooperative Training Agreement CT826513 and this abstract does not reflect EPA policy.)

233 THE IDENTIFICATION AND CHARACTERIZATION OF AN IGE-INDUCING PROTEIN IN METARHIZIUM ANISOPLIAE EXTRACT.

M. Ward[1], L. B. Copeland[1], M. J. Donohue[3] and J. A. Shoemaker[2]. *[1]NHEERL, USEPA, Research Triangle Park, NC, [2]NERL, USEPA, Cincinnati, OH and [3]Oakridge Institute for Science and Education, Cincinnati, OH.*

BALB/c mice exposed by involuntary aspiration to Metarhizium anisopliae extract (MACA), a microbial pesticide, have shown responses characteristic of human allergic lung disease. The current study was undertaken to identify and characterize fungal allergens from MACA. We have identified 4 protein bands on SDS-PAGE Western blots that bind IgE in hyperimmune serum. These proteins have an apparent molecular weight of 127.6 kDa, 114.7 kDa, 89.7 kDa, and 52.6 kDa, respectively. Immunoblots of 2-dimensional (2-D) gels shows 8 protein spots with acidic pIs ranging between pH 4.8 and pH 5.5. These protein spots were collected following alignment of 2-D gel Western blot autoradiographs with Coomassie blue stained 2-D gels. Subsequently, matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionization mass spectrometry (ESI-MS/MS) were used to analyze these isolated fungal peptides and proteins. The MS data on molecular weight, peptide profiles, and amino acid sequence is currently being used to mine databases for potential sequence or domain homology to known allergens using the MASCOT database-mining algorithm. Identification, characterization, and purification of the IgE-inducing proteins in this fungal extract will provide a tool for assessing relative potency by comparison to better characterized allergens such as house dust mite. These newly characterized fungal proteins will be added to the database of allergens, which collectively can be used to identify other structures as potential allergens. (This abstract does not reflect EPA policy.)

234 TOPICAL SENSITIZATION AND INTRANASAL CHALLENGE TO TRIMELLITIC ANHYDRIDE INDUCES AN ALLERGIC RHINITIS SIMILAR TO THAT INDUCED BY INTRANASAL SENSITIZATION AND CHALLENGE IN A/J MICE.

A. K. Farraj[1, 2], J. R. Harkema[2] and N. E. Kaminski[1]. *[1]Pharmacology and Toxicology, Michigan State University, East Lansing, MI and [2]Pathology and Diagnostic Investigation, Michigan State University, East Lansing, MI.*

Allergic airway diseases induced by low molecular weight (LMW) chemicals, including trimellitic anhydride (TMA), are mediated principally by Th2 cytokines and characterized by airway mucus hypersecretion and an influx of eosinophils and lymphocytes. The most common route of human exposure to LMW chemical respiratory allergens is inhalation. In a previous study, we demonstrated that intranasal sensitization and challenge of A/J mice with TMA induced the characteristic immunologic and pathologic responses of LMW chemical-induced allergic rhinitis. However, in many murine models of human LMW chemical-induced airway allergy, the mouse is sensitized to the chemical by topical exposure. In the present study, we hypothesized that mice topically or intranasally sensitized to TMA would develop similar immunologic and pathologic responses in their nasal airways after intranasal challenge to this LMW chemical. A/J mice were topically or intranasally sensitized and then intranasally challenged with TMA in an ethyl acetate/olive oil vehicle. Nasal and pulmonary airways were processed for light microscopic examination. Total serum IgE was measured using an ELISA. Intranasal challenge with TMA in mice that were either topically or intranasally sensitized with TMA caused a marked allergic rhinitis characterized by an influx of eosinophils, lymphocytes and plasma cells. Both topical and intranasal sensitization also induced increases in total serum IgE compared to their corresponding vehicle controls after intranasal challenge. The data suggest that topical application is effective in sensitizing mice to TMA and induces a nasal airway lesion after intranasal challenge that is qualitatively similar to intranasal sensitization and challenge. Skin exposure may be a potential route of sensitization of the respiratory tract to LMW chemicals (funded in part by American Chemistry Council #0051).

235 PERSISTENT SPECIFIC AIRWAY RESPONSIVENESS IN RATS SENSITIZED TO AND CHALLENGED WITH TRIMELLITIC ANHYDRIDE (TMA).

P. D. Siegel, X. Zhang and D. M. Lewis. *HELD/ASB, NIOSH/CDC, Morgantown, WV.*

TMA is an organic acid anhydride that is widely used in industry. It can induce specific IgE and cause occupational asthma in sensitized individuals. Persistent occupational asthma, even with antigen/hapten avoidance can be seen is approximately 20% of the cases. Duration of both circulating TMA-specific IgE and specific airway responsiveness was studied in a TMA sensitized Brown Norway rat asthma model as possible contributory factors to persistent asthma. TMA powder (4 mg) was applied to 4 anesthetized rats' backs (clipped with scissors) on days 0, 7, 14 and 21 and washed off after 4 hrs. Specific IgE analyses and airway challenge with TMA (40 mg/m^3 for 10 min) were performed on day 35 and day 386. The ELISA OD values for TMA-specific IgE were 0.46±0.07 and 0.12±0.02 on day 35 and day 386, respectively (non-specific binding from control sera OD=0.08±0.002). Dual, early- (EAR) and late-phase (LAR) airway responses (indicated by enhanced pause, an index of airway resistance) were observed on day 35. A dual response was noted upon rechallenge on day 386 in 3 rats and LAR-only in the 4th rat. These results demonstrate the persistence of specific airway responsiveness to TMA, even though circulating specific-IgE had drastically declined.

236 CROSS-REACTIVITY OF ACID ANHYDRIDES ASSESSED BY AIRWAY CHALLENGE IN RATS SENSITIZED WITH TRIMELLITIC ANHYDRIDE (TMA).

X. Zhang, J. S. Fedan, D. M. Lewis and P. D. Siegel. *HELD, NIOSH/CDC, Morgantown, WV.*

Previous studies using inhaled organic acid anhydride (OAA)-protein conjugates in studies of airway hyperreactivity have revealed immunologic cross-reactivity among OAAs. The present investigation evaluated cross reactivity between phthalic anhydride, (PA), tetrachlorophthalic anhydride (TCPA) and TMA with respect to their ability to induce asthma-like airway responses in TMA-hapten-sensitized Brown Norway rats. Four to 40 mg of dry TMA powder was applied to anesthetized rats' backs (hair removed with scissors) on days 0, 7, 14 and 21 and washed off with water 4 hours after each application. Inhalation challenge with TMA aerosol (40 mg/m^3;10 min) was performed on day 35 and Penh, an index of airway resistance, was measured. The sensitized rats developed early(EAR)- and late(LAR)-phase obstructive responses. One week after TMA inhalation exposure, rats were challenged with TMA, PA or TCPA aerosol (40mg/m^3, 10 min; n = 8 in each group). EAR developed in all rats challenged with TMA, 3 rats challenged with PA, and 7 rats challenged with TCPA. The LAR developed in all TMA-challenged, sensitized rats, but none of the TMA sensitized rats challenged with PA or TCPA developed the LAR. We have previously reported that low dose TMA airway challenge results in EAR-only similar to the airway responses observed in the present cross-reactivity studies. It is concluded that cross-reactivity exists between TMA and both PA and TCPA. These airway responses are most likely immune mediated and the reason why this cross-reactivity was manifested only by EAR may be related to lower relative antigenic potency of PA and TCPA to that of TMA in TMA-sensitized rats.

237 INHALATION EXPOSURE OF TRIMELLITIC ANHYDRIDE (TMA) AEROSOL IN A BROWN NORWAY RAT MODEL.

D. M. Lewis, X. Zhang and P. D. Siegel. *HELD, NIOSH/CDC, Morgantown, WV.*

Inhalation is one of the TMA exposure routes for sensitization and subsequent asthma. The present work reported some characteristics after inhalation exposure of TMA aerosol. Brown Norway rats (n=8/group) were exposed, once a week from day 0 to day 63 with 40 mg/m^3 TMA or to day 70 with 4 mg/m^3 TMA in a nose-only system for 10 min. Enhanced pause (Penh, an index of airway resistance) was recorded in Buxco chambers overnight (⟩12 hours). Sera were collected weekly for antibody analyses. For the group with 40mg/m^3 of TMA, specific IgE level was significantly increased after day 7 and reached the peak by day 49. By day 14 these rats developed early- (EAR) and late-phase airway responses (LAR) following exposure. For the group exposed to 4mg/m^3 of TMA, specific IgE level was significantly increased by day 21 and reached the peak by day 56; however, no obviously airway responses were noted. Two weeks after the final exposure the 4 mg/m^3 TMA exposed rats were challenged with 40 mg/m^3 TMA resulting in both an EAR and LAR response. The specific IgE levels for rats with high dose of TMA were greater than that with low dose of TMA. We conclude that high dose and short term inhalation exposure may induce specific IgE and airway responses; low dose and short term exposure can induce specific IgE production to establish sensitization, while the development of airway responses needs high dose of TMA for challenge.

238 INTERSPECIES VARIATION AND LINEAGE SPECIFICITY IN HEMATOPOIETIC TOXICITY TESTING.

C. Pereira, J. Damen and E. Clarke. *Contract Assay, StemCell Technologies Inc., Vancouver, BC, Canada.* Sponsor: M. Ehrich.

In the search for efficient and cost effective ways to screen lead compounds for hematotoxicity, the use of Colony Forming Cell (CFC) assays has received a great deal of attention. These standardized assays allow the detection of toxicity on hematopoietic progenitor subsets (erythroid, myeloid, megakaryocytic) to evaluate potential cytopenic conditions, as well as mesenchymal progenitors to evaluate potential damage to the bone and connective tissue. As these assays require the growth of primary cells in culture for up to 14 days, they offer a high level of sensitivity and allow the quantitative and qualitative evaluation of colony growth and morphology, providing high functional content. Using CFC assays, the toxic effects of three antineoplastic compounds were tested on erythroid (CFU-E, BFU-E), myeloid (CFU-GM) and mesenchymal (CFU-F) progenitor growth. 5-Fluorouracil (5-FU), Hydroxyurea (HU) and Paclitaxel were incubated in culture with both human and murine bone marrow progenitor cells for 10-14 days, after which colonies were scored and assessed. Results indicate that each compound displays a unique spectrum of toxicity on each progenitor lineage; erythroid, myeloid and mesenchymal lineages showing different relative susceptibility to toxicity depending on the compound tested. In addition, up to 10-fold differences were seen between human and murine progenitor sensitivity to each compound. Our data highlights the multifaceted nature of bone marrow and unique specificity of action of individual compounds. The results also illustrate the importance of using the most appropriate species model in preclinical studies to improve predictivity and clinical success.

239 A HIGH THROUGHPUT CELL-BASED ASSAY FOR ASSESSMENT OF HEPATOTOXICITY USING CRYOPRESERVED HUMAN HEPATOCYTES.

M. Chen, M. Hann and K. Zhang. *Pharmacology, Novartis Institute for Functional Genomics, San Diego, CA.*

Screening for hepatotoxicity during early stage of drug discovery may add another important tool, among others, to improve the quality of drug candidates advancing into lead optimization. We report here a high throughput cell-based assay (384-well plate) capable of detecting acute hepatotoxicity. Test compounds were exposed to cryopresevered primary human hepatocytes for 18 hours and cytotoxicity was measured using a luciferin/luciferase assay that quantifies cellular ATP levels. In a proof of concept study, we evaluated 30 reference compounds with known hepatotoxicity along with 8 non-hepatotoxicants using human hepatocytes obtained from three donors. Dose-dependent cytotoxicity was observed for 23 of the potential hepatotoxicants, such as aflatoxin B, tamoxifen, troglitazone, ketoconazole and flutamide, with measurable TC50 values (<115 uM) within the test concentration range. No apparent cytotoxicity was observed for the other 6 compounds (TC50 >500 uM) including phenytoin, Carbamazapine and crotaline. However, all of these six except carbon tetrachloride were reported hepatotoxic as a result of combination therapy with other drugs. For the 8 non-hepatotoxicants tested (Buspirone, Theophyline and Ibuprofen etc), none of them showed cytotoxicity (TC50 >500 uM). This data indicates that our assay is reasonably predictive in detecting acute hepatotoxicity of drug candidates and can be employed in the high throughput mode during lead optimization process. It should also be of interest for retrospective mechanistic studies of heptotoxicity observed *in vivo*.

240 PHASE I AND II RESULTS OF A VALIDATION STUDY TO EVALUATE *IN VITRO* CYTOTOXICITY ASSAYS FOR ESTIMATING RODENT AND HUMAN ACUTE SYSTEMIC TOXICITY.

M. Paris[1,2], J. Strickland[1,2], W. Stokes[1], S. Casati[3], R. Tice[1,2], H. Raabe[4], C. Cao[5], R. Clothier[6], J. Harbell[4], G. Mun[4], A. Sizemore[4], G. Moyer[4], J. Madren-Whalley[5], C. Krishna[5], M. Owen[6], N. Bourne[6], J. Haseman[7], P. Crockett[8], M. Wenk[9], M. Vallant[7] and A. Worthj[3]. *[1]NICEATM, NIEHS, Research Triangle Park, NC, [2]ILS, Inc., Research Triangle Park, NC, [3]ECVAM, Ispra, Italy, [4]IIVS, Gaithersburg, MD, [5]US Army, Aberdeen Proving Ground, MD, [6]University of Nottingham, Nottingham, United Kingdom, [7]NIEHS, Research Triangle Park, NC, [8]ASI, Research Triangle Park, NC and [9]BioReliance, Rockville, MD.*

Studies have identified a correlation between *in vitro* cytotoxicity and acute oral toxicity. NICEATM and ECVAM initiated a three-phase multi-laboratory validation study to evaluate the usefulness of two standardized *in vitro* basal cytotoxicity assays for estimating rodent and human acute toxicity and the extent that they may reduce animal use. Seventy-two coded chemicals (12 from each of five acute oral hazard categories; 12 unclassified/nontoxic chemicals) will be tested in mouse 3T3 fibroblasts and in normal human epidermal keratinocytes (NHK) using neutral red (NR) uptake assays. Protocols were optimized after each of the first two phases to minimize intra- and inter-laboratory variation prior to testing 60 chemicals in Phase III. Phase Ia established the historical databases for the positive control chemical, sodium laurel sulfate (SLS), for each of three laboratories. Three chemicals were tested in Phase Ib and nine in Phase II. Technical challenges arose in Phases Ia/Ib (i.e., formation of NR dye crystals; uneven growth of NHKs; slow growth of 3T3 cells) that were resolved with Phase II protocols. Significant variation in NHK growth occurred in Phase II with various lots of media and supplements. The optimized final protocols are being tested in Phase III. Rodent oral LD50 values were estimated using prediction models based on the Registry of Cytotoxicity data and Phase I/II results. Human toxicity will be estimated using a prediction model based on data from human poisoning reports and the Multicentre Evaluation of *In Vitro* Cytotoxicity (MEIC). Supported by: N01-ES-35504, N01-ES-75408; EPA IAG DW-75-93893601-0; European Commission 19416-2002-04 F2ED ISP GB.

241 DATA COLLECTION AND ANALYSIS SYSTEMS FOR AN *IN VITRO* CYTOTOXICITY VALIDATION STUDY.

J. Strickland[1, 2], M. Paris[1, 2], H. Raabe[3], J. Haseman[4], S. Casati[5], R. Clothier[6], C. Cao[7], P. Crockett[8], R. Tice[1, 2] and R. Stokes[2]. *[1]ILS, Inc., Research Triangle Park, NC, [2]NICEATM, NIEHS, Research Triangle Park, NC, [3]IIVS, Gaithersburg, MD, [4]NIEHS, Research Triangle Park, NC, [5]ECVAM, Ispra, Italy, [6]University of Nottingham, Nottingham, United Kingdom, [7]US Army, Aberdeen Proving Ground, MD and [8]ACI, Research Triangle Park, NC.*

NICEATM and ECVAM designed a multi-laboratory validation study to evaluate two *in vitro* basal cytotoxicity test methods using 72 coded chemicals with a wide range of acute oral toxicity. The study was designed in three phases to allow adjustments in the cytotoxicity protocols and data collection and evaluation procedures before the majority of the chemicals are tested in Phase III. An Excel template was distributed to the participating labs for collection of raw data, analysis for outliers among dose replicates, documentation of materials and procedures, simple graphical analysis of dose-response, and transformation of data to the proper format for further analysis. Rather than applying a linear interpolation technique to the dose response to calculate the IC50, GraphPad Prism software was used to perform a Hill function analysis. IC20, IC50, and IC80 values and associated 95 percent confidence limits were calculated, and the data and fitted model were graphed. Initial criteria for an acceptable dose-response for individual tests included one data point between 10 & 50 percent *via*bility, one data point between 50 & 90 percent *via*bility, and r2>0.8. A Prism template was distributed to the laboratories to automate and provide uniformity of analysis. To increase the speed of data collection and evaluation of the analyses by the Study Management Team (SMT) and consulting biostatisticians, the laboratories submitted Excel and Prism data files by e-mail. Results compiled by the SMT were returned to the originating laboratories for audit to ensure accurate transmission of data. Implementation of these procedures shows that automated data collection in relatively common, easy-to-use electronic formats can facilitate uniformity of data collection and analysis. Supported by: N01-ES 35504; EPA IAG DW-75-93893601-0; European Commission 19416-2002-04 F2ED ISP GB.

242 MANAGING TOXIC SYNERGISM IN HYPOCHLORITE-CONTAINING CLEANERS USING THE BOVINE CORNEAL AND PERMEABILITY (BCOP) ASSAY, PART II.

J. E. Swanson[1], W. M. Rees[1], D. S. Hilgers[1], J. C. Merrill[2] and J. W. Harbell[2]. *[1]SC Johnson & Son, Inc., Racine, WI and [2]Institute for In Vitro Sciences, Inc., Gaithersburg, MD.*

Consumers continue to seek products that will both clean and combat mold and mildew stains on various surfaces in the home. Such products are commonly formulated as alkaline hypochlorite and surfactant-containing aqueous solutions and usually have significant ocular irritation potential. Previous studies have shown the applicability of the BCOP Assay to evaluate this class of cleaners (Rees et al., 2001). In this study, a standard cleaner containing 0.25% sodium hypochlorite, anionic surfactant and alkali (pH 13, 0.24% active chlorine content) was compared to a 2.5% stabilized hypochlorite cleaner containing the same surfactant (sodium N-chlorosulfamate salts, pH 5, 2.4% active chlorine content). A worst possible case human exposure time of 5 minutes was utilized in the assay. Treatment and scoring followed the standard BCOP protocol (Sina et al., 1995). However, longer post-exposure incubation times of 4 and 20 hours were found to be necessary for detecting the irritation potential of oxidizing/reactive chemistries in earlier work. Results show that the pH 5, 2.5% stabilized hypochlorite cleaner is much less irritating than the standard alkaline 0.25% sodium hypochlorite cleaner. The BCOP *In Vitro* Scores were 5.2 and 4.3 for the stabilized hypochlorite cleaner compared to 55.6 and 59.7 for the sodium hypochlorite cleaner with 4 and 20 hour exposure times, respectively. Histology confirmed these results. These data suggest that ocular irritation and tissue damage potential for active chlorine-containing cleaners may be markedly reduced when alternative chemical forms of hypochlorite are employed, relative to conventional sodium hypochlorite-based compositions.

243 *IN VITRO* CYTOTOXICITY TESTING WITH CULTURED IMMORTAL HUMAN COLON CELLS.

R. Konsoula and F. A. Barile. *Department of Pharmaceutical Sciences, St. John's University College of Pharmacy, Jamaica, NY.*

An *in vitro* study was conducted to test the toxicity of 20 representative chemicals on immortal human colon cells (Caco-2). The study aims to develop a cell culture model that compares gastrointestinal absorption (GIA) with cytotoxicity. The chemicals were selected from the guidance document prepared by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). Cultured Caco-2 cells have been traditionally used as an *in vitro* model of intestinal epithelium. Caco-2 cells were seeded in 96-well plates and cytotoxicity was determined using cell *via*bility assays (MTT and NRU). Confluent monolayers were exposed to increasing concentrations of chemicals for 24- and 72-hrs. In addition, cells were seeded on polycarbonate filter membranes (Millicell® culture plate inserts). After 12 to 14 days, the differentiated monolayers were exposed to increasing concentrations of the chemicals from the apical side for 24- and 72-hrs. Transepithelial electrical resistance (TEER) was used to monitor paracellular permeability in the inserts. Inhibitory concentrations 50% (IC50s) were extrapolated from concentration–effect curves after linear regression analysis. The data suggest that the *via*bility assays were more sensitive indicators of chemical exposure than TEER measurements. Comparison of the IC50s for the cytotoxicity data indicates that there was no significant difference between 24- and 72-hr exposures. In addition, the results from the MTT and NRU assays were statistically similar (paired students' t-test, P=0.05). Comparison of the IC50s in this model allows for the differentiation between the concentrations necessary for cytotoxicity and those needed to interfere with paracellular permeability. Development of an *in vitro* test system for GIA, in combination with acute toxicity, improves the predictive ability of *in vitro* acute cytotoxicity assays for *in vivo* lethality. (Supported by NIH/NIEHS/AREA R15 ES012170-01).

244 INDUCTION OF FIBROSIS BY BLEOMYCIN AND CARMUSTINE IN RAT LUNG SLICES.

H. P. Behrsing, K. Amin, C. Ip and C. A. Tyson. *Toxicology Laboratory, SRI International, Menlo Park, CA.*

At present, there are no generally accepted *in vitro* models for identifying and studying chronic effects of lung toxicants. Due to recent advances in slice culture techniques, precision-cut slices were evaluated as a potentially feasible option for this purpose. The slices were prepared from Fischer 344 rat lungs and placed on HATF-covered titanium inserts in a dynamic roller system. The incubations were conducted in supplemented M-199 medium for up to 28 days under a 5% CO_2/air atmosphere in the presence or absence of bleomycin and carmustine, agents known to produce pulmonary toxicity. Tissue *via*bility and functionality was assessed weekly by measuring LDH and alkaline phosphatase content and histological examination with H&E and Masson trichrome stains. Control slices showed intact, adequately preserved parenchymal architecture with high *via*bility and minimal or no fibrosis over the 28-day period. Exposure to bleomycin resulted in damage to tissue and loss of *via*bility reflected in decreased enzyme levels and histological changes. Fibrosis was evident at the highest concentration at Day 8 and additionally at lower concentrations at Days 16 and 28. The fibrosis was characterized by a predominantly focal pattern present at the slice margin and around the bronchioles. These lesions mimicked the subpleural and peribronchiolar pattern of fibrosis observed by other investigators in intact lungs after systemic administration of bleomycin. The toxicity was less dramatic with carmustine. Mild-to-moderate damage was seen in bronchiolar and alveolar epithelium. An interesting finding was the development of fibrosis at Day 28 which is consistent with the fact that carmustine causes delayed fibrosis clinically. These results show that precision-cut lung slices can be successfully cultured in the dynamic roller system for at least 28 days and serve as a candidate model *in vitro* system for studying lung toxicants. This work was supported by NCI grant CA09748.

245 INDUCTION OF A ZONE OF CELL DEATH IN MULTI-WELL PLATES BY REFEEDING.

H. A. Raabe, G. O. Moyer, G. C. Mun, A. M. Sizemore, J. W. Harbell and J. C. Merrill. *Institute for In Vitro Sciences, Inc., Gaithersburg, MD.*

Multi-well plates provide an efficient format for cell-based bioassays. As the number of wells increases per plate, the surface area and number of cells per well decreases. Consequently, the use of the small-well format can present some problems. We have observed cell cultures in 96-well plates where significant populations of cells begin to die shortly after refeeding the cell cultures (dumping the spent medium by inverting the plate and adding fresh medium). The zones or areas of cell death appear to occur within a predictable ring just inside of the periphery of the multi-well plate wells, coinciding with the formation of a meniscus formed by the residual medium in the well following removal of the spent culture medium. This effect appears in larger well formats (e.g., 24-well plates) but has more impact in smaller well formats where the ratio of wall circumference to cell area is greater. The impact of these effects tends to be more pronounced when cultures are refed at relatively low confluence, resulting in areas devoid of cells. The zone is also more evident with cell types where cell migration is limited, presumably since those cell types are less apt to migrate to establish new colonies in the deserted areas. Cells cultured in the presence or absence of serum show qualitatively similar results. When uniform, 30% confluent lawns of human keratinocytes in 96-well plates were refed, the cells within the zone rapidly lost the ability to take up neutral red and showed nuclear condensation. After 48 hours, the neutral red uptake (OD_{550}) was significantly reduced in the refed wells (0.830 ± 0.058, mean ± standard de*via*tion, n=6 assays) compared to wells that were not refed (1.066 ± 0.034 where $p \leq$

0.0001). These results were confirmed in 3T3 cells cultured in serum-containing medium. These observations show the impact of the medium change in the 96-well plate format, and suggest that protocols should be designed to minimize this cell loss.

246 ARE PRIMARY RAT HEPATOCYTE CULTURES APPROPRIATE FOR PREDICTIVE HEPATOTOXICITY TESTING OF PHARMACEUTICALS.

H. Powell, A. Quigley, J. Hopwood, J. Eakins and G. Kenna. *Safety Assessment, AstraZeneca, Macclesfield, Cheshire, United Kingdom.* Sponsor: T. Orton.

Liver toxicity is a significant cause of project attrition within the pharmaceutical industry and an important class of adverse drug reactions. This reflects an unmet need for approaches that aid selection of non-hepatotoxic compounds during drug discovery. Although cultured hepatocytes have been used extensively to explore mechanisms of toxicity, their predictive value is unclear. The aim of the present study was to investigate this issue. Hepatocytes from male Han Wistar rats (192-372g) were isolated by collagenase perfusion and cultured in collagen-coated 24-well plates in monolayer or sandwich (i.e. Matrigel™ overlay) configuration. The monolayers exhibited variable basal ATP levels and LDH leakage, and a progressive decrease in *via*bility in culture that were not observed for sandwich cultures. Treatment of monolayer cultures with 5 non-hepatotoxic compounds (streptomycin, physostigmine, metformin, acyclovir, *N*-acetyl-*m*-aminophenol [AMAP]) resulted in IC_{50} values for LDH leakage at 24 hr that exceeded 1.7 mM. More potent toxicity was observed with 9 hepatotoxic compounds (α-naphthylisocyanate, diclofenac, ibuprofen, chlorpromazine, amiodarone, tetracycline, methapyrilene, tacrine, gentamicin) but not with a further 8 hepatotoxins (acetaminophen [APAP], thioacetamide, valproate, methotrexate, phenobarbital, zidovudine, acetylsalicylate, vancomycin,). Similar, albeit more reproducible, results were obtained when sandwich cultures were exposed to 3 of the non-hepatotoxins (streptomycin, metformin, AMAP) and 7 of the hepatotoxins (diclofenac, chlorpromazine, methapyrilene, tacrine, APAP, valproate, methotrexate). The most striking feature of these results is the absence of false positives amongst the non-hepatotoxic compounds. The sensitivity for detection of hepatotoxins (9 of 17; 53%) was modest, although this could be related to our choice of compounds. Use of additional endpoints of toxicity may improve predictivity, as may further assay refinement (e.g. repeat dose studies in sandwich cultures).

247 HIGH-THROUGHPUT ASSAY FOR ASSESSING LIVER TOXICITY.

P. McGrath, C. Parng, C. Ton and C. Zhang. *Phylonix Pharmaceuticals, Inc., Cambridge, MA.* Sponsor: P. Mayeux.

The goal of this study was to develop a high-throughput whole animal bioassay to evaluate liver/gastrointestinal (GI) toxicity of potential therapeutics. We used the level of biotin as an *in vivo* toxicity marker. The sole known function of biotin in human cells is to act as cofactor of five biotin-conjugated carboxylases. These biotin-dependent carboxylases catalyze key reactions in gluconeogenesis, fatty acid metabolism, and amino acid catabolism. Decreased levels of these biotin-conjugated enzymes have been reported in infants with severe liver dysfunction and increased enzyme levels were observed in transgenic mice with massive liver enlargement. Using the well-characterized mechanism of the strong interaction of biotin with avidin conjugated with reporter enzymes, we measured enzyme levels indirectly based on the stochiometric relationship between the enzyme and biotin molecules throughout early liver/gut development. Since the zebrafish develops rapidly, and the liver is functional at 48 hpf (hours post-fertilization), a relative short experimental time is required to assess toxicity. This study demonstrated the utility of using zebrafish embryos to evaluate drug induced toxicity in a quantitative 96-well microplate format. Using soluble substrate and end product, we measured changes in total biotin content, which reflected carboxylase activity and other biotin-related functions. Next, we showed a clear dose response for several test drugs, and we correlated drug-induced changes in biotin content in zebrafish with liver damage in humans. We also demonstrated the pathology of liver disease such as fatty liver and the pharmacological response of CYP in zebrafish are comparable to those in human. Assessment of drug effects in a convenient vertebrate model, prior to proceeding to evaluation in a complex system, such as mouse, can potentially streamline drug development and dramatically reduce costs.

248 CHANGES IN ARGININE UPTAKE, GLUTATHIONE LEVELS, UREA AND NITRIC OXIDE SYNTHESES IN RAT LIVER SPHEROIDS AFTER EXPOSURE TO PROPRANOLOL.

M. Ma, J. Xu and W. Purcell. *Faculty of Applied Sciences, University of the West of England, Bristol, United Kingdom.* Sponsor: C. Atterwill.

Propranolol is an adrenergic β-blocker, used clinically in the treatment of cardiovascular disorders and hypertension. It can cause liver injury in patients and hepatic cytotoxicity *in vitro*. The mechanism of its hepatotoxicity is however unclear. This study examined the changes in arginine uptake, glutathione (GSH and GSSG) levels and urea and nitric oxide (NO) syntheses in rat liver spheroids after exposure to propranolol. Rat liver spheroids were prepared by a gyrotatory-mediated method in a 6-well plate format and exposed to propranolol at 62.5, 125, 250 and 500 μM respectively. The results showed that propranolol caused a concentration-dependent increase in arginine uptake ($p < 0.01$) and significantly increased NO synthesis ($p < 0.01$) and GSH levels ($p < 0.05$) at 250 μM but decreased both endpoints at 500 μM ($p < 0.01$). GSSG levels were similar across all concentrations of propranolol tested. Urea secretion was increased significantly ($p < 0.05$) at 125 μM of propranolol and decreased at 500 μM ($p < 0.01$) but no significant change was observed at the other concentrations tested. It is concluded that propranolol can cause extensive functional and biochemical changes in hepatocytes. The increase in urea secretion relates to the increase in arginine uptake. The increases in NO synthesis and GSH levels after exposure to 250 μM propranolol represent cells actively responding to the stressful challenge. Beyond a certain point cells may be seriously injured or die as indicated by urea, NO synthesis and GSH levels, which significantly decreased after exposure to higher concentrations of propranolol. It suggests that the changes in NO synthesis and GSH levels after exposure to propranolol may reflect the severity of cell injury.

249 A TWO-STEP PROTOCOL TO DETERMINE LIVER SPHEROID CELL SPREADING INHIBITION CONCENTRATION (SCSIC) OF TOXICANTS.

J. Xu and W. M. Purcell. *Faculty of Applied Sciences, University of the West of England, Bristol, United Kingdom.* Sponsor: C. Atterwill.

Liver spheroid culture has been shown to be a valuable *in vitro* model in toxicology studies. An hepatocyte is a complex unit responding to challenges induced by chemicals and functional changes are dependent upon the nature of the toxicity induced. To elucidate the concentration dependent relationship between a challenge and a particular functional change, a suitable range is essential. Therefore, a reference concentration for deciding the 'dosing regimen' for *in vitro* studies is needed. We previously developed a new method, the Spheroid Cell Spreading Inhibition Test (SCSIT), to determine the minimum concentration causing Spheroid Cell Spreading Inhibition (SCSIC). SCSIC can be used as a reference concentration for subsequent experiments. This study further refined the protocol for SCSIT to determine the SCSIC of a toxicant more precisely by a two-step protocol. Rat liver spheroids were prepared from a Sprague rat liver by a gyrotatory spheroid culture method. Three hepatotoxicants, chlorpromazine, (2-chloro-10-[3-dimethylaminopropyl]phenothiazine), 2-propylpentanoic acid (valproic acid) and ethanol, which represent different levels of relative toxicity were selected. SCSIT was performed in a 24-well plate format. Five spheroids were used for each concentration. The first step covered a wide concentration range from 1 μM to 1 M (1, 25, 50, 100, 500 and 1000 μM, and 10, 50, 100, 500 and 1000 mM). The second step was based on the results of the SCSIC from the first step. The minimum SCSIC of each test compound from the first step was further diluted down to the highest SCSIT negative concentration. The results from the first step showed that the SCSIC for chlorpromazine was at 50 μM, for 2-Propylpentanoic acid at 50 mM and for ethanol at 1000 mM. The more precise results were obtained from the second step in that the SCSIC for chlorpromazine was at 30 μM, for 2-Propylpentanoic acid at 8 mM and ethanol at 600 mM. It is concluded that the two step protocol for SCSIT greatly improved the precision in determining the SCSIC of a test compound.

250 EVALUATION OF LIVER SPECIFIC FUNCTIONS AS HEPATOCYTOTOXIC ENDPOINTS USING A LIVER SPHEROID MODEL.

W. M. Purcell and J. Xu. *Faculty of Applied Sciences, University of the West of England, Bristol, United Kingdom.* Sponsor: C. Atterwill.

Liver specific functions such as albumin and urea syntheses, glucose secretion and galactose uptake are important in normal physiology. These functions reflect the status of hepatocytes in terms of protein synthesis, amino acid and energy metabolism and their changes can be easily detected in the blood. Therefore, they are ideal parameters to measure to assess liver functionality. How these functions respond to acute exposure to toxicants has not been evaluated in detail before. Previous studies have shown that mature spheroids (6 days) perform these specific functions much better and are more stable than immature ones (1-5 days). This study using the rat liver spheroid model investigated the changes of liver specific functions in the acute phase after exposure to model hepatotoxicants, diclofenac (100, 500 and 1000 μM), galactosamine (5, 10 and 20 mM), isoniazid (5, 10 and 20 mM) and paracetamol (1, 5 and 25 mM). The results show that diclofenac, isoniazid and paracetamol significantly ($p < 0.01$) decreased albumin secretion, whereas galactosamine significantly ($p < 0.05$) increased albumin release at 5 mM and caused no significant change at other concentrations tested. The test toxicants significantly ($p <$

0.01) reduced galactose uptake except for isoniazid, which did not cause any significant detectable effect on galactose uptake. The changes in urea secretion after exposure to the four test toxicants were unpredictable. Diclofenac (1000 μM) and paracetamol (25 mM) significantly ($p < 0.01$) increased urea secretion, galactosamine (10 mM) significantly ($p < 0.05$) decreased secretion and isoniazid had no apparent effect on urea secretion. Glucose secretion was significantly ($p < 0.01$) decreased in a concentration-dependent manner after exposure to each of the four toxicants. It is concluded that the test toxicants variously affected the different functional endpoints in liver spheroids with glucose secretion being the most sensitive indicator.

251 *IN VITRO* ANALYSIS OF MULTIPARAMETRIC CYTOTOXICITY AT THE INDIVIDUAL CELL LEVEL.

V. Abraham, B. Samson, O. Lapets and J. Haskins. *Cellomics, Inc., Pittsburgh, PA.* Sponsor: E. McGuire.

We have developed an automated image analysis algorithm for automated classification of individual cells *in vitro* according to their responses across multiple indicators of cytotoxicity. Measurements of each indicator are automatically computed in individual cells *via* analysis of high-resolution digital images captured using automated fluorescence imaging microscopy. Classification is achieved through defining 'Events' by combining individual indicator measurements with standard Boolean operators in a manner that is biologically meaningful. This functionality may be used to rank toxic effects and determine mechanism of toxicity (e.g. differentiating apoptosis from necrosis). Two sets of experiments were performed to validate this approach. In the first, the dose-dependent effects of valinomycin (K+ ionophore) and CCCP (protonophore) on HepG2 cells were evaluated. Cellular targets evaluated were mitochondrial transmembrane potential, membrane permeability and nuclear morphology. Events were defined to classify cellular responses according to acuteness of toxicity. This approach allowed analysis of dose-dependent effects across multiple relevant parameters and rapid comparison of multiparametric toxicity of valinomycin and CCCP on a molar basis. Secondly, we implemented an assay for phosphatidylserine (PS) translocation to detect apoptosis in Jurkat cells treated with camptothecin. Changes in nuclear morphology (indicative of late stage apoptosis) and onset of membrane permeability (indicating necrosis) were also monitored and incorporated in Event definitions. Events were defined to detect the frequency of occurrence of apoptosis in the absence of necrosis at the individual cell level. These results demonstrate the applicability this approach for rapid, automated analysis of individual cellular cytotoxicity profiles. In addition, this approach is particularly valuable in providing a method of data reduction for high-content cellular investigations that typically generate greater than 10^5 individual relevant cellular measurements per 96-well microplate.

252 ALLERGEN-INDUCED CHANGES IN CYTOKINE EXPRESSION BY CULTURED DENDRITIC CELLS: RELATIONSHIP WITH CYTOTOXICITY.

C. J. Betts[1], M. Cumberbatch[1], B. Hulette[2], G. Gerberick[2], C. A. Ryan[2], R. J. Dearman[1] and I. Kimber[1]. [1]*Syngenta CTL, Macclesfield, Cheshire, United Kingdom and* [2]*Procter & Gamble, Cincinnati, OH.*

Epidermal Langerhans cells (LC) play pivotal roles in cutaneous immune responses, including skin sensitization. Analyses of these cells have been facilitated by the development of methods for the generation in culture of dendritic cells (DC) with an LC-like phenotype from murine bone marrow-derived precursors. Such cells may provide a basis for identifying alternative approaches for skin sensitization hazard identification. Bone marrow cells were isolated from the femurs and tibiae of female BALB/c strain mice and cultured in the presence of murine granulocyte/macrophage-colony stimulating factor over a period of 8 days. These cells acquired an immature DC-like phenotype with expression of low levels of MHC class II determinants and high levels of intracellular adhesion molecule 1 and CD11c, but were negative for CD80 and CD86. We have analyzed the cytokine secretion profiles during 24 hr culture with the water-soluble allergen dinitrobenzene sulfonic acid (DNBS). Control cells were cultured with medium alone, or with 1μg/ml bacterial lipopolysaccharide (LPS). DC cultured in the presence of medium alone secreted measurable levels of interleukin (IL)-1α, IL-1β, IL-6 and IL-12 (p40 subunit). IL-12 (p70) was not detectable. Culture in the presence of 0.5mM DNBS, a dose that did not impact on cell *via*bility, had relatively little impact on cytokine secretion, with modest increases only in IL-12 (p40 and p70). However, treatment with 1mM DNBS, a concentration that routinely caused a marked loss in cell *via*bility (0-20% *via*ble), resulted in increased production of IL-1α, IL-1β, IL-6 and both forms of IL-12. Culture of mouse DC with LPS (*via*bility greater than 90%) resulted in marked up-regulation of tumor necrosis factor α, IL-10 and IL-17 in addition to those cytokines mentioned above. It may prove that optimal allergen-induced cytokine secretion by cultured DC requires a second signal in the form of cellular trauma

253 HEAT SHOCK PROTEIN RESPONSES IN TETRAFLUOROETHYLCYSTEINE-INDUCED CYTOTOXICITY.

H. Ho[1], Y. Jia[1], Z. Hu[3], D. M. Hockenbery[4], N. Fausto[2], S. D. Nelson[1] and S. A. Bruschi[1]. [1]*Medicinal Chemistry, University of Washington, Seattle, WA,* [2]*Pathology, University of Washington, Seattle, WA,* [3]*Amgen Inc., Seattle, WA and* [4]*Fred Hutchinson Cancer Research Center, Seattle, WA.*

Tetrafluoroethylecysteine (TFEC), a metabolite of the industrial gas tetrafluoroethylene, initiates nephrotoxicity by the covalent modification of a relatively few mitochondrial proteins. The use of murine TAMH cell line faithfully reproduces TFEC bioactivation and renal cell injury with dose- and time-dependent inhibitions of both mitochondrial aconitase and α-ketoglutarate dehydrogenase. A cytotoxic dose of TFEC (e.g. 250 μM; 8 h) significantly elevated the levels of inducible HSP70 (HSP70i). Alterations to stress protein HSP60, mtHSP70 and HSP25-related levels were also observed following TFEC treatment. Prior heat shock treatment induced levels of HSP70i, HSP40 and HSP25 and displayed a cytoprotective effect on TFEC toxicity. However, heat shock cross-tolerance and cytoprotection was more effective with diquat or rotenone treatment in comparison to TFEC. Supporting earlier evidence of BAX translocation and cytochrome c release, we also observed that constitutive overexpression of BCL-xL suppressed HSP70i induction but failed to inhibit the induction of stress-responsive transcription factor, ATF3. This suggests that participation of BCL-xL in cytoprotection precedes proteotoxicity and some cellular stress responses like HSP70i upregulation, but not others like ATF3. Overall, the results implicate a role for heat shock proteins in the modulation of TFEC-induced cytotoxicity. (Supported by NIEHS Center Grant P30ES07033 and Pfizer Inc.)

254 APPLICATION OF QSARs TO EVALUATE THE CYTOTOXICITY OF p-BENZOQUINONES TO RAT PRIMARY HEPATOCYTES AND PC12 CELLS.

A. G. Siraki, T. S. Chan and P. J. O'Brien. *Pharmaceutical Sciences, University of Toronto, Toronto, ON, Canada.* Sponsor: J. Uetrecht.

Quinones are found ubiquitously, and are involved in homeostasis, anti-cancer treatment, but are environmental contaminants or reactive metabolites that can cause cytotoxicity and induce carcinogenesis. Consequently, a better understanding of the molecular characteristics of quinones that induce cytotoxicity is desirable. We have performed a comparative study, where we apply quantitative structure-activity relationships (QSARs) to evaluate the cytotoxic effects of p-benzoquinone (BQ) congeners in rat PC12 cells and primary rat hepatocytes. Hepatocytes and PC12 cells were cultured and maintained at 37 °C, 5% CO_2/Air. Cytotoxicity was determined by the MTT assay, and reactive oxygen species (ROS) formation was determined by 2′, 7′-dichlorofluorescein after treatment with BQ derivatives. We derived robust QSARs for cytotoxicity which were dependent on the solvent accessible surface area (SA) and the Hammett substituent constant (σ, correlates to electron affinity) of the BQ: log LD50(hepatocytes) = 0.0146(±0.00154)SAS - 0.562(±0.0461)σ + 0.694(±0.149). The PC12 equation was similar. The most cytotoxic compounds were the halogenated BQs and the least toxic were fully substituted alkyl BQs such as duroquinone and Coenzyme Q1. The relative order of BQ cytotoxicity in both cell types was similar, suggesting a similar mechanism of cytotoxic action. Since BQs are readily reduced by the two electron NADPH:Quinone Oxidoreductase (NQO), we evaluated cytotoxicity in NQO-inhibited (dicumarol treated) cells and derived a robust QSAR with only SA as a parameter. Finally, ROS formation was found to correlate with cytotoxicity, but BQ treatment in PC12 cells produced more ROS per dose than in hepatocytes. In conclusion, BQ cytotoxicity was dependent on SA and electrophilicity (σ). Second, BQ cytotoxicity with NQO-inhibition was dependent on SA suggesting that one-electron reductases have a structural requirement for reduction. Lastly, the greater amounts of ROS produced in PC12 cells than hepatocytes is likely a result of lower catalase levels compared to hepatocytes.

255 RELATIONSHIP BETWEEN CD86 EXPRESSION, CYTOTOXICITY AND EXPOSURE OF DENDRITIC CELLS TO CHEMICAL ALLERGEN.

B. Hulette[1], C. Ryan[1], L. Gildea[1], I. Kimber[2], R. Dearman[2] and F. Gerberick[1]. [1]*Procter & Gamble, Cincinnati, OH and* [2]*Syngenta Central Toxicology Laboratory, Macclesfield, Cheshire, United Kingdom.*

Human peripheral blood derived dendritic cells (DC) respond to a variety of chemical allergens by up-regulating expression of the co-stimulatory molecule CD86. It has been postulated that this measure might provide the basis for an *in vitro* alternative approach for the identification of skin sensitizing chemicals. We recently reported that DC, exposed in culture to the highest non-cytotoxic concentrations of various chemical allergens, displayed marginal up-regulation of membrane CD86

expression; the interpretation being that such changes were insufficiently sensitive for the purposes of hazard identification. For the work presented here, immature DC were derived from human monocytes and treated with the chemical allergens 2, 4-dinitrobenzenesulfonic acid, nickel sulfate, p-phenylenediamine, oxazalone, Bandrowski's base, hydroquinone and propyl gallate for 48 hrs at concentrations which induced both non-cytotoxic and slight to moderate cytotoxicity. For comparison, DC were treated with the irritants sodium dodecyl sulfate (SDS), benzoic acid (BA), and benzalkonium chloride (BZC) at concentrations resulting in comparable levels of cytotoxicity. CD86 expression, as measured by flow cytometry, was consistently up-regulated (ranging from 190-386 % control) on DC treated with concentrations of chemical allergens that induced 10-15% cytotoxicity. The irritants SDS, BA, and BZC did not induce up-regulation of CD86 expression (ranging from 87-100 % control) when tested at concentrations that induced similar levels of cytotoxicity. Our results confirm that chemical allergens up-regulate CD86 expression on blood-derived DC and illustrate further that up-regulation of CD86 surface marker expression is more robust when DC are treated with concentrations of chemical allergen that induce slight to moderate cytotoxicity. We conclude that an association between CD86 expression and cytotoxicity appears evident, but the mechanism underlying this phenomenon have yet to be determined but is under investigation.

256 COMPARATIVE TOXICITY OF DIFFERENT EMISSION PARTICLES IN MURINE PULMONARY EPITHELIAL CELLS AND MACROPHAGES.

T. Stevens[1], P. Singh[2], M. Daniels[2] and M. Gilmour[2]. *[1]Toxicology, UNC, Chapel Hill, NC and [2]NHEERL, USEPA, Research Triangle Park, NC.*

Epidemiological studies have shown a correlation between levels of particular matter (PM) and hospital admissions and mortality due to respiratory illness. The chemical components of PM responsible for these effects are not known. This study investigated the ability of diesel exhaust particles (DEP) and fine coal fly ash (CFA) from different sources to cause injury and activation of lung cells. Murine pulmonary macrophages and epithelial cell lines were exposed for 1, 4, or 24 hours with varying doses of NIST 2975, Japanese DEP, German DEP, W Kentucky fine CFA, or Montana fine CFA (10 μg/ml, 50 μg/ml, or 150 μg/ml) as well as vehicle control and endotoxin (0.2 μg/ml, 1 μg/ml, or 5 μg/ml). After exposure, biochemical markers of cytotoxicity, lactate dehydrogenase (LDH) and ATP production, and pro-inflammatory cytokines (TNF-α, MIP-2, IL-6, and IL-1β) were analyzed. Overall, the DEPs caused more cytotoxicity than the CFA samples, and macrophages were more sensitive than epithelial cells. ATP effects were stronger and seen at earlier time points demonstrating this assay to be more sensitive than LDH. No differences in cytokine levels were observed in the epithelial cells of any treatment group. Each cytokine was greatly increased in LPS-exposed macrophages but showed variable patterns with the PM exposure treatments across time and concentration. While some increases were noted, there was a general decrease in cytokine output as exposure concentrations increased which may be related to either the decrease in ATP levels, or binding of cytokines by the particulates. We conclude that measurement of ATP levels in the murine macrophage cell line was the most sensitive endpoint of PM effects and that DEP samples are more potent than CFAs. Measurement of cell products such as cytokines may be confounded by changes in metabolic activity or physical interaction with the materials under study. (Supported by NIEHS T32ESO7126-21A, this abstract does not reflect EPA policy.)

257 PRESENCE OF TIGHT AND ADHERENS JUNCTION PROTEINS IN AN IMMORTALIZED Z310 CHOROID PLEXUS CELL LINE.

W. Zheng[1], L. Shi[1], J. Li[1], J. Szmydynger-Chodobska[2] and A. Chodobski[2]. *[1]School of Health Sciences, Purdue University, West Lafayette, IN and [2]Clinical Neurosciences, Brown University, Providence, RI.*

The blood-CSF barrier (BCB), located mainly in the epithelium of the choroid plexus, has been demonstrated to play an important role in brain development, maturation, aging, endocrine regulation, and pathogenesis of certain neurodegenerative diseases. To facilitate the *in vitro* investigation of material transport on the BCB, we have recently developed an immortalized Z310 murine choroidal epithelial cell line (Zheng and Zhao, Brain Res, 958:371, 2002). This study further characterized the tight junction properties of this BCB cell model. The presence of tight and adherens junction proteins was investigated by Western blotting, immunocytochemistry, and RT-PCR. The expression of occludin, E-cadherin, and claudin-1, but not claudin-2, was confirmed by Western blot analyses. While ZO1 was detectable on immunoblots, the intensity of bands was low even with the high protein loading (40 ug per lane). The cellular localization of occludin and E-cadherin was investigated by immunocytochemistry; the presence of transcripts for these proteins was confirmed by RT-PCR. Transmission electron microscopy (TEM) was used to examine the intercellular tight junctions in Z310 cells grown on a semipermeable membrane in a Transwell device. TEM showed a tight junction-type of structures located immediately below the apical surface between two adjacent cells. TEM also revealed the presence of microvilli on the apical domain of Z310 cells, suggesting a secretory function of these cells. The initial permeability rate constants, obtained with a paracellular permeability marker [^{14}C]sucrose, were 862, 432, and 538 fmol/min for devices without cells (control), with primary choroidal epithelial cells, and with Z310 cells, respectively. These data indicate that the Z310 cells possess the tight junction properties and may be suitable for the barrier transport studies. [Supported by: NIH/NIEHS R01 ES-08146 (WZ) and NIH/NINDS R01 NS-39921 (AC)]

258 DETERMINATION OF ENERGY AND REDOX STATES IN CELL CULTURE FOLLOWING CADMIUM EXPOSURE.

L. Yu[1], R. C. Gupta[2] and M. S. Yang[1]. *[1]Biology, Hong Kong Baptist University, Hong Kong, China and [2]Toxicology Department, Murray Sate University, Breathitt Veterinary Center, Hopkinsville, KY.*

The present investigation was undertaken to determine energy and redox states by measuring the levels of ATP, ADP, AMP, GSH, and GSSG in HepG2 hepatoma and C6 glioma cell lines. Perchloric acid extracts of these cells were analyzed for ATP, ADP, and AMP using an isocratic RP-HPLC method and for GSH and GSSG using a fluorescence method. The values of these metabolites were used to calculate the energy charge potential (ECP=[ATP+0.5ADP]/TAN), total adenine nucleotides (TAN=ATP+ADP+ AMP), total glutathione (TG=[GSH+GSSG]/TAN) and the redox state (GSH/GSSG ratio). Findings revealed that ATP:ADP:AMP was 0.76:0.11:0.13 for the HepG2 cells and 0.80:0.11:0.09 for the C6 glioma cells. ECP was 0.81±0.01 and 0.85±0.01 for the HepG2 and the C6 glioma cells, respectively. TG was 3.2±0.54 and 2.03±0.05 for HepG2 and C6 glioma cells, respectively, indicating that the level of TG is 2-3 fold higher than the levels of energy metabolites in the two cell lines. GSH/GSSG was 2.66±0.16 for the HepG2 cells and 2.03±0.23 for the C6 glioma cells. Following a 3 hr incubation, CdCl2 at a low level (0.2 mM) caused some increases in TG in both cell lines. GSH/GSSG in the C6 glioma cells rapidly decreased at a dose of 0.1 mM CdCl2. The HepG2 cells on the other hand, showed no significant change in GSH/GSSG ratio until CdCl2 concentration reached 0.6mM. These results indicate that energy and redox states differ in these two cell lines and that the cell lines respond differently to cadmium exposure.

259 BASAL GENE EXPRESSION PROFILES AND EFFECTS OF HEPATOCARCINOGENS ON GENE EXPRESSION IN PRIMARY HUMAN HEPATOCYTES AND HEPG2 CELLS.

A. J. Harris[1], S. L. Dial[1] and D. A. Casciano[2]. *[1]Center for Hepatotoxicity, NCTR, Jefferson, AR and [2]Office of the Director, NCTR, Jefferson, AR.*

Toxicogenomics is a relatively new discipline of toxicology. Microarrays and bioinformatics tools are being used successfully to understand the effects of toxicants on *in vivo* and *in vitro* model systems, and to gain a better understanding of the relevance of *in vitro* models commonly used in toxicological studies. In this study, cDNA filter arrays were used to determine the basal expression patterns of cultured primary human hepatocytes from 3 male donors; compare the gene expression profile of HepG2 to that of primary human hepatocytes; and analyze the effects of 3 genotoxic hepatocarcinogens; aflatoxin B1 (AFB_1), 2-acetylaminofluorene (2AAF), and dimethylnitrosamine (DMN), as well as acetaminophen (APAP) a non-genotoxic hepatotoxicant, on gene expression in both *in vitro* systems. Real-time PCR was used to verify differential gene expression for selected genes. Of the approximately 31, 000 genes screened, 3-6 % were expressed in primary hepatocytes cultured on matrigel for 16 hr. Of these genes, 870 were expressed in cultured hepatocytes from all donors. HepG2 cells expressed about 98% of the genes detectable in human hepatocytes, however, 31% of the HepG2 transcriptome was unique to the cell line. There was considerable variability in the response to chemical carcinogen exposure in primary hepatocytes from different donors. The expression of transcription factor genes, E2F1 and ID1, were increased 3-fold and 6-fold ($P < 0.05$, $P < 0.01$), respectively in AFB_1 treated primary human hepatocytes but were not altered in HepG2. ID1 expression was also increased by DMN, 2AAF and APAP in both primary hepatocytes and HepG2. This study suggests that identification of biomarkers of exposure to some chemicals may be possible in the human through microarray analysis, despite the variability in responses.

260 GENE EXPRESSION MICROARRAY-BASED HYPOTHESIS FOR CADMIUM-INDUCED BONE LOSS.

M. H. Bhattacharyya[1], A. Regunathan[1], D. A. Glesne[1] and A. K. Wilson[2]. *[1]Argonne National Laboratory, Argonne, IL and [2]Benedictine University, Lisle, IL.*

To identify a mechanism of Cd-induced bone loss that did not rely on guessing the pathways involved, a whole-mouse genome gene-expression microarray was used to determine bone cell changes after a single Cd gavage to mice. To identify early pathways, time points were 2h and 4h after gavage, for a dose (200 ug Cd/mouse) at

which calcium release from bone starts at 8h. Three mouse strains [CF1, metallothionein-normal (MTN), and MT1, 2-deficient (MT1, 2KO)] were studied to identify a robust mechanism that applied across mouse strains. Results showed that ~18 genes increased significantly in mRNA concentration in bone cells in all 4 or in 3 of the 4 microarrays. High to low in Cd responsiveness, these were: cysteine-rich protein 61 (Cyr61), metallothionein 2 (MT2), transferrin receptor (TfR), glutamine synthetase pseudogene 1, MT1, acidic chitinase, RIKEN cDNA 3930, src-like adaptor protein, vacuolar proton pump ATPase, integrin alpha v, aquaporin 1, and p38 MAP Kinase. No genes showed analogous decreases. Concentration increases were small but validated by Northern analyses. Gene changes fit into the following hypothesis: Cadmium could, by currently unknown mechanisms, increase expression of cysteine-rich protein 61 (Cyr61) in osteoblasts and of p38 MAP kinase in osteoclast precursor cells. Cyr61, high in osteoblasts, is a secreted extracellular matrix-associated protein that could attract osteoclasts and bind to their surface integrin receptor, stimulating osteoclast migration, adhesion and activation. Activation would stimulate expression of other osteoclast genes, including integrin alpha-v, vacuolar proton pump ATPase (provides acid to dissolve bone calcium and activate acid proteases that degrade bone matrix), and transferrin receptor (provides needed iron). Osteoclast precursor cells contain high concentrations of p38 MAP kinase compared to other bone cells, and this kinase is required for osteoclast formation. The latter pathways could explain existing evidence that cadmium stimulates bone loss by increasing both osteoclast formation and activation.

261 BISMUTH-INDUCED RESISTANCE AGAINST CISPLATIN NEPHROTOXICITY AND GENE EXPRESSION PROFILE IN CULTURED TUBULAR EPITHELIUM.

F. A. de Wolff[1], B. T. Leussink[1], J. J. Baelde[2], T. M. Broekhuizen-van den Berg[2], E. de Heer[2], A. Slikkerveer[3], G. B. van der Voet[1] and J. A. Bruijn[2]. *[1]Toxicology Laboratory, Leiden University Med. Ctr, Leiden, Netherlands, [2]Department of Pathology, Leiden University Med. Ctr, Leiden, Netherlands and [3]Research Laboratories, Yamanouchi Europe BV, Leiderdorp, Netherlands.*

Introduction Nephrotoxicity is the most important dose-limiting factor in cisplatin-based anti-neoplastic treatment. Pretreatment with bismuth salts, used as pharmaceuticals to treat gastric disorders, has been demonstrated to reduce cisplatin-induced renal cell death in clinical settings and during *in vivo* and *in vitro* animal experiments. *Aim* To investigate the genetic basis of the renoprotective effect of bismuth against cisplatin nephrotoxicity. *Methods* We exposed NRK-52E cells, a cell line of proximal tubular epithelial origin, to 33 μM Bi^{3+} for 12 h, which made them resistant against cisplatin-induced apoptosis. Genes differentially expressed in treated and untreated NRK-52E cells were detected with subtraction PCR and microarray technology. *Results* Genes found to be down-regulated (0.17 to 0.31 times) were cytochrome-*c* oxidase subunit I, BAR (an apoptosis regulator), heat-shock protein 70-like protein, and 3 proteins belonging to the translation machinery (ribosomal proteins S7 and L17, and S1, a member of the elongation factor 1-α family). The only gene found to be up-regulated was glutathione S-transferase subunit 3A (1.89 times). *Conclusions* Glutathione has been demonstrated earlier to play a key role in protecting proximal tubular epithelial cells from cisplatin induced cell death. Local upregulation may therefore explain the renoprotective effect of bismuth. Guided by the expression levels of these genes, it may be possible to improve renoprotective treatments in the context of anti-neoplastic therapies.

262 TOXICOGENOMIC ANALYSIS OF ABERRANT GENE EXPRESSION IN NEWBORN MOUSE LIVER INDUCED BY TRANSPLACENTAL EXPOSURE TO CARCINOGENIC DOSES OF INORGANIC ARSENIC.

Y. Xie[1], J. Liu[1], B. A. Diwan[2], J. M. Ward[3], D. L. Logsdon[3] and M. P. Waalkes[1]. *[1]Inorganic Carcinogenesis Section, LCC, NCI at NIEHS, Research Triangle Park, NC, [2]SAIC, NCI at Frederick, Frederick, MD and [3]Office of Laboratory Animal Science, NCI at Frederick, Frederick, MD.*

We previously found that brief exposure of pregnant mice to arsenite in the drinking water induced hepatocellular carcinoma (HCC) in the offspring when they reached adulthood. Furthermore, adult mice exposed to carcinogenic doses of arsenite *in utero* show remarkable gene expression changes that could be related to oncogenesis. To help determine the onset of these changes, this study focused on aberrant gene expression in newborn mice induced by *in utero* arsenic (As) exposure at a dose shown in our prior work to induce HCC. Thus, groups of pregnant mice were exposed to drinking water containing 85 ppm As (as sodium arsenite) or unaltered water (control) from gestation day 8 to 18. The dams were allowed to give birth, and liver samples were taken from the newborn pups for analysis of As content, global DNA methylation status and altered gene expression. This dose was well tolerated and there was no evidence of maternal or fetal toxicity. In the neonatal liver As was detectable after *in utero* exposure (57.2 ng/g on average) but not present in the controls, suggesting that As had crossed the placenta and accumulated in the fetal liver. Global hepatic DNA methylation status was not altered by As exposure. In As-treated neonates, 70 out of the 600 genes analyzed by microarray showed aberrant expression, which was confirmed for selected genes by PCR analysis. IGF (insulin-like growth factor)-related genes as well as genes encoding cytochrome P450 and GST showed particularly noticeable alterations. This included the reduced expression of IGF-1, IGF-2, IGFBP-1, IGFBP-2 and CYP1A2, 2B9, 2D9, 2E1, 2F2, 3A11, 3A13, 3A25, 4A10 and 4A14, and the enhanced expression of GST 5, GST P1 and GST theta 1. This study revealed that *in utero* exposure to As at non-toxic doses induces remarkable aberrant gene expression changes in the newborn liver, a target of As carcinogenesis.

263 FURTHER STUDIES ON GENE EXPRESSION CHANGES ASSOCIATED WITH TRANSPLACENTAL ARSENIC CARCINOGENESIS.

J. Liu[1], Y. Xie[1], B. A. Diwan[2], J. M. Ward[3], D. L. Logsdon[2] and M. P. Waalkes[1]. *[1]Inorganic Carcinogenesis, LCC, NCI at NIEHS, Raleigh, NC, [2]SAIC, NCI-Frederick, Frederick, MD and [3]Office of Laboratory Animal Sciences, NCI -Frederick, Frederick, MD.*

Our prior work shows arsenic alone is a transplacental carcinogen in male mice. In a follow-up study, pregnant C3H mice were given drinking water containing 85 ppm arsenic as sodium arsenite or unaltered water from day 8 to 18 of gestation. In order to promote arsenic-initiated tumors, mice received arsenic *in utero* and then 12-O-teradecanoyl phorbol-13-acetate (TPA), a tumor promoter, was applied to the skin of offspring (2 mg/0.1 ml acetone, 2 times/week) from 4-25 weeks. The administration of TPA did not promote skin tumors but significantly increased liver tumors in females. Thus, arsenic exposure *in utero* plus TPA at weaning produced liver tumors in both male and female mice. Liver tumors and non-tumorous tissues were taken at necropsy. Total RNA was isolated and purified to perform microarray analysis using a 600-gene custom array. Arsenic plus TPA treatment resulted in increased expression of a-fetoprotein, c-myc, cyclin D1, CYP2A4, cytokeratin-8, cytokeratin-18, glutathione S-transferases and insulin-like growth factor binding proteins, as well as decreased expression of betaine-homocysteine methyltransferase, CYP7B1, CYP2F2 and insulin-like growth factor-1 in liver tumor samples and arsenic-exposed non-tumorous surrounding tissue samples, as compared to controls. Regardless of sex, the expression of several of these genes, such as a-fetoprotein, c-myc, and cyclin D1, is associated with liver tumor formation. Microarray data were confirmed by real-time RT-PCR analysis on selected genes. The gene expression changes agree well with our prior genomic analysis on mice exposed to arsenic only *in utero*. Thus, inorganic arsenic exposure *in utero* plus inclusion of TPA after weaning caused liver tumor formation and remarkable alterations in gene expression in adult mice despite ending arsenic exposure during gestation. These aberrant expression changes may be associated with the formation of arsenic-induced tumors in the liver.

264 MODULATION OF AHR-REGULATED GENE EXPRESSION BY ARSENITE, CADMIUM, AND CHROMIUM.

R. H. Elbekai and A. O. El-Kadi. *University of Alberta, Edmonton, AB, Canada.*
Sponsor: J. Koropatnick.

AHR ligands and heavy metals are environmental co-contaminants and their molecular interaction may disrupt the coordinated regulation of AHR-dependent gene expression. The aim of this study was to determine the effect of arsenite (As^{3+}), cadmium (Cd^{2+}) and chromium (Cr^{6+}), on the constitutive and inducible expression of the AHR-regulated genes: cyp1a1, NAD(P)H: quinone oxidoreductase (QOR) and glutathione S-transferase Ya (GST Ya). For this purpose, murine hepatoma Hepa 1c1c7 cells were treated with increasing concentrations of As^{3+} (1, 5, and 10 μM), Cd^{2+} (1, 5, and 25 μM) and Cr^{6+} (1, 5, and 25 μM) with or without one of the AHR ligands: 2, 3, 7, 8-tetrachlorodibenzo -p-dioxin (0.1 nM), 3-methylcholanthrene (0.25 μM), β-naphthoflavone (10 μM), or Benzo[a]pyrene (1 μM). Our results show that metals or AHR ligands alone increased the catalytic activities and the mRNA levels of all AHR-regulated genes. When metals were co-administered with AHR ligands, all three metals inhibited the induction of cyp1a1 activity by the AHR ligands but potentiated inducible mRNA expression of cyp1a1 in a dose dependent manner. In addition, all metals enhanced QOR constitutive activity and mRNA transcript levels in a dose-dependent manner but modulated its induction by AHR ligands in a dose, metal, and AHR ligand-dependent manner. Generally, Cr^{6+} inhibited while As^{3+} and Cd^{2+} potentiated the induction of QOR activity and mRNA levels. Maximal elevations in GST Ya catalytic activity by As^{3+} or Cd^{2+}, alone and with an AHR ligand co-treatment, occurred at the highest doses tested and were accompanied by increased mRNA transcript levels. Cr^{6+}, however, de-

pressed GST Ya catalyzed detoxification capacity of the cells at the mRNA and activity levels. The three metals enhanced the expression of heme oxygenase-1 (HO-1) in a dose-dependent manner suggesting that HO-1 is involved in the modulation of AHR-regulated gene expression by heavy metals. The ability of metals to alter the capacity of AHR ligands to induce the bioactivating phase I and the detoxifying phase II enzymes will influence the carcinogenicity and mutagenicity of the AHR ligands.

265 DIFFERENTIAL EFFECTS OF HEAVY METALS ON ARYL HYDROCARON RECEPTOR-REGULATED GENES.

H. M. Korashy and A. O. El-Kadi. *Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.*

Both simultaneous and sequential exposure to heavy metals and aryl hydrocarbon receptor (AHR) ligands potentially occur in human populations, yet there have been relatively few studies of combined effects of heavy metals and AHR ligands on AHR-regulated genes. The aim of this work was to investigate the potential effects of heavy metals, particularly mercury (Hg^{2+}), lead (Pb^{2+}), and copper (Cu^{2+}), on the constitutive and the inducible expression of AHR-regulated genes; cytochrome P450 1a1 (cyp1a1), NAD(P)H:quinone oxidoreductase (QOR) and glutathione S-transferase Ya; (GST Ya) at the activity and mRNA levels. For this purpose, murine hepatoma hepa 1c1c7 wild-type cells were incubated with increasing concentrations of Hg^{2+} (2.5, 5 and 10 μM), Pb^{2+} (10, 25 and 100 μM), and Cu^{2+} (1, 10 and 100 μM) alone or with the AHR-ligand, 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (0.1 nM), 3-methylcholanthrene (0.25 μM), β-naphthoflavone (10 μM), or Benzo[a]pyrene (1 μM). Our results clearly showed that metals alone did not significantly alter the cyp1a1 activity, whereas a significant reduction in AHR ligand-mediated induction of cyp1a1 activity was observed by all metals in a concentration-dependent manner. Interestingly, the decrease in cyp1a1 activity was associated with an increase in cyp1a1 mRNA level. With respect to QOR, the activity was strongly increased dose-dependently by all metals in the absence or presence of an AHR-ligand, with the exception of Cu^{2+} which significantly decreased the activity. Changes in QOR at the mRNA levels were in accordance with the activity results. Differently, GST Ya activity was significantly increased by Cu^{2+} and Pb^{2+} and inhibited by Hg^{2+}. All metals significantly increased the expression of stress protein, heme oxygenase-1 (HO-1). We conclude that heavy metals differentially modulate the constitutive and the inducible expression of AHR-regulated genes. Chronic exposure of heavy metals/AHR-ligands mixture would be less effective in inducing AHR-mediated toxicity than exposure to metals or AHR-ligand alone.

266 ARSENITE INITIATES AH RECEPTOR-INDEPENDENT REPRESSION OF CYP1A1 INDUCTION BY TCDD.

J. A. Bonzo[1], A. Galijatovic[1], S. Chen[1] and R. H. Tukey[1, 2]. *[1]Pharmacology, University of California San Diego, La Jolla, CA and [2]Chemistry & Biochemistry, University of California San Diego, La Jolla, CA.*

Arsenite (As^{3+}) alters the expression and function of several drug-metabolizing enzymes, including cytochrome P450 1A1 (CYP1A1). In addition, it has been shown that *CYP1A1* expression can be regulated in a cell cycle dependent manner. Interestingly, As^{3+} interrupts cell cycle control by initiating G2/M arrest. To examine the impact of As^{3+} on TCDD initiated induction of CYP1A1, HepG2 cells were treated with sub-cytotoxic (0.5 μM to 50 μM) doses of As^{3+}. Exposure of HepG2 cells to TCDD and As^{3+} revealed an As^{3+} dose-dependent increase in G2/M phase arrest. Vinblastine causes G2/M arrest and was shown to decrease TCDD-induced *CYP1A1* gene expression. Examination of the catalytic activity of CYP1A1 as measured by EROD assay in HepG2 cells demonstrated that As^{3+} led to a dose-dependent decrease in TCDD-induced EROD activity. This reduction correlated with a comparable reduction in CYP1A1 as determined by Western blot analysis. The addition of As^{3+} to microsomal preparations generated from TCDD treated cells did not influence EROD activity, suggesting that inhibition occurs upstream of translation. This was verified by quantitative analysis of CYP1A1 mRNA by real-time RT-PCR. The lowest concentration of As^{3+} (0.5 μM) resulted in a 61% reduction in TCDD-induced CYP1A1 mRNA. Using HepG2 cells that express the human *CYP1A1*-luciferase reporter gene, it was demonstrated that As^{3+} did not alter TCDD induction of *CYP1A1*-luciferase. Gel shift analysis confirmed activation of the AhR and subsequent binding to the XRE following co-treatment with TCDD and As^{3+}. Combined, *CYP1A1* initiated transcription does not appear to be a target for the inhibitory properties of As^{3+}. These results indicate that G2/M phase arrest may be a factor in As^{3+} inhibition of TCDD initiated induction of CYP1A1. Moreover, the mechanism of inhibition does not impact on activation of the AhR. (Supported by USPHS grant ES10337).

267 ARSENIC-TRANSFORMED HUMAN PROSTATE EPITHELIAL CELLS SHOW CHANGES IN ANDROGEN METABOLISM AND ESTROGEN RECEPTOR EXPRESSION.

L. Benbrahim-Tallaa[1], M. M. Webber[2] and M. P. Waalkes[1]. *[1]Inorganic Carcinogenesis Section, NCI at NIEHS, Research Triangle Park, NC and [2]Michigan State University, East Lansing, MI.*

Inorganic arsenic (As) is an important environmental toxicant, with natural and industrial sources. Human As exposure is linked to cancers in various tissues including the prostate. However, the molecular mechanisms of As carcinogenesis are still poorly defined. Steroid hormones play a critical role in stimulating the growth and differentiation of normal prostate epithelial cells, as well as in the initial growth of prostate cancer cells. Furthermore, genes involved in the prostatic steroid signaling pathways have been implicated in cancer development. Variation in the androgen or estrogen receptor genes, as well as in the steroid metabolizing enzymes, such as, the steroid 5α-reductase, have been linked to prostate cancer causation and progression. We have developed an *in vitro* model of As-induced prostate cancer by inducing malignant transformation of the immortalized, non-tumorigenic human prostate epithelial cell line RWPE-1 by chronic As exposure. To help understand the molecular mechanisms underlying As-induced malignant transformation, we performed gene expression comparisons between these chronic As transformed prostate epithelial cells (designated CAsE-PE) and control RWPE-1 cells. RT-PCR and western blot were used to examine expression of ER-α and ER-β. Our studies revealed an increase in ER-β (1.6-fold) and a decrease in ER-α (45%) transcript in As transformed cells compared to control. However, the correlate receptor protein levels were significantly elevated from 20 and 100% in CAsE-PE cells. 5α-reductase transcript, which encodes for the enzyme that converts testosterone to its active metabolite, DHT, was markedly increased in CAsE-PE cells. These data suggest altered expression of steroid receptors and altered steroid metabolism occurs in CAsE-PE cells. These changes in steroid metabolism and sex steroid signaling pathways may be associated with As-induced malignant transformation and prostate cancer.

268 EXPOSURE TO SOLUBLE NICKEL ALTERS IRON-MEDIATED GENE TRANSCRIPTION AND ENZYME ACTIVITY IN A549 CELLS.

T. L. Davidson, H. Chen, T. Kluz and M. Costa. *Department of Env. Med., New York University, Tuxedo, NY.*

Nickel compounds have been identified as human carcinogens/cocarcinogens using both *in vivo* studies and epidemiological evaluations. Nickel is known to stabilize the hypoxia inducible factor-1α (HIF-1α) protein, which is thought to be important in tumor progression. Since nickel affects HIF-1α, which is controlled by an iron dependent enzyme, we designed *in vitro* experiments to look at the effect of nickel on iron dependent processes in A549 cells. Using the Affymetrix Gene Chip, we identified several iron-regulated genes that were altered in reponse to nickel treatment. The mRNA levels of transferrin receptor and GADD45, both of which are increased in response to low iron levels, were substantially increased in response to nickel. In addition, the transcription of many HIF-1α-dependent genes was also increased. Nickel chloride was also able to block the induction of ferritin by ferrous iron. Atomic absorption was then performed to see if nickel had an effect on total iron levels in A549 cells. The levels of total iron were significantly lowered in response to 1mM nickel chloride. Furthermore, the enzyme activity of several iron dependent enzymes, including prolyl hydroxylase and aconitase, was significantly lowered when cells were exposed to 1mM nickel chloride. Surprisingly, when we treated a cell extract with concentrations of nickel chloride as low as 10 uM, prolyl hydroxylase activity was decreased. In sharp contrast, when we treated purified aconitase enzyme with concentrations of nickel chloride as high as 2mM, we observed no decline in enzyme activity. The prolyl hydroxylase enzymes that control the stability of HIF-1α appear to be very sensitive to very low concentrations of nickel. Iron dependent processes are a requirement for life and interference with these processes may be involved in nickel-induced carcinogenesis. These data may give new insight into the mechanisms of nickel induced carcinogenesis, as well as, contribute important information for the treatment and prevention of occupational diseases.

269 EUKARYOTIC TRANSLATION INITIATION FACTOR 4E (EIF4E) IS A CELLULAR TARGET FOR CADMIUM TOXICITY.

S. Othumpangat and P. Joseph. *Health Effects Laboratory Division, NIOSH, Morgantown, WV.*

Cadmium, to which large numbers of people are occupationally and otherwise exposed, has been categorized as a human carcinogen by the International Agency for Research on Cancer (IARC). Several theories have been proposed to account for the

mechanisms potentially responsible for cadmium toxicity and carcinogenesis. Recently, our laboratory has demonstrated a novel mechanism for cadmium carcinogenesis that involves the upregulation of translation initiation factor 3 and translation elongation factor-1 delta. Presently, we have investigated whether the translational proto-oncogene, eukaryotic initiation factor 4E (eIF4E) is a cellular target for cadmium toxicity and carcinogenesis. Four different human cell lines: HCT-15 (colorectal adenocarcinoma), PLC/PRF/5 (hepatocellular carcinoma), HeLa (adenocarcinoma) and Chang (likely derived from HeLa cells) cells, were exposed to 30 μM cadmium chloride for time intervals up to 24-hours and the expression level of eIF4E was determined by Western blot analysis using an antibody against human eIF4E. Exposure to 30 μM cadmium chloride resulted in significant cytotoxicity and cell death in all four cell lines tested. Furthermore, exposure to cadmium chloride resulted in significant inhibition of eIF4E in all cell lines, and the highest inhibition was noticed following the 24-hours exposure to cadmium chloride. The significance of cadmium chloride-mediated inhibition of eIF4E was further investigated by silencing the expression of eIF4E by employing small interfering RNA (SiRNA) specifically targeting eIF4E. The SiRNA mediated silencing of eIF4E resulted in significant cell death. Our results thus suggest that eIF4E is a cellular target for cadmium toxicity and that the cadmium-induced cytotoxicity and cell death may be due to the inhibition of eIF4E.

270 LEAD IS MITOGENIC TO WTHBF-6 CELLS, BUT LEAD CHROMATE (LC) INDUCES CELL CYCLE ARREST.

J. Moreland[1], S. Teufack[1, 3], S. Sandwick[1], J. Dufour[1], S. S. Wise[1], A. Holmes[1], M. Ketterer[2], W. Hartsock[2], E. Fomenchenko[1, 3], S. Katsifis[3] and J. P. Wise[1].
[1]Wise Laboratory of Environmental and Genetic Toxicology, Center for Integrated and Applied Environmental Toxicology, University of Southern Maine, Portland, ME, [2]Department of Chemistry, Northern Arizona University, Flagstaff, AZ and [3]Department of Biology, University of Bridgeport, Bridgeport, CT.

Hexavalent chromium (Cr(VI)) is a human lung carcinogen of significant public health concern. The particulate Cr(VI) compounds are more potent carcinogens than soluble ones. Preliminary reports suggest that LC, a prototypical particulate Cr(VI) compound, partially dissolves to produce ionic lead (Pb) and Cr and that while the Cr ions are damaging DNA, the lead ions are stimulating cell growth. We found that Pb induced cell growth in WTHBF-6 cells: 250, 500, 1000, 2000 uM lead glutamate (LG) induced 40, 54, 85, and 142% increases in the mitotic index. However, LC did not stimulate cell growth: 0.5, 1.0, and 5.0 ug/cm^2 reduced the mitotic index by 52.9, 52.9, and 92.35% respectively and metaphases were absent at 10.0 and 50.0 ug/cm^2. These same LC concentrations induced prolonged growth arrest. Furthermore, LC-induced arrest occurred at intracellular lead concentrations much lower than those found for growth stimulatory levels of ionic lead. We found that soluble sodium chromate (SC) also inhibited growth and the concentrations of Cr inside the cell after both LC and SC were equivalent. Further analysis of the cell cycle showed that 0.5, 1.0, 5.0 and 10.0 ug/cm^2 LC increased the percentage of cells in S-phase by 9.7, 24.3, 83.5, and 104% respectively. These increases coincided with a drop in the percentage of cells in G1 phase. SC also induced accumulation of cells in S-phase at moderate concentrations. Yet only 2000 uM LG, resulted in S and/or G2/M arrest. These results indicate that Cr, not Pb, is responsible for cell growth effects associated with LC. This work was supported by NIEHS grant 1R01 ES 10838-01 (J.P.W.).

271 MERCURY MODULATES CELL CYCLE PROGRESSION IN HUMAN LIVER CARCINOMA CELLS THOUGH INDUCTION OF C-FOS, CYCLIN-A, AND CYCLIN-D EXPRESSION, AND REPRESSION OF GADD153.

P. B. Tchounwou and D. J. Sutton. *Center for Environmental Health, Jackson State University, Jackson, MS.*

Exposure to mercury has been associated with a significant number of adverse health effects on various organ systems, and cancer in some cases. Its toxicity has been attributed to its high affinity to sulfhydryl groups of proteins. However, little is known regarding the molecular mechanisms by which mercury exerts its toxicity, mitogenesis, and/or carcinogenesis. Recent studies in our laboratory have shown that mercury is cytotoxic and transcriptionally activates stress genes in human liver carcinoma (HepG2)cells. We hypothesized that mercury-induced expression of stress proteins and cyclins may play a role in the molecular events leading to toxicity and tumorigenesis. To test this hypothesis, we performed the MTT-assay for cell *via*bility, and the Western Blot and densitometric analyses to assess the expression of specific cellular proteins including c-fos, GADD153, cyclin-A, and cyclin-D. Data obtained from the MTT-assay indicated a strong dose-response relationship with respect to mercury toxicity. The LD50 was computed to be 3.5 +/- 0.6 μg/mL upon 48 hrs of exposure. Western blot and densitometric analyses showed c-fos, cyclin-A, and cyclin-D expressions to increase in a dose-dependent manner within the dose-range of 0-3 μg/mL; showing a peak induction at 3 μg/mL and a sharp drop at 4 μg/mL, probably due to cell death at higher levels of mercury exposure. No statistically significant differences ($p > 0.05$) were found in GADD153 expression between control and mercury-treated cells. Taken together, these findings indicate that mercury is cytotoxic, and has the potential to modulate cell cycle control proteins in HepG2 cells. Induction of c-fos/cyclin-D and cyclin-A expression indicates its stimulation of cell cycle progression, respectively through the G1/S and G2/M transitions. The ramifications of these findings to our understanding of merucy-induced toxicity and tumorigenesis are important. [Research supported by NIH-RCMI Grant No. 1G12RR13459, and DoA-MACERAC Grant No. DACA-42-02-C-0057].

272 URANIUM IS CYTOTOXIC AND GENOTOXIC TO HUMAN LUNG CELLS.

W. Diaz, S. Wise and J. P. Wise. *Wise Laboratory of Environmental and Genetic Toxicology, Center for Integrated and Applied Environmental Toxicology, University of Southern Maine, Portland, ME.*

Maine has some of the highest rates in the nation for bladder and lung cancer. It is also a state that relies heavily on well water, and many of these wells are contaminated with uranium. In addition, uranium has also recently become a specific concern for the health of our soldiers, as well as for those exposed through fragments left behind in the environment, because of the heavy use of depleted uranium in military armor and munitions. Uranium potentially has both chemotoxic and radiotoxic effects. Previous investigations have largely focused on the radiotoxic effects of uranium and its decay products; much less is known about uraniums potential chemical toxicity. One major chemotoxic effect that has been characterized is nephrotoxicity, and there is reason to consider uranium a potential bladder carcinogen because chronic exposures have been shown to be carcinogenic. Uranium has also been associated with human lung cancer. As a first step to understanding the potential carcinogenic mechanisms of uranium, we treated WTHBF-6 cells, a human lung fibroblast cell line with concentrations ranging from 1- 200 uM uranium acetate for 24 h and determined its cytotoxic and genotoxic effects. We found that uranium was moderately cytotoxic over this range with 89.9%, 57.2%, and 40.6% relative survival at 25, 50 and 100 uM. We also found that uranium was clastogenic inducing multiple observable lesions in chromatid structure. At concentrations of 50, 100 and 200uM, 3%, 4% and 19% damage of mitophases was noted in preliminary data. The spectrum of chromosome damage included chromosome breaks, which are consistent with the types of karyotypic alterations seen in many neoplasms. Future research is aimed at understanding the genotoxic mechanisms involved in uranium-induced neoplastic transformation

273 INTERFERON-α INDUCTION OF METALLOTHIONEIN IN RAT LIVER IS NOT LINKED TO INTERLEUKIN-1, -6 OR TUMOR NECROSIS FACTOR-α.

E. Brambila[1], A. Leon[1], J. Guevara[1], O. Castellanos[1], M. P. Waalkes[2] and W. E. Achanzar[2]. *[1]University of Puebla, Puebla, Mexico and [2]Inorganic Carcinogenesis Section, NCI at NIEHS, Research Triangle Park, NC.*

Synthesis of metallothionein (MT) is induced by interferon-α (IFN-α) *in vitro* and *in vivo*. In addition, IFN-α promotes redistribution of zinc (Zn) from the plasma to the liver in mice. However, it is not clear if IFN-α induces hepatic MT synthesis directly or indirectly *via* liberation of other cytokines. In order to address this issue, we determined hepatic MT levels, Zn concentration in plasma, liver and urine, and plasma levels interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in rats following intramuscular injection of human IFN-α (1.5 X 106 UI/m2). Animals were housed in metabolic cages and sacrificed at various times after IFN-α administration. Zn concentrations in serum, urine and hepatic tissue were determined by atomic absorption spectrophotometry. MT protein was measured using the MT silver saturation method and Western-blot analysis. Plasma levels of IL-1, IL-6, and TNF-α were determined using an ELISA method. Hepatic MT levels began to increase at 2 h following IFN-α administration and reached maximum levels at 12 h post-treatment. IFN-α also produced biphasic increases in hepatic Zn, with levels peaking at 2 h, the time-point when MT levels are first increased, and again at 18 h. Concurrently, there were decreases in plasma Zn levels at these time points, suggesting IFN-α-induced movement of Zn from the blood to hepatic tissue. The decrease in plasma zinc was not due to increased excretion since urinary Zn levels were unaffected following IFN-α treatment. IFN-α administration had no effect on plasma IL-1, IL-6 and TNF-α levels. These results show that IFN-α promotes the increase of hepatic MT levels and plasma/liver redistribution directly, without IL-1, IL-6 or TNF-α participation

274 PROLYL HYDROXYLASES AS TARGETS FOR CARCINOGENIC NICKEL.

K. Salnikow[1], A. Zhitkovich[2], S. P. Donald[1], J. Phang[1] and K. Kasprzak[1].
[1]National Cancer Institute, Frederick, MD and [2]Brown University, Providence, RI.

Nickel is a well established human carcinogen, however mechanisms of its carcinogenicity are not understood. Recently, we performed a microarray analysis of cells exposed to soluble nickel and observed strong induction of hypoxia-inducible

genes. These data suggested that nickel activated HIF-1 transcription factor. It was shown previously that under normal conditions, hydroxylation of proline-564 in HIF-1 alpha leads to its interaction with von Hippel Lindau (VHL) tumor suppressor protein and rapid proteosomal degradation. In our experiments we found that nickel exposure resulted in increased stabilization of HIF-1 alpha protein suggesting that hydroxylation of this protein was diminished. Here we tested the hypothesis that inactivation of prolyl hydroxylase activity by nickel causes stabilization of HIF-1 alpha transcription factor. Using a reporter plasmid containing the oxygen-dependent degradation domain (ODD) of HIF-1 alpha with proline-564, fused with luciferase protein (ODD-Luc), we showed that exposure to nickel significantly stabilized this reporter when transfected into human airway epithelial (HAE) cells. Cobalt, deferoxamine and dimethyloxoglycine, known inducers of HIF-1 transcription factor, also stabilized the reporter in HAE cells. Additionally, we have shown that stabilization of ODD-Luc reporter by nickel takes place only in cells with normal, but not with mutated VHL. The induction of endogenous HIF-1 alpha protein by nickel was also confirmed by both induction of HRE-Luc plasmid and by induction of hypoxia-inducible protein NDRG-1/Cap43. Using cell extracts from nickel-exposed and control cells, we demonstrated that ODD-dependent conversion of 2-oxoglutarate into succinate was suppressed by nickel exposure. In conclusion, these data strongly indicate that nickel blocks hydroxylation of proline-564, which leads to the stabilization of HIF-1 alpha protein

275 LEAD INHIBITS OSTEOBLAST DIFFERENTIATION BY BLOCKING SMAD SIGNALING.

K. O. Hochberg, T. J. Sheu, E. M. Schwarz, M. J. zuscik, R. J. O'Keefe, R. N. Rosier and J. E. Puzas. *Orthopedics, University of Rochester, Rochester, NY.*

Rationale: For many years lead has been recognized as an environmental toxicant that adversely affects bone metabolism, especially osteoblast function. Prior studies have shown that lead interferes with the ionic milieu of cells; however, no studies have linked its effect to a direct control of gene expression. Our results indicate that lead inhibits osteoblast differentiation by affecting TGFb activation of the transcription factor(s), Smad2/3. Methods: In these experiments we used primary calvarial osteoblasts. Alkaline phosphatase (AlkPhos) activity, as measured by the hydrolysis of pNPP, was used as an indicator of cell differentiation. Transfection of the cells by electroporation with two reporters for the TGFb pathway (i.e. p3TP-luc and 4xSBE-luc) was used to measure TGFb pathway activity. Overexpression of a constitutively active Smad3 was accomplished with transfection of Smad3D-His-SV40. Cells were exposed to lead ranging from 0.08uM to 50uM for 24-72 hours. Results: Lead exposure resulted in a dose dependent decrease in osteoblast differentiation as demonstrated by a 50% decrease in AlkPhos activity after 24 hours. Transfection of cells with either Smad reporter occurred at an efficiency of 100% of surviving cells. Lead caused a decrease in activity for both reporters of between 25 and 80% at concentrations of lead ranging from 0.08 to 0.4uM after 24 hours. In order to determine if the effect of lead was upstream or downstream of Smad3 activation, we co-transfected the cells with a constitutively active Smad3 construct and the 4xSBE-luc reporter. Under these conditions, lead was unable to affect the Smad signaling pathway. Discussion: Our data support the hypothesis that lead interferes with osteoblast differentiation by inhibiting components of the TGFb/Smad signaling pathway. Since expression of a constitutively active Smad3 was able to rescue Smad signaling in lead exposed osteoblasts, we conclude that lead's effect is upstream of Smad3 activation. Our work will continue to elucidate the effects of lead on TGF-beta signaling.

276 *IN VIVO* ACTIVATION OF METALLOTHIONEIN ISOFORM 3 EXPRESSION IN HUMAN CANCER CELLS.

D. A. Sens[1], V. Gurel[2], S. H. Garrett[2], S. Somji[2] and M. Sens[2]. *[1]Surgery, University of North Dakota, Grand Forks, ND and [2]Pathology, University of North Dakota, Grand Forks, ND.*

This laboratory has shown that the third isoform of metallothionein is overexpressed in human breast, bladder and prostate cancer. In breast cancer, MT-3 overexpression was confined to a subset of cancers and expression was associated with ductal carcinoma in situ lesions having a poor prognosis. In prostate cancer, MT-3 was also overexpressed in a subset of prostate cancers and expression correlated with Gleason score. In bladder cancer, MT-3 was shown to be overexpressed in all cancers with the degree of overexpression correlating to tumor grade. Despite the widespread occurrence of MT-3 overexpression in these cancers, most cell lines derived from these cancers have no or very low expression of MT-3 mRNA and protein. The goal of the present study was to determine if a feature of the *in vivo* environment could activate MT-3 expression in tumor cell lines having no *in vitro* expression of MT-3. This was tested by assessing the MT-3 expression of human tumor cell lines before heterotransplantation into immunocompromised nude mice and in the tumors produced by these heterotransplanted cell lines. The results demonstrated that tumorigenic cultures resulting from the treatment of the UROtsa bladder cell line with cadmium or arsenite had no *in vitro* expression of MT-3 mRNA or protein, but had high levels of expression in the tumors formed in the nude mouse. Likewise, it was shown that the commonly utilized PC-3 prostate cancer cell line had no *in vitro* expression of MT-3 mRNA or protein, but that the tumors produced in nude mouse heterotransplants had high levels of MT-3 mRNA and protein. This finding was further extended by an analysis of MT-3 mRNA and protein expression of the MCF-7, T-47D, Hs578t and MDA-MB-231 breast cancer cell lines under *in vitro* and *in vivo* conditions of growth. These results demonstrate that a factor associated with *in vivo* tumor growth can activate the expression of MT-3 mRNA and protein.

277 EXPRESSION OF METALLOTHIONEIN 3 PROTEIN IS RESTRICTED IN THE NORMAL BREAST EPITHELIAL CELL LINE, MCF-10A.

M. Sens[1], V. Gurel[1], S. H. Garrett[1], S. Somji[1] and D. A. Sens[2]. *[1]Pathology, University of North Dakota, Grand Forks, ND and [2]Surgery, University of North Dakota, Grand Forks, ND.*

The third isoform of metallothionein (MT-3) is not expressed in normal breast epithelial cells, but is overexpressed in some breast cancers and its expression is associated with a poor disease outcome. The goal of the present study was to determine the effect that overexpression of MT-3 would have on the immortal, but non-tumorigenic, MCF-10A human breast epithelial cell line. The MCF-10A cells were stably transfected with the MT-3 coding sequence and MT-3 mRNA was determined by RT-PCR and MT-3 protein by immunoblot. It was demonstrated that the wild type MCF-10A cell had no basal expression of MT-3 mRNA or protein. Transfection of the MCF-10A cells with the MT-3 coding sequence resulted in a high level of expression of MT-3 mRNA but no MT-3 protein. Exposure of the MT-3 transfected MCF-10A cells to lethal and sub-lethal levels of Cd+2 did not cause expression of the MT-3 protein, but did result in enhanced expression of the MT-1 and MT-2 isoform proteins. As a control, the MT-3 gene was also transfected in the normal bladder epithelial cell line UROtsa, and in the human proximal tubule cell line, HK-2. Treatment of these cell lines with cadmium resulted in an increase in MT-3 protein in the HK-2 cells but not in the UROtsa cell line. These studies demonstrate that the normal human breast epithelial cell and the bladder epithelial cell can restrict the expression of the MT-3 protein and that MT-3 protein expression can be controlled at the post-transcriptional level.

278 EFFECT OF CADMIUM ON THE EXPRESSION OF METALLOTHIONEIN 1 AND 2 PROTEIN IN THE NORMAL BREAST TISSUE AND THE CELL LINE MCF-10A.

V. Gurel[1], D. A. Sens[2], S. Somji[1], S. H. Garrett[1] and M. Sens[1]. *[1]Pathology, University of North Dakota, Grand Forks, ND and [2]Surgery, University of North Dakota, Grand Forks, ND.*

The protein representing the first and second isoforms of metallothionein (MT-1/2) has been shown to be overexpressed in a sub-set of human breast cancers and that overexpression correlates to poor prognosis, type of tumor histology, estrogen receptor status and lymph node involvement. The goals of the present study were to determine the expression of MT-1 and MT-2 mRNA and MT-1/2 protein in normal human breast tissue and in the MCF-10A cell line, and to determine the effect cadmium exposure would have on MT mRNA and protein expression in the MCF-10A cells. It was shown that normal breast tissue expressed very high levels of MT-1X and MT-2A mRNA, but only low amounts of the MT-1/2 protein. Microdissection of normal breast tissue demonstrated that these mRNAs were present in high abundance in ductal breast epithelial cells. The MCF-10A cells were shown to have a very similar pattern of expression of the MT mRNAs and MT-1/2 protein to that found in normal breast tissue. Treatment of the MCF-10A cells with cadmium at both lethal and sub-lethal levels for up to 16 days was shown to result in negligible increases in MT mRNA, but in a large increase in the accumulation of MT-1/2 protein. Treatment of confluent MCF-10A cells with 7 micromolar cadmium increased MT-1/2 protein from 3.2 nanogram/microgram total cell protein at the time of addition to 52.8 nanogram/microgram total cell protein following 10 days of exposure. These studies provide evidence that cadmium can increase the accumulation of MT-1/2 protein in the human breast epithelium through a mechanism involving post-transcriptional control.

279 METALLOTHIONEIN AND GLUTAMYL-CYSTEINE LIGASE GENE EXPRESSION IN METAL-EXPOSED DEER MICE.

H. LaVire[1], S. Srinouanprachanh[1], M. Hooper[2], S. McMurry[2], G. Cobb[2] and T. Kavanagh[1]. *[1]University of Washington, Seattle, WA and [2]Texas Tech University, Lubbock, TX.*

Site studies conducted at the Anaconda Smelter Site (Montana) show varying tissue concentrations of As, Pb, Cd, Cu, and Zn in small mammal tissues. This provides a unique opportunity to study heavy metal mixture dose-responses for gene expression in tissues from wild rodent populations. Glutamyl-cysteine ligase (GCL, the

initiating enzyme in glutathione synthesis) and metallothionein (MT) levels are known to increase with heavy metal exposures in mammalian tissues; this increase in GCL expression after semi-chronic heavy metal exposures suggests oxidative stress, however few field-based studies have been conducted. Here, GCL and MT expression levels in livers of *Peromyscus maniculatus* collected from Anaconda Smelter were measured with real-time quantitative PCR (RT-PCR) and Western blot analysis. Analyses of RT-PCR data indicate a significant correlation between gene expression of the normalizing gene *Gapdh* and Cd exposure ($p<0.001$), several endpoint correlations remain significant when normalized by other factors (i.e. RNA loaded, β-actin). These include correlations between *Gclc* and Cd and between *Gclm* and Cd, Cu, and Pb in females ($p<0.05$). No correlation was found for MT and any of the metals. A correlation was found between *Gclc* and *Gclm* expression for males and females ($p<0.05$), suggesting possible co-regulation. Also, Western blot analyses reveal several significant post-transcriptional trends. Most notable are an overall increase in GCLm protein level with Pb exposure ($p<0.01$), and increases in both GCL subunits in males with Pb exposure ($p<0.05$). The lack of correlations between MT and heavy metals could be due to post-transcriptional regulation or due to adaptation. In conclusion, under chronic exposure of small mammals to heavy metal mixtures, correlations between heavy metals (particularly Cd and Pb) and GCL subunits suggest that it may be a better biomarker of chronic heavy metal exposure under these conditions. Sponsored by NIEHS grants P42ES04696 and P30ES007033.

280 ACUTE CADMIUM EXPOSURE ENHANCES AP-1 DNA BINDING AND INDUCES CYTOKINES EXPRESSION AND HEAT SHOCK PROTEIN 70 IN HEPG2 CELLS.

V. Souza[1], C. Escobar[1], L. Gomez-Quiroz[1], L. Bucio[1], E. Hernandez[1], E. Chavez Cossio[2] and C. Gutierrez-Ruiz[1]. [1]*Cs de la Salud, UAM-I, Mexico, DF, Mexico and* [2]*Bioquimica, Instituto Nacional Cardiologia Ignacio Chavez, Mexico, DF, Mexico.*

Cadmium has been regarded as one of the inflammation-related xenobiotics. This study was undertaken to examine the effects of low cadmium concentrations in HepG2 cells in the IL-1β, TNF-α, IL-6 and IL-8 expression, production of Hsp70 and the activation of nuclear factors AP-1 and NF-κB. Also, the participation of TNF-α and oxidative stress in AP-1 activation was evaluated. RNA was isolated from HepG2 cells after 0.5, 1, 3 or 6 h incubation with 1, 5 or 10 μM CdCl2. TNF-α and IL-1β presented a maximum response after 1 h treatment, while IL-6 and IL-8 maximum response was after 3 h treatment. The Hsp70, determined by Western blot, was constitutively produced, and it increased after 3 h Cd treatment. NF-κB activation, determined by EMSA, was not increased as a result of Cd treatment. DNA binding of AP-1 was detected and increased, with time up to 4 h with an increment of 24 times control value with 5μM CdCl2. The HepG2 cells were pretreated with anti-TNF-α antibody or 1 mM N-acetyl cysteine (NAC) one hour before Cd treatment. Anti-TNF-α treatment reduced 67% AP-1 activation, while NAC 47.5%. These data indicate that, Cd induced TNF-α and IL-1β, that probably, activate AP-1 transcription factor and IL-6 and IL-8 were induced. Anti-TNF-α and NAC partially inhibited AP-1 activation. All imply that, a number of factors participate in AP-1 cadmium-induced activation. The Hsp70 is produced by the HepG2 cells after cadmium treatment, and probably has a role in the non-participation of NF-κB in the cellular response.

281 PARTICULATE HEXAVALENT CHROMIUM-INDUCED CLASTOGENESIS IS MEDIATED BY EXTRACELLULAR DISSOLUTION THAT DOES NOT REQUIRE PARTICLE-CELL CONTACT.

H. Xie[1], A. Holmes[1], S. Wise[1], N. Gordon[2] and J. P. Wise[1]. [1]*Wise Laboratory of Environmental and Genetic Toxicology, Center for Integrated and Applied Environmental Toxicology, University of Southern Maine, Portland, ME and* [2]*Department of Chemistry, University of Southern Maine, Portland, ME.*

Hexavalent chromium (Cr(VI)) is a well-established human lung carcinogen commonly encountered in occupational settings and in the environment. Water solubility is a key factor in the carcinogenicity of Cr (VI), with the particulate compounds more potent carcinogens. We investigated the role of particle dissolution in WTHBF-6 cells, a human lung cell line, after exposure to particulate lead chromate (LC). We found that LC was clastogenic in a concentration-dependent manner with 0.1, 0.5, and 1 ug/cm^2 LC damaging 10, 27 and 37% of metaphases respectively, while 5, and 10 ug/cm^2 caused complete cell cycle arrest. We further found concentration-dependent increases of intracellular Cr levels of 1.3, 5.3, 8.4 and 16.7 uM/cell and extracellular Cr levels of 0.3, 1.2, 1.9 and 4.7 uM at these concentration. We investigated particle internalization using electron microscopy and found that in as little as 1 h, LC particles were internalized in tight vacuoles after cells were exposed to the four highest concentrations. In addition, there was an apparent relative increase with concentration; however, there was no particle internalization at the lowest dose (0.1 ug/cm^2) even after 24 h. Further, we found no lysosomal association with the vacuoles containing particles suggesting that intracellular dissolution may not have occurred. Co-treating the cells with LC and vitamin C decreased extracellular dissolution of the particles and eliminated both uptake of ionic chromium and the clastogenic activity of the particles, but had no effect on internalization of the particles. Thus these data indicate that LC clastogenesis is mediated by the extracellular dissolution of particles and not intracellular dissolution or internalization. This work was supported by NIEHS grant 1R01 ES10838-01 (J.P.W.)

282 CHARACTERIZATION OF DNA DAMAGE INDUCED BY DEPLETED URANIUM.

M. Yazzie[1], C. Salanga[1], A. M. Hays[2], R. Ahmad[2], E. R. Civitello[1], R. C. Lantz[2] and D. M. Stearns[1]. [1]*Chemistry and Biochemistry, Northern Arizona University, Flagstaff, AZ and* [2]*Cell Biology and Anatomy, University of Arizona, Tucson, AZ.*

The overall goal of this research is to measure the chemical genotoxicity of uranium. Previous work has shown that depleted uranium as uranyl acetate produced single strand breaks in plasmid DNA in the presence of vitamin C (ascorbate) *in vitro*. The purpose of the current study was two-fold: (1) to characterize the molecular mechanism of UA-induced strand breaks *in vitro*; and (2) to measure the production of DNA strand breaks in cultured cells. Incubation of depleted uranium as uranyl acetate with ascorbate in the presence of thymidylyl (3'-5')-2'-deoxyadenosine (dTpdA) produced hydrolysis of the dinucleotide by HPLC; however, the reaction was not as efficient as that of cerium(IV) with dTpdA. These data are consistent with the interpretation that uranyl ascorbate-induced strand breaks are not completely radical-mediated *in vitro*. Because either metal-catalyzed DNA hydrolysis or radical-induced DNA fragmentation may be readily repaired in cultured cells, the presence of DNA damage was sought in the Chinese hamster ovary (CHO) EM9 line, which is sensitive to DNA strand breaks. The presence of DNA strand breaks was detected by alkaline single cell gel electrophoresis (the comet assay). Greater tail moments were observed in the CHO EM9 line relative to the CHO AA8 line for all doses of H2O2 (40 - 80 uM) and uranyl acetate (50 - 300 uM) tested. Understanding the molecular mechanisms through which depleted uranium reacts with DNA will help to elucidate mechanisms of lung and other cancers in populations exposed to uranium. Supported by NIH grants CA096320 and CA096281.

283 HPRT MUTATIONS INDUCED BY URANYL ACETATE IN CHINESE HAMSTER OVARY AA8 AND EM9 CELLS: EFFECT OF DNA REPAIR INHIBITION.

D. M. Stearns[1], V. H. Coryell[1], A. Bradley[1], A. M. Hays[2], N. Denipah[2] and R. C. Lantz[2]. [1]*Chemistry and Biochemistry, Northern Arizona University, Flagstaff, AZ and* [2]*Cell Biology and Anatomy, University of Arizona, Tucson, AZ.*

Questions about possible adverse health effects from environmental and occupational exposures to uranium have arisen as a result of uranium mining, residual mine tailings, and the use of depleted uranium in the military. Previous work has shown that depleted uranium as uranyl acetate (UA) produced DNA strand breaks in the presence of vitamin C. The purpose of the current study was to measure the toxicity of depleted uranium as UA in mammalian cells. The cytotoxicity and genotoxicity of UA was compared in the parental CHO AA8 line, as well as the CHO EM9 line, in which the repair of DNA strand breaks is impaired. Cytotoxicity was measured by clonogenic survival. A dose of 200 uM UA over 24 hr produced 1.8-fold greater cell death in the CHO EM9 than the CHO AA8 line, and a dose of 300 uM was 3-fold more cytotoxic. By comparison the CHO EM9 line was 2-fold more sensitive to 100 uM H2O2 than the CHO AA8 line. Mutagenicity at the hypoxanthine (guanine) phosphoribosyltransferase (hprt) locus was measured by selection with 6-thioguanine. A dose of 200 uM UA produced 10-fold higher induced mutation frequency in the CHO EM9 line relative to the CHO AA8 line. The hypothesis that UA was more cytotoxic and genotoxic under conditions of DNA repair inhibition was also supported by the observation that CHO AA8 cells exposed to UA in the presence of sodium arsenite were more sensitive to University(VI) than cells exposed to uranium in the absence of arsenic. These data may have implications for humans exposed to drinking water containing both uranium and arsenic. Supported by NIH grants CA096320 and CA096281.

284 MOLECULAR ANALYSIS OF HPRT MUTATIONS INDUCED BY CHROMIUM PICOLINATE IN CHO AA8 CELLS.

V. H. Coryell and D. M. Stearns. *Chemistry and Biochemistry, Northern Arizona University, Flagstaff, AZ.*

The dietary supplement chromium picolinate (CrPic) has previously been shown to induce mutations at the hypoxanthine guanine phosphoribosyl transferase (hprt) locus in Chinese hamster ovary (CHO) cells; however, the molecular mechanism

underlying this genotoxicity is unknown. The purpose of the current work was to characterize the nature of the mutations caused by this dietary supplement. Cells were exposed to 80 ug/cm2 chromium picolinate for 48 hr, and clones resistant to 6-thioguanine were expanded. The hprt mRNA from 42 mutants was amplified, using reverse-transcriptase polymerase chain reaction (RT-PCR) with a nested PCR, and this amplified cDNA was sequenced. Genomic DNAs of the cDNA deletion mutants were also amplified, using hprt exon-specific primers, and visualized on agarose gels. Thirteen of the mutants had point mutations evident in their cDNA; specifically, 11 had an A-to-T substitution at nucleotide 217 changing amino acid 73 from lysine to a termination signal, and 2 had a T-to-A mutation at nucleotide 220 resulting in a change at amino acid number 74 from phenylalanine to isoleucine. Four mutants were missing exon 6 (one of these was also missing exons 2-3 in its cDNA). Analysis of the genomic DNA of these mutants indicated that the deletion of exon 6 from the cDNA was due to a true genomic deletion of exon 6. Twenty seven other mutations were inferred to be splice site mutations due to the lack of evidence of genomic deletions; specifically one lacked exon 8, one was missing exon 2 (as well as having a point mutation); five mutants were missing exons 2 and 3, seventeen mutants lacked exons 2, 3, and 4; and three mutants lacked exons 2, 3, 4, 5, and 6 in their cDNA but not in their genomic DNA. At this time, the independence of identical mutants is not assumed; however, of the eight different types of mutations observed, 7/8 were putative point mutations. Elucidating the molecular pathways of chromium picolinate mutagenesis will aid in the evaluation of the safety of this human dietary supplement. Supported by NIH grant # CA75298.

285 CARCINOGENIC HEAVY METALS, AS^{3+} AND CR^{6+}, INCREASE THE AFFINITY OF NUCLEAR LIPOCORTIN I HETEROTETRAMER FOR DAMAGED DNAS.

A. Hirata, Y. Hou, G. B. Corcoran and F. Hirata. *Pharmaceutical Sciences, Wayne State University, Detroit, MI.*

Heavy metals such as As^{3+} and Cr^{6+} induce gene mutations. Accumulated evidence suggests that while they alone may not be mutagenic, they promote the mutagenic effects of DNA damaging agents. Therefore, these heavy metals apparently modify the DNA replication machinery to promote the translesion DNA synthesis bypassing damaged DNA sites, thereby causing mutations. We have recently demonstrated that purified nuclear lipocortin/annexin I heterotetramer can exhibit both ssDNA binding and helicase activities, essential for the translesion DNA synthesis. In this communication, we investigated its DNA binding and helicase activities with damaed DNA oligonucleotides as templates. Selected individual G*s in the P1, $^{5'}$GTCCACTATTAAAG*AACGTGGACTCCAACG*TCAAAGG*GCGAAAAACG*TCTATC-AGGGCGATGGCCCACTACGTGAACCA$^{-3'}$, was replaced in each template by 8-oxo-G. Over various concentrations of the 8-oxo-G DNAs (14G, 30G, 37G and 48G), lipocortin I was found to have higher affinities for these damaged polynucleotides. To examine if heavy metals alter the specificity of lipocortin I for nucleotide sequence at the damage sites or if they alter the affinity of lipocortin I for damaged DNAs, we investigated effects of heavy metals on the DNA binding and DNA annealing activities of lipocortin I with 14G, 30G, 37G and 48G in the presence of various concentrations of As^{3+} and Cr^{6+}. Concentrations of these heavy metals required for half maximal activities were essentially same with those (3 and 2.5 μM, respectively) for the naive oligonucleotide template, P1. When concentrations of 14G were varied, they apparently increased the affinity for the template rather than stimulated the maximal activity. From these results, we concluded that the carcinogenic heavy metals, As^{3+} and Cr^{6+}, increased the translesion helicase activity of lipocortin I by replacing Ca^{2+}, thus facilitating translesion DNA synthesis. (Supported in parts by ES10814 from NIEHS, NIH.)

286 CHROMOSOMAL INSTABILITY AS CONSEQUENCES OF MICROTUBULE INJURY BY CADMIUM.

I. Chou[1], Y. Zhao[1] and W. Li[2]. *[1]Microbiology, Boston University School of Medicine, Boston, MA and [2]Biochemistry, Boston university School of Medicine, Boston, MA.*

Cadmium[Cd(II)], a known human carcinogen, induces depolymerization of microtubules (MT), a major element of the cytoskeleton. MT are fundamental in regulation of cell division. Spindle MT organize chromosomes and are critical in pulling chromatids toward the spindle poles in mitosis. Thus, MT structural damage by Cd(II) could translate into functional deficiencies resulting in chromosomal abnormality. We tested this possibility by examining Cd(II) effects on cell cycle progression, cell division aberrations and chromosomal damage in cultured rat lung fibroblasts (RFL6). FACS analysis showed that serum-stimulated cell cycle progression was inhibited by Cd(II) in a dose (0, 2, 4 and 8 μM)-dependent manner. Fluorescent double staining of spindle MT and chromosome showed that Cd(II)-exposed cells exhibited various cell division aberrations manifested by a defect in or the absence of spindle MT accompanied by chromosomal lagging, scattering, clustering, etc. (in 60% of 300 cells counted), as compared to control cells (in only 2% of 300 cells counted). Furthermore, chromatid structural damage revealed by Giemsa-staining in hypotonically prepared chromosomes, e.g., gaps, breaks, deletions, etc., occurred at metaphase in 25% of 8 μM Cd-treated cells compared to 3% in the control. In addition, Cd(II) treatment markedly increased the number of cells with anuploidy and polyploidy, reaching 25% and 18% in 8 μM-Cd(II) exposed cells in comparison to 12% and 11% in controls, respectively. These results suggest that chromosomal instability manifested as disorientations and structural/numerical aberrations are closely linked to Cd-induced spindle MT damage with relevance to mechanisms of Cd carcinogenesity. (supported in part by NIH grants R01-ES 11164 and R01-ES 11340 and Philip Morris external research program).

287 ARSENITE MEDIATES GENE EXPRESSION IN HUMAN BLADDER EPITHELIUM.

X. Zheng[1], T. G. Bredfeldt[1], G. S. Watts[2], S. E. Vaught[2], A. G. May[2] and A. Gandolfi[1]. *[1]Pharmacology/Toxicology, University of Arizona, Tucson, AZ and [2]Arizona Cancer Center, University of Arizona, Tucson, AZ.*

Epidemiological studies have demonstrated an association between elevated arsenic levels in drinking water and the incidence of urinary bladder transitional cell carcinomas. The mechanism(s) of this toxicity are not defined, but should involve genetic alteration. We therefore profiled the genes regulated by arsenite in uroepithelia cells using microarray. UROtsa (human urothelium) cells were exposed to 30 μM sodium arsenite (AsIII) for 6 hr. The total RNA was isolated from treated and untreated cells followed by labeling with Cy3 or Cy5. The labeled probes were hybridized to a human oligonucleotide microarray (18, 861 genes) fabricated on site by the microarray core service. Hybridizations were performed three times using independent total RNA preparations to ensure reproducibility. The differential expressed genes were identified based on 2-fold cut off up or down and statistical (t-test analysis, $p<0.05$, after multiple testing correction) significance. A total of 109 altered genes were reported, among them 63 genes were up-regulated and 46 genes were down-regulated. About 20 percent of the up-regulated genes are stress response genes (HMOX1, MT and HSP gene families, etc). Together the altered genes were involved in regulation of cell cycles (CDC10, CCND1), transcription (KRML, SFRS1), translation (ERF4G2), signal transduction (ASIP, MCP), and DNA damage (DDIT3), oncogene (DEK, RPN1). These results are consistent with our previous array study on As(III) on HEK293 cells. Clearly, our array study suggests that arsenite induces complex cellular injury and the cellular adaptation to As(III) is associated with gene expression changes (NIEHS 04940, 06694 and NCI 023074).

288 CADMIUM AND HYDRAZINE TOXICITY IN BRL 3A CELLS AND PRIMARY RAT HEPATOCYTES BASED ON BIOCHEMICAL AND GENE EXPRESSION ANALYSIS.

S. Hussain[1], K. Geiss[2], J. Schlager[2], V. Chan[1] and J. Frazier[2]. *[1]Air Force Research Laboratory, Mantech Environment, Dayton, OH and [2]AFB, Dayton, OH.*

The present study was conducted to compare the toxicity of cadmium and hydrazine in primary hepatocytes and the rat liver cell line (BRL 3A) in order to select the best model cell line for initial toxicity screening of chemical compounds. A comparison of the gene expression pattern of primary rat hepatocytes and BRL 3A cell line was also investigated to further define cell similarities. To obtain similar growth states of primary cells and cell lines, the BRL cell line was cultured until confluent in 6-well plates. DNA synthesis was evaluated by thymidine (^{3}H) incorporation assay and showed a minimum rate of DNA synthesis at confluency suggesting contact inhibited cell stasis. After obtaining similar culture density, the primary hepatocytes and BRL 3A cells were exposed separately to cadmium (Cd) or hydrazine (HzN) for 2 h and further incubated in normal media for 24 h in the absence of test chemical. Biochemical end points for toxicity evaluation were LDH leakage, MTT reduction, GSH, and total ATP content. The BRL cells were found to be more resistant to Cd using all biochemical analysis whereas no difference was found with HzN compared to primary cells. The fluorescence probes YO-PRO-1 and propidium iodide were used to detect apoptotic, necrotic and *via*ble cells in Cd exposed cells. Cd induced apoptosis in BRL 3A cells at 50 μM while a much lower concentration (1 μM) was required in primary rat hepatocytes. The results of DNA fragmentation indicated that BRL cells are more resistant to Cd-induced apoptosis than HzN. Baseline gene expression analysis (Affymetrix Rat Genome U34A) indicated significant differences in gene expression profiles between confluent BRL cells and rat primary hepatocytes. The differences in basal gene expression observed between primary and immortalized BRL 3A cells appear to support the increased sensitivity of primary cells to Cd exposure.

289 CHROMIUM INHIBITS TRANSCRIPTION FROM PAH-INDUCIBLE PROMOTERS BY BLOCKING THE RELEASE OF HDAC AND PREVENTING THE BINDING OF P300 TO CHROMATIN.

Y. Wei[1, 3], M. Huang[1], M. Sartor[1], K. Tepperman[2] and A. Puga[1]. [1]*Center for Environmental Genetics and Department of Environmental Health, University of Cincinnati, Cincinnati, OH,* [2]*Department of Biological Sciences, University of Cincinnati, Cincinnati, OH and* [3]*MPH Program, Fort Valley State University, Fort Valley, GA.*

Chromium exposure has been shown to alter inducible gene expression, possibly due to the formation of chromium-DNA adducts, chromium-DNA cross-links, or to the disruption of transcriptional activator/coactivator complexes. Earlier studies from this laboratory have demonstrated that exposure of mouse hepatoma Hepa-1 cells to chromate inhibits the induction of the Cyp1a1 and Nqo1 genes by dioxin. To test the hypothesis that chromium blocks gene expression by interfering with the assembly of productive transcriptional complexes in the promoters of inducible genes, we have studied the effects of Cr on the expression of genes induced by B[a]P, another AHR agonist, and used chromatin immunoprecipitation assays to characterize the disruption of Cyp1a1 gene expression by chromium at the promoter level. Real-time RT-PCR and luciferase reporter gene assays showed that exposure of Hepa-1 cells to chromium caused a dose-dependent inhibition of both B[a]P-induced Cyp1a1 mRNA and luciferase activity. High density microarray analysis revealed that the inhibitory effect of chromium on B[a]P-dependent gene induction was generalized, affecting the inducibility of over 50 different genes involved in a variety of signaling transduction pathways. Immunoprecipitation of chromatin with anti-histone deacetylase-1 (HDAC-1) and anti-p300 antibodies showed that chromium prevented the B[a]P-dependent release of HDAC from Cyp1a1 chromatin, blocking the binding of p300 to the Cyp1a1 transcriptional complex and inhibiting gene transcription. These results provide a mechanistic explanation to the well-known observation that chromium inhibits inducible, but not constitutive gene expression. (Supported by NIH grants ES010807, P42 ES04908, P30 ES06096 and by a grant from Phillip Morris USA).

290 C-TERMINAL DELETION MUTANT OF MRE-BINDING TRANSCRIPTION FACTOR-1 INHIBITS MRE-DRIVEN GENE EXPRESSION.

T. Kimura[1], N. Itoh[2], T. Sone[1], K. Tanaka[2] and M. Isobe[1]. [1]*Department of Toxicoligy, Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Japan and* [2]*Department of Toxicoligy, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Japan.* Sponsor: M. Cherian.

MRE-binding transcription factor (MTF)-1 activates transcription in response to oxidative stress and regulates the expression of some cytoprotective factor genes, such as metallothionein, γ-glutamyl systeine synthetase and Cu/Zn-SOD. Therefore, it is thought that MTF-1 plays a role in cellular stress response. However, the physiological role of MTF-1 remains unclear because of lacking MTF-1 specific activator and/or inhibitor. To obtain MTF-1 specific inhibitor, we construct C-terminal deletion mutant of MTF-1 (MTFΔC). The MTFΔC could bound MRE, and compete MTF/MRE complex formation. Transient expression of MTFΔC could inhibit MRE-driven gene expression in HepG2 cells. Thus, MTFΔC was dominant-negative mutant of MTF-1. HepG2 cells stably expressing MTFΔC showed increased susceptibility to the cytotoxic effects of *tert*-butyl hydroperoxide (*t*BH). Furthermore, we constructed the recombinant adenovirus Ad5MTFΔC, contains an MTFΔC construct. Infection of the virus inhibited endogeneous MTF-1 and increased susceptibility to the cytotoxic effects of *t*BH. These results indicated that MTF-1 might help to control cellular redox state. Two other regultors of cellular stress response, namely c-Jun and NF-κB, could inhibit by the regulator specific inhibitors, such as a deletion mutant of c-Jun which acts as dominant-negative mutant, a mutated form of IκBα which acts as a superrepressor of NF-κB activity. The specific inhibitors were of utility value for clarify the role of c-Jun and NF-κB. The MTF-1 specific inhibitor, MTFΔC, might be useful tool to investigate the role of MTF-1.

291 THE TWO ISOFORMS OF RAT METALLOTHIONEIN ARE COORDINATELY REGULATED *IN VIVO*.

D. M. Todd[1, 2], N. DelRaso[2], J. Gearhart[3, 2], V. Chan[3, 2], D. Mahle[3, 2] and J. M. Frazier[2]. [1]*Wright State University, Dayton, OH,* [2]*AFRL/HEST, Wright-Patterson AFB, OH and* [3]*ManTech Environmental Technology, Inc., Dayton, OH.*

Metallothionein (MT) is an inducible protein whose unique structure contributes to its ability to detoxify heavy metals. Cadmium (Cd), which is an environmental pollutant and a hazard to both humans and animals, is detoxified by binding to MT. This study focuses on only MT I and MT II and limited information has been reported about their pattern of mRNA expression. This study investigated the hypothesis that the two isoforms of rat metallothionein are coordinately regulated *in vivo*. Untreated rats were compared with treated rats that were exposed to 0.5, 1.0 or 2.0 mg/kg Cd acetate and the MT levels were determined by Real-Time PCR at various time points (0, 0.5, 1, 3, 6, 9, 12, 18 and 24 hours). The fold increase in MT expression was determined using the comparative C_T method and plotted versus time. MT I exhibits a similar induction pattern at the 0.5, 1.0 and 2.0 mg/kg doses up to the 6 hour time point. By 12 hours both the 0.5 mg/kg and 1.0 mg/kg doses decline to baseline levels whereas the 2.0 mg/kg dose remains elevated until 24 hours. MT II's pattern of induction is similar for all three doses up to 9 hours. At 12 hours, the 2.0 mg/kg dose increases slightly and then decreases at 24 hours. The 0.5 mg/kg and 1.0 mg/kg doses of MT II both decline at the 12 hour time point and increase up to 24 hours. In comparing MT I and MT II expression, the results demonstrate that in all doses MT II peaks first followed by a decline at 3-6 hours at which time MT I peaks. As MT I decreases at 9 hours, MT II peaks at 9 hours. At the 0.5 mg/kg and 1.0 mg/kg doses both MT I and MT II decrease at 12 hours followed by an increase through to 24 hours. Even though MT I and MT II show similar induction patterns after 9 hours across all doses, the initial time points (<6 hours) exhibit a much different response. After comparing MT I and MT II mRNA patterns of induction, it is evident that the two isoforms are not coordinately regulated *in vivo*.

292 TOXICOGENOMICS OF DRINKING WATER ARSENIC *IN VIVO*: EFFECTS OF REPLICATES ON MICROARRAY ANALYSIS.

J. C. Davey[1], A. S. Andrew[1], A. Barchowsky[2], N. V. Soucy[1], D. D. Mayka[1], R. Lantz[3], A. Hays[3] and J. W. Hamilton[1]. [1]*Pharmacology & Toxicology, Dartmouth Medical School, Hanover, NH,* [2]*Environmental & Occupational Health, University of Pittsburgh, Pittsburgh, PA and* [3]*Cell Biology & Anatomy, University of Arizona, Tucson, AZ.*

Chronic exposure to low levels of arsenic in drinking water is associated with increased risk of lung cancer, cardiovascular disease and other diseases. The mechanisms underlying these various adverse effects are not known. As one approach, we have examined the effects of arsenic on gene expression in various target tissues using a mouse model. Affymetrix arrays of differential gene expression in lung tissue were used to examine the statistical impact of biological replication in deciphering functional genomics data. Three adult male C57BL/6 mice each were treated with or without 50 ppb arsenic(III) trioxide in drinking water for 5 weeks. Lung RNA from individuals was hybridized separately to Affymetrix mouse 430(A) 22, 000 gene probe arrays. With an n=1, comparison of individual control v. treated arrays for differential expression gave a range of 61 to 759 genes differing by 1.5 fold or greater, depending on the data pairs analyzed. Increasing the replication from n=1 to n=2 for control and treated and using coefficient of variance analysis narrowed the scope and range to 22-37 differentially expressed genes but with only partial overlap of resulting gene lists. Increasing replication to n=3 using the same analysis resulted in 25 differentially expressed genes with partial overlap to n=2 lists. A similar statistical trend was seen with analysis of heart array data. Differential gene expression was further statistically analyzed with the n=3 data sets using normalization with dCHIP/MBEI and Robust Multichip Analysis (Iobion) followed by statistical analysis using Bayesian statistics, SAM (Significance Analysis of Microarrays), ANOVA and simple fold change variation with similar results. These results indicate the importance of biological replication and multiple statistical comparisons in functional genomics studies. Supported by NIEHS SBRP grants ES07373 and ES04940.

293 DOSE-DEPENDENT ALTERATION OF OXIDATIVE STRESS AND DNA REPAIR GENE EXPRESSION BY DIMETHYLARSINIC ACID [DMA(V)] IN TRANSITIONAL EPITHELIUM OF URINARY BLADDER FROM FEMALE F344 RATS.

B. Sen[1, 2], A. Wang[3], S. D. Hester[1], J. L. Robertson[3] and D. C. Wolf[1]. [1]*Environmental Carcinogenesis Divison, USEPA, Research Triangle Park, NC,* [2]*NRC, Research Triangle Park, NC and* [3]*VA-MD College of Veterinary Medicine, Blacksburg, VA.*

Millions of people are at risk from drinking arsenic (As) contaminated water and exposure is associated with an increased incidence of urinary bladder cancer. DMA(V) is a major methylated metabolite of inorganic As and results in dose-dependent increase in urinary bladder tumors in F344 rats. The tumors arise from the transitional epithelium. Study of this target cell population is critical in understanding molecular events that develop following exposure. Enhanced cell proliferation, oxidative damage, and loss of DNA repair fidelity are implicated in As mediated toxicity and carcinogenicity. Simultaneous interactions of these effects may be important in As mediated neoplasia in the transitional epithelium of the urinary bladder. Female F344 rats were treated with DMA(V) (1, 4, 40 or 100 ppm) in the drinking water continuously for 28 days. After treatment, rats were euthanized, the bladder removed, flushed with saline, infused with Trizol TM and the contents were removed and frozen. Total RNA from the transitional epithelium was ex-

tracted and gene expression analysis using a custom spotted array with 4, 362 rat genes was performed. Analysis (GeneSpringTM) showed that 6.5% of all genes passed the statistical filter (1 way ANOVA, $p<0.05$). Genes involved in DNA repair and cellular response to oxidative stress were significantly altered. DMA(V) was also modulated expression of genes associated with protein degradation and several oncogenes. There was a dose-dependent response to the pattern of gene expression. As compared with controls, expression levels of most genes were up to 3-fold increased after 4ppm and decreased after 40ppm. Detection of genome wide dose-dependent effects in the target cell population may result in the development of reliable and sensitive markers of As toxicity and carcinogenicity. This abstract does not reflect EPA policy.

294 AGE-RELATED SUSCEPTIBILITY: A GENOMICS APPROACH.

J. E. Royland. *Neurotoxicology Division, USEPA, Durham, NC.* Sponsor: S. Padilla.

By the year 2030 more than 70 million Americans will be over the age of 65. These older adults are a subpopulation that may have special susceptibility to toxic insult due to critical characteristics of their life-stage. Current EPA testing guidelines do not identify the elderly as a special at risk group, however new initiatives have been implemented that focus research on this segment of the population. Monitoring global patterns of gene expression by microarray analysis allows one to examine multiple physiological mechanisms simultaneously. Our efforts are focused on pathways important in toxicological assessment. That is, pathways involved in ROS generation and elimination, enzymes involved in DNA maintenance and repair and genes that have wide ranging function in regulation of cell processes such as cell adhesion molecules, transcription factors and proteins in key signal transduction pathways. We used a commercial rat toxicology array (Atlas Rat Toxicology 1.2 from Clontech) to examine steady state expression levels of genes that have the potential to contribute to susceptibility. Hippocampus and striatum, brain areas associated with deficits in the aged, from the brains of non-dosed young (3 mos) and aged (23 mos) male Fisher rats were compared. Over 150 genes were found to be differentially expressed between the two age groups. Our data analysis concentrates on identifying and interpreting expression profiles in the different brain areas to determine potential region specific, age-related sensitivities. This abstract does not necessarily reflect USEPA policy.

295 THE ROLE OF HEME-OXYGENASE (HO-1) AND THIOREDOXIN IN RAT HIPPOCAMPAL ASTROCYTES PRETREATED WITH EBSELEN.

D. Hardej and L. D. Trombetta. *Pharmaceutical Sciences, St. John's University, New York, NY.*

Ebselen is a synthetic seleno-organic compound shown to act as a potent antioxidant and anti-inflammatory agent. It has been suggested that increases in thioredoxin(Trx) may play a role in cytoprotection by ebselen. Thioredoxins are a class of small 12KDa redox proteins involved in protection against oxidative stress and scavenging of free radicals. Heme-oxygenase 1 (HO-1) is a 32KDa protein that is induced by toxic insults such as exposure to metals, and UV radiation. Induction of HO-1 has been demonstrated to decrease cytotoxicity and deficiencies to exacerbate toxicity. Previous work in this laboratory has shown that ebselen induces HO-1 in rat hippocampal astrocytes in a dose dependent manner. Transcriptional sites NF-κB and AP-1 have been demonstrated to be involved in regulation of both HO-1 and Trx. The purpose of this study was first, to determine if ebselen induces Trx in a dose dependent manner as was seen with HO-1. Secondly, to determine the role HO-1 induction might play in cytoprotection by ebselen. Previously, 24h pretreatment with 30umμM ebselen was demonstrated to significantly protect against 250 and 500μM cisplatin toxicity in such cells. Since HO-1 and Trx have similar transcriptional regulation, this study examines the relationship of ebselen on HO-1 and Trx induction. Cells were pretreated with ebselen and HO-1 antisense oligonucleotides to block transcription of HO-1 to determine if inhibition would affect ebselen's ability to protect against cisplatin toxicity. Results indicate that treatment of rat astrocytes with 60 and 120μM ebselen results in a significant dose dependent increase in Trx. Blocking of HO-1 with antisense oligonucleotides does not decrease ebselen's cytoprotection against cisplatin toxicity. We conclude that ebselen induces both Trx and HO-1 and such induction may be related to cytoprotection by ebselen.

296 ACUTE AND REPEATED INHALATION OF TOLUENE BY RATS PERFORMING A SIGNAL DETECTION TASK LEADS TO BEHAVIORAL TOLERANCE ON SOME PERFORMANCE MEASURES.

W. M. Oshiro and P. J. Bushnell. *Neurotoxicology Division, USEPA, Research Triangle Park, NC.*

Previous work showed that trichloroethylene (TCE) impairs accuracy and latency in a signal detection task (SDT) in rats, and that these effects abate during repeated exposures if rats inhale TCE during SDT testing. The present experiment compared the effects of acute and repeated exposure to toluene, another commonly-used solvent. Sixteen male, Long-Evans rats were trained to perform the SDT. Upon completion of training, rats were divided into 2 groups (n=8) with equivalent accuracies and response latencies. In Phase 1, concentration-effect functions were determined for toluene (0, 1200, 1600, 2000, 2400 ppm) in both groups. In this phase, toluene reduced the proportion of hits, P(hit); did not affect the proportion of false alarms, P(fa); and increased response times (RT). In Phase 2, Group 1 was exposed to 1600 ppm toluene during 11 daily SDT sessions, while Group 2 performed the SDT in air. The effect of toluene on P(hit) abated in Group 1 during this phase, indicating the development of tolerance to toluene. In Phase 3, concentration-effect functions for P(hit) were re-determined and showed a shift to the right in both groups, indicating that both groups had developed tolerance to toluene. Although there was a trend for Group 1 to show more tolerance on P(hit) measures than Group 2, it was not significant. However, subsequent analysis of corrected P(hit), a measure of overall accuracy, indicated that Group 1 was less sensitive to toluene in Phase 3 than was Group 2. Thus, repeated inhalation of toluene can lead to behavioral tolerance, which can develop after as few as 4 exposures (as occurred in Group 2 during Phase 1). Unlike TCE, however, toluene did not increase P(fa), nor did tolerance develop on the RT measure, indicating a possible difference in the mode of action of these two solvents. (This abstract does not necessarily reflect EPA policy.)

297 CARBONYL SULFIDE INHALATION PRODUCES BRAIN LESIONS IN F344 RATS. DL MORGAN, PB LITTLE, VC MOSER, DW HERR, AND RC SILLS. NIEHS, Research Triangle Park, NC; PATHOLOGY ASSOCIATES, INC., Research Triangle Park, NC; ORD/NHEERL, USEPA, Research Triangle Park, NC.

D. Morgan[1], P. B. Little[2], V. C. Moser[3], D. W. Herr[3] and R. C. Sills[1]. *[1]NIEHS, Research Triangle Park, NC, [2]Pathology Associates, Inc., Research Triangle Park, NC and [3]ORD/NHEERL, USEPA, Research Triangle Park, NC.*

Carbonyl sulfide (COS) is an intermediate in the production of pesticides and herbicides, and is a metabolite of the neurotoxicant carbon disulfide. The potential neurotoxicity of inhaled COS was investigated in F344 rats. Male rats were exposed to 0, 75, 150, 300, or 600 ppm COS for 6h/d. After 2 exposures to 600 ppm rats exhibited signs of neurotoxicity. Primary lesions included necrosis in the parietal cortex, thalamus, posterior colliculus and anterior olivary nucleus. Male and female rats were exposed to 0, 300, 400, or 500 ppm COS for 12 exposures (6h/d, 5d/w). All 10 males and 4/10 females exposed to 500 ppm were moribund. Rats exposed to 400 ppm had slight gait abnormalities, hypotonia, decreased motor activity and grip strength. Lesions at 400 and 500 ppm included necrosis in the parietal cortex and putamen, and at 500 ppm included bilateral necrosis in the retrospenial cortex, thalamus, posterior colliculus, and olivary nucleus. In a 12-week (6h/d, 5d/wk) study, male and female rats were exposed to 0, 200, 300, or 400 ppm COS. Microscopic lesions were present primarily in the parietal cortex and posterior colliculus of rats exposed to 400 ppm. Occasional areas of necrosis were present in the putamen, thalamus and anterior olivary nucleus. Decreases in brain stem auditory-evoked responses were detected in the posterior colliculus, lateral meniscus and auditory olivary nucleus in rats exposed to 400 ppm COS for 12 weeks. A concentration related decrease in cytochrome oxidase activity was detected in the parietal cortex and posterior colliculus. Cytochrome oxidase activity was decreased at COS concentrations that did not cause detectable lesions, suggesting that brain lesions may be preceded by disruption of the mitochondrial respiratory chain by COS with a subsequent decrease in cellular ATP. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

298 INDUCTION OF C-FOS GENE EXPRESSION IN DIFFERENT RAT BRAIN LOCATIONS, AS AN EARLY RESPONSE OF NEURONAL ACTIVATION, AFTER TREATMENT WITH NMDA ANTAGONISTS.

B. P. de Wergifosse[1], B. Vanrossomme[1], W. Dewe[1], T. Murray[2], M. J. O'Neill[2] and K. Kramer[1]. *[1]Toxicology / Drug Disposition, Eli Lilly and Company, Mont-Saint-Guibert, Belgium and [2]Neurosciences Division, Lilly Research Centre, Windelsham, United Kingdom.* Sponsor: C. Thomas.

Introduction N-methyl-D-aspartate (NMDA) antagonists such as ketamine and MK-801 produce behavioural activation syndrome in experimental animals and can precipitate psychotic syndromes in stabilized schizophrenic patients. Immunohistochemical data in cingulate/frontal cortex showed a MK-801-dependent increase of glucose utilisation that coincided with the induction of the immediate early c-fos gene expression. The aim of this study was to use real-time quantitative RT-PCR to study baseline c-fos expression levels and its major sites of induction in different regions of the rat brain following a subcutaneous injection of ketamine or MK-801. Methodology Male Sprague-Dawley rats (n=8) received one

subcutaneous injection of MK-801 (1 mg/kg), ketamine (25 mg/kg) or vehicle (5% glucose). Two hours after treatment, anterior and posterior cingulate cortex, striatum, and hippocampus were dissected from each brain. After total RNA extraction and reverse transcription, cDNA templates were amplified with an in-house optimised ABI Taqman assay (PDAR 4327115). Results Baseline c-fos mRNA levels were found to be brain location specific (frontal cingulate cortex > anterior cingulate cortex > striatum > hippocampus), which may reflect the presence of various cell populations and specific neurotransmitter receptors. MK-801 (1 mg/kg) produced a significant 2.0 to 2.5-fold increase of c-fos gene expression levels in anterior cingulate cortex ($p<0.0001$). A trend of induction was also observed in frontal cortex and hippocampus ($p=0.07$ and $p=0.08$, respectively). Ketamine produced a trend in c-fos induction in hippocampus ($p=0.08$). No effect was observed in striatum. Conclusions These RT-PCR-based results are in agreement with c-fos immunohistochemistry data in similar brain locations and set baseline data for studies to measure neuro-toxicity or efficacy of similar class of compounds.

299 DEVELOPMENT OF A RELIABLE MOUSE MODEL OF VINCRISTINE-INDUCE NEUROPATHY.

F. A. Wininger, S. A. Steinberg and C. Massicotte. *Small Animal Clinical Sciences, University of Pennsylvania, Philadelphia, PA.*

Vincristine, one of the most frequently used chemotherapeutic agents in humans, predictably produces peripheral neuropathy. In mice, the degree of neuropathy varies significantly with dosing regimen, treatment interval and mouse strain. Moreover, the dose necessary to produce neuropathy can be debilitating, even fatal. Till now, a reliable mouse model of vincristine-induced neuropathy has been lacking. Therefore we chose to develop such a model, both for its own value, and to study its mechanism of neurotoxicity. Vincristine was administered at 1.8mg/kg twice weekly to 25 FVB-1 mice over 8 weeks. To assess the degree of neuropathy induced, a battery of motor function tests including footprint analysis, videotaped gait scoring, and tail nerve conduction velocity (TNCV) were performed at the sixth, seventh and eighth week. Test mice were sacrificed and their sciatic nerves harvested for histopathology. Of 25 mice dosed, 19 displayed the neuropathy, 3 showed no signs of the neuropathy within 8 weeks, and 3 died presumably of other toxic side effects. Videotape gait scoring was an accurate method of detecting the neuropathy with 95 % sensitivity and 100 % specificity. Footprint analysis was also an effective means of detection. Control mouse average stri width (3.45 ± .30cm) and stride length (10.03 ± 1.5 cm) differed greatly from experimental mouse average stride width (3.98 ± .42 cm) and stride length (7.97 ± .19 cm). TNCV of affected mice were significantly decreased in comparison to control mice. The average TNCV of control mice was 31.64 m/s (SD 6.55), while the average TNCV of experimental mice was 16.68 m/s (SD 3.70). Mild axonopathy with intact myelin sheaths was detected with light microscopy in the sciatic nerves of affected mice. Vincristine peripheral neuropathy is inducible in the mouse in a short dosing period and is easily detectable, thus making it an ideal model for examining underlying toxic mechanisms. Alteration of Na^+, K^+ -ATPase activity is being studied in our laboratory as a possible cause of vincristine toxicity.

300 ACRYLAMIDE-INDUCED REDUCTIONS OF AXONAL SODIUM AND POTASSIUM CHANNELS IN PROXIMAL AND DISTAL CNS AXONS: COMPARISON WITH PNS AXONS.

D. W. Sickles, J. Porter, T. Angela and D. Kumiski. *Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA.*

Inhibition of the motor protein kinesin and reductions in fast anterograde axonal transport are proposed critical actions of acrylamide (ACR) induced neurotoxicity (Sickles, Neurotoxicology 23:223-251, 2002). To determine whether these effects produce distal protein content deficits, we have used semiquantitative immunofluorescence of Na and K channels in proximal and distal CNS as well as PNS axons of male, Sprague-Dawley rats following high (50 mg/kg/d ip x 10d n=4;80-100 axons each) and low (20 mg/kg/d in drinking water x 48-50d; n=4;80-100 axons each) dosing rates of ACR. Behavioral analysis demonstrated ataxia with bothe high and low dosing rates; hindlimb paralysis was seen at the end of the chronic (49d)treatment. Both high and low dosing rates produced panoramic reductions (-20 to -60%)in K channel fluorescence in the nodes of Ranvier of sciatic and tibial nerve as well as the cuneate (proximal axons) and gracile (distal axons) fasciuli. Na channels were primarily reduced by low chronic dosing, especially in the distal axons of the tibial nerve and gracile fasciculus. Sodium channels were more affected by the low doses (-43% in tibial nerve, -40% in gracile fascicle) while higher doses generally reduced K channels more than Na channels. The results demonstrate reductions in both CNS and PNS axonal proteins following two different dosing rates of ACR known to produce different spectrum of pathologies. For both dosing rates, for both CNS and PNS axons, and for Na and K channels, distal axons were consistently reduced more than proximal ones. The greater effect on ion channels of distal axons is consistent with inhibition of fast transport as the cause of the reduction. The reductions in K channels were correlated with ataxia of animals in both high and low dosing rates. The reduction in Na channels was correlated with hindlimb paralysis. The differential response on Na and K channels indicates selectivity in the effect of ACR on the dynamics of delivery and/or turnover of ion channels. (Supported by NIEHS # ES 11223)

301 DEXTROMETHORPHAN DOES NOT CAUSE NEURONAL VACUOLATION OR DEGENERATION IN THE POSTERIOR CINGULATE/RETROSPLENIAL CORTEX OF RATS.

D. L. Shuey[1], T. P. O'Neill[2], R. Carliss[1] and R. J. Gerson[1]. *[1]Endo Pharmaceuticals Inc., Chadds Ford, PA and [2]WIL Research Laboratories, Ashland, OH.*

Dextromethorphan (DX) is a noncompetitive NMDA receptor antagonist. Potent NMDA antagonists (e.g., MK-801) produce specific neuropathologic changes in rodents, characterized by vacuolation and degeneration of neurons in the posterior cingulate/retrosplenial cortex after short-term dosing, and neurodegeneration in other limbic structures after chronic administration. In cultured cerebrocortical neurons, the neurotoxicity of NMDA antagonists is highly correlated with binding to the high affinity PCP receptor site. The affinity of DX and its metabolite dextrorphan for this site, based on Ki, are ~0.007x and 0.06x that of MK-801, respectively. This study was conducted to determine whether DX causes neuropathologic changes characteristic of potent NMDA antagonists. DX was administered orally for 30 days to Crl:CD®(SD)IGS BR rats at doses of 150, 275, or 400 mg/kg (males) and 120 mg/kg (females). The high doses were selected as maximum nonlethal doses, based on a range-finding study, although mortality occurred at the high doses in this study. MK-801, given as a single dose of 9 mg/kg (sc), was the positive control. Animals were killed 4-6, 24-28, or 48-52 h after the final dose. The brains were preserved by in situ perfusion fixation at necropsy, with subsequent immersion fixation. The brains were serial sectioned and evaluated using either amino cupric silver staining of thick frozen sections (right half) or hematoxylin and eosin (left half). Brains of animals given MK-801 showed expected histologic changes, including cytoplasmic vacuolation in neurons of the posterior cingulate/retrosplenial cortex 4-6 h after dosing, and argyrophilia and degeneration 24-48 h after dosing. Neuronal argyrophilia and degeneration in the piriform cortex, indisium griseum, and dentate gyrus were also observed in some animals. There were no findings in any animals given DX. Thus, DX does not produce histologic changes characteristic of potent NMDA antagonists in the brain, even after repeated administration of toxic doses.

302 THROMBIN PRECONDITIONING PROTECTS AGAINST 6-HYDROXYDOPAMINE, WHILE LARGE DOSES RESULT IN BEHAVIORAL DEFICITS.

J. Cannon, G. Xi, Y. Hua, T. Schallert and R. Keep. *Neurosurgery, University of Michigan, Ann Arbor, MI.*

Recent research suggests large doses of thrombin lesion dopaminergic neurons of the rat substantia nigra. However, low doses are protective against stroke models when administered prior to lesioning, termed thrombin preconditioning (TPC). Here, the ability of low (1U) and high dose (5U) thrombin to induce behavioral deficits and the ability of TPC to protect against behavioral deficits caused by 6-hydroxydopamine (6-OHDA) were evaluated. Male Sprague-Dawley rats (250-300g) were used. Thrombin groups received 1 (n=7) or 5 (n=7) University of thrombin in 50µL saline onto the medial forebrain bundle (MFB). 6-OHDA animals received stereotaxic injection of either saline (50 µL; n=10) or thrombin (TPC; 1U in 50 µL; n=12) onto the MFB 3 days before administration of 6-OHDA (10 µg/4 µL saline+0.01% ascorbic acid). Behavioral deficits were assessed prior to injection and for 3 weeks after. Vibrissae-elicited placing was measured for both sides and the percentage of unsuccessful placings determined. A forelimb asymmetry score was calculated by observing the use of the left, right or both forelimbs, during rearing. Thrombin (5 University) resulted in significant placing deficits on days 3 (93±7; mean±%SE), 7 (69±18), 14 (69±18) and 21 (14±14), while 1U did not (0±0% at all days). Similarly, significant asymmetry differences were observed on days 3 (87±8 vs. 14±13), 7 (65±13 vs. 5±9), 14 (73±7 vs. 6±7), and 21 (75±6 vs. 17±10). Neither TPC or saline induced deficits prior to 6-OHDA. The TPC group exhibited a significantly ($p<0.05$) lower placing deficit on days 7 (22±11 vs. 62±13; mean±%SE) and 21 (4±4 vs. 53±15%) than the saline pretreated group. The asymmetry score for TPC was also significantly ($p<0.05$) lower than the 6-OHDA group on days 3 (32±7 vs. 67±7), 7 (24±9 vs. 71±12), 14 (28±10 vs. 73±9), and 21 (24±10 vs. 67±11). The results indicate that 5U thrombin causes behavioral deficits and TPC provides protection against 6-OHDA. Investigation of the role of thrombin as both a neuroprotective and neurodegenerative agent in Parkinson's disease is warranted.

303 LONG-TERM SURVIVAL, MIGRATION AND PHENOTYPIC EXPRESSION OF MARROW STROMAL CELLS TRANSPLANTED INTO THE ADULT RAT BRAIN.

T. M. Coyne[1, 2], D. Woodbury[2] and I. Black[2]. *[1]Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ and [2]Department Neuorsi. & Cell Bio, UMDNJ Robert Wood Johnson Med. Sch., Piscataway, NJ.* Sponsor: K. Reuhl.

Neurotoxic injury in the adult is generally considered irreversible. However, recent findings provide hope that restoration of neuronal function can be fostered through adult stem cell transplantation. We recently differentiated adult bone marrow stromal cells (MSC) differentiate into neurons *in vitro*. We have now transplanted MSCs *in vivo* to define their behavior in the intact, adult brain. BrdU-labeled MSCs were grafted into the hippocampus, a region critical for working memory, and the striatum, a region which regulates motor programs. At 2, 6 and 12 weeks post-operatively, MSC survival, migration, and expression of neural genes were evaluated. At 12 weeks MSC survival was robust in both the hippocampus and striatum. At 2 weeks, the majority of cells transplanted into the hippocampus remained within the dentate hilus injection site with an apparent migratory subpopulation detected more than 1 mm from the graft core. Migration in the hippocampus appeared directed: at 6 and 12 weeks, BrdU-labeled cells were present predominantly along the dorsal leaf of the dentate gyrus. In the striatum, the majority of BrdU-labeled cells were present within the graft core, with apparent migration evident at 2 weeks. Migration within the striatum appeared non-directional, and continued with time: at 6 and 12 weeks, the number of BrdU-labeled cells distal to the graft core increased. Furthermore, small percentage of BrdU labeled cells in both graft regions expressed phenotypic markers of neurons, β-III tubulin and NeuN, and glia, GFAP. Our results suggest that adult MSCs exhibit neuronal ontogenetic processes, surviving, migrating, and expressing neuroglial-specific genes, after transplantation into the adult brain. Moreover, these characteristics encourage the development of neural transplantation strategies utilizing MSCs in the treatment of neurotoxic and neurodegenerative conditions. Support Contributed By: NIEHS (ES67148), NIH (HD23315), NJCSCR, & Zofchak Fund.

304 NEUROBEHAVIORAL EVALUATION AND KINETICS OF PEAK AND CONSTANT INHALATORY EXPOSURE TO TOLUENE IN HUMAN VOLUNTEERS.

J. H. Lammers, W. Meuling, L. van der Horst-Groeneveld, R. Pels Rijcken, D. de Groot and V. Feron. *TNO Nutrition and Food Research, Zeist, Netherlands.*

The health risks of inhalatory exposure to volatile organic solvents may not only depend on the total external dose, but also on the pattern of exposure. It has been suggested that exposure to regularly occurring peak concentrations (peak exposure) may have a stronger impact on the brain than constant exposure. Recent animal experimental studies conducted in our laboratory using relatively high concentrations of toluene have shown different effects on discrimination performance and motor activity during and after exposure, depending on the exposure scenario. Relevance of these findings for humans was evaluated in a double-blind, single dose crossover volunteer study. In two sessions with a one week interval in between, twelve healthy men (age 20-50) were exposed by inhalation for 4 hours to a constant concentration of 40 ppm toluene or to three 30-minutes exposure peaks at 110 ppm during a 4 hours period. Neurobehavioral testing using selected tests from the Neurobehavioral Evaluation System (NES) was performed repeatedly during and after exposure. Blood concentrations of toluene were measured at relevant time points. Toluene concentration in blood increased during constant exposure. Peak concentrations in the test atmosphere were reflected by fluctuating concentrations in blood, with the highest concentration measured at the end of the third exposure peak. No clear changes were observed on neurobehavioral measures of motor performance, attention, perceptual coding and memory, or on measures on mood and affect. Although peak concentrations relevant to occupational settings induce fluctuating concentrations in the blood (and presumably in the brain), these exposure conditions do not seem to induce significant changes in central nervous system function similar to those observed at high concentrations in animals.

305 NEUROPROTECTION AGAINST ENDOGENOUS OXIDATIVE STRESS IN AGING PROSTAGLANDIN H SYNTHASE-1 (PHS-1) KNOCKOUT MICE.

P. G. Wells[1, 2], A. Ramkissoon[1] and W. Jeng[1]. *[1]Pharmacy, University of Toronto, Toronto, ON, Canada and [2]Pharmacology, University of Toronto, Toronto, ON, Canada.*

PHS is implicated in neurodegeneration in aging, but the mechanism is unclear. To determine if PHS-1 expression is associated with increased oxidative DNA damage, we examined various brain regions of aging wild-type (+/+) PHS-1- normal, and heterozygous (+/-) and homozygous (-/-) PHS-1-deficient knockout mice for endogenous DNA oxidation evidenced by 8-oxo-2-deoxyguanosine formation, characterized both by high-performance liquid chromatography coupled with electrochemical detection, and by immunohistochemistry. Compared to congenic +/+ PHS-1-normal mice, PHS-1 -/- mice had lower DNA oxidation in the cortex ($p<0.002$), hippocampus ($p<0.02$), cerebellum ($p<0.002$) and brainstem ($p<0.04$). DNA oxidation was similarly lower in the cortex, ($p<0.02$) hippocampus ($p<0.01$) and cerebellum ($p<0.001$) of PHS-1 -/- than +/- mice ($p<0.02$, $p<0.001$), the latter of which had consistently less oxidative damage than +/+ mice. This PHS-1 gene dose-dependent effect was corroborated immunohistochemically, with PHS-1 -/- mice exhibiting less localized DNA oxidation than +/+ mice. To determine the potential mechanism for PHS-dependent reactive oxygen species (ROS) formation, purified PHS-1 was incubated *in vitro* with 2-deoxyguanosine and various endogenous neurotransmitters. Dopamine ($p<0.03$), L-DOPA ($p<0.04$) and epinephrine ($p<0.04$) but not glutamate or norepinephrine were excellent PHS substrates, resulting in PHS-dependent ROS formation that initiated DNA oxidation. These results provide the first evidence that endogenous ROS generated by PHS-catalyzed bioactivation of endogenous neurotransmitters may produce oxidative DNA damage in the CNS. This is consistent with the enhanced neurodegeneration observed in aging mutant mice deficient in the antioxidative enzyme glucose-6-phosphate dehydrogenase (Toxicologist 54: 178, 2000), and provides a novel hypothesis potentially relevant to the mechanisms and risk factors for age-related neurodegeneration. (Support: CIHR; SOT Covance Fellowship)

306 EVIDENCE FOR POSSIBLE STRAIN DIFFERENCES IN RESPONSE TO CLONIDINE IN THE ACCELERATING ROTAROD TEST OF MOTOR CO-ORDINATION IN SPRAGUE-DAWLEY AND HAN WISTAR RATS.

I. Strang[1], S. Palethorpe[1], K. Pitts[2], W. S. Redfern[1], J. Valentin[1] and T. G. Hammond[1]. *[1]Safety Pharmacology SAUK, AstraZeneca, Alderley Park, Cheshire, United Kingdom and [2]Drug Discovery DMPK, AstraZeneca, Alderley Park, Cheshire, United Kingdom.*

Introduction: The accelerating rotarod is an established test of motor co-ordination in rodents, frequently used in safety pharmacology studies. *Objective:* To validate this model for use in the evaluation of effects of compounds on motor co-ordination in the rat. Clonidine was used for the pharmacological validation - an alpha-2 adrenoceptor agonist which impairs motor skills in humans [1]. *Method:* Male Han Wistar (207-256 g) and Sprague-Dawley rats (233-268 g) were given 2 fixed-speed training sessions, followed by 1 accelerating session prior to dosing. Animals were then dosed with one of the following (n = 8 per group): vehicle, clonidine 0.1, 0.3 or 1.0 mg/kg po, and tested 45 minutes later. Blood samples were taken after rotarod testing (1 h 15 min post-dose), and clonidine concentrations in plasma analysed by LCMS-MS. *Results:* % Impairment of rotarod performance (normalised for vehicle) were as follows: (clonidine 0.1, 0.3 and 1.0mg/kg respectively for each strain) Han Wistar: -30±13% (NS), -11±13% (NS), 24±13% (NS); Sprague-Dawley: 48±13% ($p < 0.05$), 79±10% ($p < 0.01$), 99±9% ($p < 0.01$). Plasma analysis values were: (clonidine 0.1, 0.3, 1.0mg/kg respectively for each strain) Han Wistar: 24±2 ng/ml, 36±1 ng/ml, 50±7 ng/ml; Sprague-Dawley: 17±2 ng/ml, 12±1 ng/ml, 21±3 ng/ml. *Conclusion:* Despite higher plasma exposure to clonidine in Han Wistar rats, less impairment of motor co-ordination was demonstrated compared to Sprague-Dawley rats. This suggests a potential strain difference in sensitivity to clonidine in this test. Further work is required to assess whether this apparent difference is specific to this pharmacological class. [1] Frith CD (1989) Psychopharmacol 98: 120-5.

307 REAL-TIME RT-PCR MONITORED SELECTIVE ALTERATIONS OF GENE EXPRESSION IN MICE INDUCED BY MPTP.

Z. A. Xu, K. McCastlasin, D. Cawthon, W. Slikker and S. F. Ali. *Neurochemistry Lab., Division of Neurotoxicology, NCTR, Jefferson, AR.*

1-methyl-4-phenyl-1, 2, 4, 6, -tetrahydropyridine (MPTP) is a selective neurotoxin which produces dopamine depletion and causes parkinsonism like symptoms in humans and is used to generate animal models for Parkinson's disease (PD). In this study, C57BL/6N mice were treated with MPTP acutely (3×20mg/kg, 2-h interval) and subacutely (30mg/kg, 5 consecutive days). Mice were then sacrificed at 24 hours (acute study) and 72 hours (subacute study), and brain tissue was collected. Specific genes of dopaminergic system and parkinsonism-related were examined using real time RT-PCR in substantia nigra. Dopamine (DA) concentration and its metabolites were examined using HPLC analysis in striatum. In both dose models, MPTP significantly suppressed expression of tyrosine hydroxylase (TH), dopamine transporter (DAT), and vesicle monoamine transporter (VMAT), coinciding with the pattern of dopamine concentration changes with MPTP treatment. The decrease of DAT was much higher in the acute model (5 folds) than that in the subacute (1.6 fold), which may suggest a recovery of DAT 72 hours after MPTP treatment. Although significant decrease of DA metabolites were found in striatum, there were no change in the expression of monoamine oxidases (MAOa, MAOb) nor catechol O-methyltransferase (COMT) in either model, indicating that the

changes of these enzymes were at the protein level as previously reported. The decrease of a-synuclein expression was only observed in the acute dose model, suggesting different mechanisms between the acute and subacute MPTP treatment. In addition, b-synuclein and parkin were not altered in either animal model. Our study using real-time PCR indicate that MPTP selectively alters gene expression and provide information for clinical studies in PD.

308 PROTEIN/DNA ARRAYS INDICATE SELECTIVE ALTERATIONS OF TRANSCRIPTION FACTORS IN MPP$^+$-INDUCED NEUROTOXICITY IN PC12 CELLS.

S. F. Ali, Z. A. Xu, K. McCastlain and W. Slikker. *Neurochemistry Lab., Division of Neurotoxicology, NCTR, Jefferson, AR.*

MPP+ is the active metabolite of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), a compound causing parkinsonism in human and animal models. MPP$^+$-treated PC12 cultured cells are frequently used to study dopaminergic neurotoxicity and are a possible model for Parkinson's disease. MPP$^+$ depletes dopamine content and elicits cell death in these cells. Recently, differentiated PC12 cells have been widely used because they represent a more sensitive model than undifferentiated cells. However, the underlying mechanism of MPP$^+$-induced neurotoxicity in PC12 cells is still unclear. In this study, the dose response and time course of MPP$^+$-induced DA depletion as well as decreased cell *via*bility were determined at various time points. Protein/DNA-binding arrays were used subsequently to evaluate alterations of the PC12 cellular transcription factors (TF). Thirty-three TFs were identified in untreated cells with Panomics protein/DNA array I; many of them are related to neurotransmission, apoptosis, and oxidative stress. The data were analyzed with the bioinformatic software, Spotfire 7.1. K-means clustering identified five different patterns of protein/DNA-binding alterations, and modified Spotfire line charts were used to show the fold (%) changes from control with MPP$^+$ treatment. 6 of the 33 TFs increased and 5 decreased above 50% of control with MPP$^+$ treatment. In addition, 3 TFs decreased within the range of 25-50%. The alterations of these TFs were correlated to the changes of DA concentration and cell *via*bility, indicating a complex mechanism of MPP$^+$-induced neurotoxicity in PC12 cells. These data indicated that MPP$^+$ induced selectively altered TFs in PC12 cells, and may provide mechanistic information as a basis for animal studies with MPTP and clinical studies for Parkinson's disease.

309 THE INJURED NEURON / PHAGOCYTIC MICROGLIA RATIO "R" REVEALS THE PROGRESSION AND SEQUENCE OF NEURODEGENERATION.

R. L. Jakab and J. F. Bowyer. *Neurotoxicology, NCTR/FDA, Jefferson, AR.*

Is an observed neurodegenerative process in its initial, peak or declining phase? Which region did it start in, and where did secondary degeneration occur? Here we present an experimental approach that can reveal the temporal progression and regional sequence of neurodegeneration. The method is based (n> 100 rats) on the finding that degenerated neurons and phagocytic microglia form pairs and are detected at a 1:1 ratio at the peak of neurodegeneration induced by amphetamine or ephedrine, if the drug treatment does not disrupt the blood brain barrier. One (Fluoro-Jade labeled; FJ+) injured neuron activates one (isolectin B4-labeled; IL+) microglia, and the activated microglia will then phagocytose the neuron. As the phagocytic clearance progresses, the value of the injured neuron/phagocytic microglia (FJ+/IL+) ratio "R" moves from R>1 (<2 days after start of neurodegeneration) to R~1 (2-3 days after start) to R<1 (= 4 days after start), because the degenerating neuron appears and disappears earlier than its phagocytic counterpart. Using histological sections from animals sacrificed at 5 days after an amphetamine-induced neurotoxic insult as an example, it can be ascertained that neurodegeneration starts first in the piriform cortex, later in the parietal cortex and last in specific thalamic nuclei. This was determined by the fact that the Rthalamus > Rparietal cortex > Rpiriform. Furthermore, the R values for the regions indicate that, neurodegeneration occurred at 1-2, 1-3 and 2-4 days, respectively in the 3 regions. This was verified by assessing the first appearance of FJ+ neurons from histological sections obtained 1, 2, 3, 4 and 5 days after insult. Because FJ+ counts drop to near zero within 7 to 10 days and IL+ phagocytes within 10 to 14 days, and the fact that severe neurotoxic insults often disrupt the blood brain barrier, our approach is most applicable to mild to moderate neurotoxic insults and is most suitable for acute or subchronic neurotoxic exposures. Supported by the USFDA/NCTR.

310 DERMAL EXPOSURE TO JP8 JET FUEL: DISRUPTION OF AUDITORY FUNCTION, AND INDUCTION OF DERMATITIS IN RATS.

L. D. fechter[1] and R. Gallucci[2]. [1]*Research (151), Loma Linda VA Medical Center, Loma Linda, CA and* [2]*Pharmaceutical Sciences, University Oklahoma Hlth Sciences Ctr, Oklahoma City, OK.*

Impairment of auditory function can result both from exposure to specific chemical contaminants and, more commonly, from contaminants in the presence of noise. Among agents that produce such hearing loss are several aromatic hydrocarbons that are present in JP-8 fuel. This project was designed to determine whether JP-8 given by dermal exposure could produce impaired auditory function by itself or potentiate noise-induced hearing loss. The rationale for this work stems from the frequent association of exposure to both jet fuel and to noise. Rats were exposed to JP-8 alone by dermal exposure, an octave band of noise designed to produce a very limited degree of permanent hearing loss in a specific frequency region of the rats audiogram, both agents in combination, and no experimental treatment. Pure-tone auditory thresholds were assessed 4 weeks later in order to measure permanent impairments in hearing. Additionally, analysis of JP-8 treated skin indicated that the expression of a number of cytokines/chemokines were significantly induced by JP-8 exposure including; IL-6, Eotaxin, Mip 1a, Mip-2, MCP-1, RANTES, and IP-10. Histology of skin biopsies showed mild to moderate inflammatory infiltrate, characterized primarily by neutrophils, and macrophages. The results indicate that JP-8 exposure alone and noise exposure alone both were able to produce hearing loss. The effect of combined exposure to these agents was an additive impairment in hearing. Dermatitis associated with JP-8 exposure is characterized by the induction of multiple cytokines, and primarily involved neutrophilic infiltrate. (supported in part by a grant from AFOSR through the DEPSCoR program F49620-02-1-0274)

311 TETRALIN AND METABOLITES: PROTEIN REACTIVITY, CHROMOGENICITY AND NEUROTOXICITY.

V. S. Palmer[2], D. Tshala-Katumbay[1], S. B. Hashemi[1], M. I. Sabri[1, 2] and P. Spencer[1, 2]. [1]*Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR and* [2]*Department of Neurology, School of Medicine, Oregon Health & Science University, Portland, OR.*

We have reported the protein-reactive, chromogenic, and neurotoxic properties of the aromatic and aliphatic solvent metabolites, 1, 2-diacetylbenzene (1, 2-DAB) and 2, 5-hexanedione (2, 5-HD) (Kim et al., Toxicol. Appl. Pharmacology 183:55, 2002). These agents react with lysine-rich cytoskeletal proteins and induce neurofilamentous axonal swellings with secondary demyelination distally (2, 5-HD) or proximally (1, 2-DAB), respectively. Proximal demyelination also characterized rats treated with acetylmethyltetramethyltetralin (AETT) (Spencer et al., Science 204, 633, 1979), which causes excretion of green urine comparable to that seen in 1, 2-DAB-treated rodents. Tetralin, a component of paints and varnishes that induces neurological disturbances (headache, restlessness, seizures, lethargy, and coma) in children and adults, is also reported to cause excretion of green urine in humans and rodents (Spencer et al., Int. J. Environment Hlth. 205:131, 2002). Neither tetralin nor its alpha-tetralol metabolite formed a chromogen when reacted *in vitro* at high concentrations (100-500 mM) with bovine serum albumin, but alpha-tetralol formed a non-chromogenic protein adduct. The spinal cord, spinal ganglia, and sciatic nerves of 12-week-old C57Bl/6 male mice (~25 g) treated i.p. daily 5 days/week with saline containing 10 percent acetone were indistinguishable at 14 and 35 days from tissue removed from animals comparably treated with 3 mmol/kg/d tetralin or 0.34 or 0.67 mmol/kg/d alpha-tetralol, the doses of the latter two increasing 50 percent every two weeks up to 35 days. Animals showed dose-related drowsiness, acute respiratory distress, lethargy, and body weight changes, with greater acute toxicity in alpha-tetralol-treated animals. In sum, neither tetralin nor alpha-tetralol formed colored pigments with proteins nor did they trigger axonal neuropathy under the conditions studied. Supported by NIEHS grants ES10338 & ES11384 & the Oregon Workers Benefit Fund.

312 COMPARATIVE ANALYSIS OF NEUROPSYCHOLOGICAL TOXICITY OF BIOLOGICAL, CHEMICAL, AND PHARMACEUTICAL AGENTS.

M. Peterson and R. C. Pleus. *Intertox, Inc., Seattle, WA.*

Neuropsychological tests are often used in a clinical setting to assess potential damage from exposure to various agents including diseases, therapeutic and environmental agents. Toxicologists are often tasked with determining the cause and effect relationships for exposure to chemical and biological agents. When evaluating specific cases where an exposure to an alleged agent has occurred, it is necessary to evaluate all possible agents when determining the most probable cause of neuropsychological effects. A comprehensive review of the literature was conducted in relation to three different types of agents that can influence neuropsychological test interpretation: biological, chemical, and pharmaceutical agents. Our initial review focused on three representative agents: hepatitis C (biological), tetrachloroethylene (chemical), and cocaine (pharmaceutical). We present the results of this work with an emphasis on the comparative effects of these agents considering exposure and dose (e.g., chemical and pharmaceutical) and pathogenicity (e.g., biological). Our research indicates that it is critical to consider medical histories, temporal relationships, sign and symptom progression, normative values, and consistency of test results with the neuropsychological literature when assessing cause and effect from these agents.

313 DECREASED NEUROLOGICAL SIDE EFFECTS WITH ARIPIPRAZOLE: A RESULT OF FUNCTIONALLY SELECTIVE ACTIVATION OF DOPAMINE D_2 RECEPTORS.

J. D. Urban[1], E. A. Gay[2] and R. B. Mailman[2, 1]. [1]*Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC and* [2]*Department of Psychiatry, University of North Carolina, Chapel Hill, NC.*

High affinity D_2 dopamine receptor antagonism has long been implicated in the development of neurological side effects associated with the chronic administration of a number of antipsychotics. The recently marketed atypical antipsychotic aripiprazole demonstrates a very low incidence of these extrapyramidal side effects (EPS) despite the unusually high affinity the drug has for the D_2 receptor. We hypothesize that the low incidence of EPS caused by aripiprazole is due, in part, to its ability to activate certain functions linked to the D_2 receptor differentially, a mechanism known as functional selectivity or agonist trafficking. Our previous studies using two D_2-mediated functional endpoints (GIRK channel activation and GTPγS binding) showed aripiprazole to be an antagonist (Shapiro et al., 2003), although the compound was a partial agonist against adenylate cyclase (Lawler et al., 1999; Burris et al., 2002; Shapiro et al., 2003). The current study investigated the actions of aripiprazole at the D_{2L}-mediated activation of MAP kinase. Using CHO cells stably transfected with the hD_{2L} receptor, we measured MAP kinase activation by means of an ELISA. Relative to the intrinsic activity of the D_2 full agonist quinpirole, aripiprazole was determined to be a partial agonist. The data from effects on D_{2L}-mediated effects on adenylate cyclase and MAPK are being compared to D_2-mediated effects on phospholipase A_2 activation (i.e., $[^3H]$arachidonic acid release) in the same stably transfected CHO cell line. Together, these studies will offer insight into the D_{2L}-mediated molecular mechanisms of G protein functional selectivity, and their role in decreasing neurotoxic side effects of the atypical aripiprazole.

314 IS THERE A LINK BETWEEN FREE RADICAL FORMATION AND CELL DEATH.

F. Fonnum[1], T. Reistad[1], A. Dreiem[1] and E. Mariussen[2]. [1]*Protection, Forsvarets Forskningsinstitutt, Kjeller, Norway and* [2]*Norwegian Institute of Air Research, Kjeller, Norway.*

The purpose of this study is to discuss whether there is a correlation between free radical formation and cell death in cultured rat cerebellar granule cells. Free radical formation is assayed by the use of the fluorescent probe DCFH, and cell death is either monitored by trypan blue exclusion or by release of lactate dehydrogenase. Toxic compounds that are investigated are polychlorinated biphenyls, brominated flame retardants, perfluorinated compounds and hydrocarbon solvent. The results show that there is a correlation between free radical formation and cell death for PCB, although the higher PCB seems to be more toxic than expected from their DCFH-fluorescence. For brominated flame retardants tetrabromobisphenyl yields a high degree of DCFH-fluorescence and substantial cell death. The brominated diphenylethers are less active in DCFH-fluorescence, but active in killing cells. For the perfluorated compounds there is not a good correlation between cell death and DCFH-fluorescence. Aromatic and naphtenic hydrocarbons yield DCFH-fluorescence and cell death, whereas aliphatic hydrocarbons only produce free radicals.

315 COMPARISON OF RAT HIPPOCAMPAL GENE EXPRESSION UTILIZING LASER CAPTURE MICRODISSECTION (LCM), RNA AMPLIFICATION AND OLIGONUCLEOTIDE MICROARRAYS.

A. C. Kant[1], S. A. Ferguson[1], P. M. Douglass[2] and T. A. Patterson[1]. [1]*Neurotoxicology, NCTR/FDA, Jefferson, AR and* [2]*Agilent Technologies, Germantown, MD.*

The hippocampus has a crucial role in transferring short-term memory to long-term memory, with diminished function associated with aging. Previously, spatial learning and memory in water-reinforced complex maze and escape-reinforced Morris water maze tasks for three rat strains were assessed in our laboratory. Distinctive learning differences were observed across strains and within strain. To determine if memory and learning performance are correlated to differential gene expression, hippocampi from fast-learning (FL) and slow-learning (SL) Sprague-Dawley, Spontaneously Hypertensive and Wistar-Kyoto aged rats (15 months) were examined. Frozen brain sections were mounted on glass slides, and then stained and dehydrated. Cells from the CA regions of the hippocampus were collected using a PixCell IIe LCM system and total RNA was extracted. First round RNA amplification was performed using a RiboAmp OA RNA amplification kit. Briefly, from total RNA, double-stranded (ds) cDNA was synthesized prior to *in vitro* transcription to generate aRNA, which was used in second round amplification to generate additional ds cDNA. The ds cDNA was introduced into an Agilent low RNA input fluorescent linear amplification kit to generate labeled antisense cRNA. The FL and SL RNA samples within strain were labeled using Cy3 and Cy5 dyes, and were hybridized to Agilent rat oligo microarrays. Microarray slides were scanned and the data analyzed using the Rosetta Resolver data analysis system. Data analysis suggests minimal gene expression differences within strain between FL and SL rats, with approximately one dozen genes of interest. These expression differences will be verified using real-time PCR and the hippocampal gene expression between these aged rats and younger counterparts are currently being examined. By further exploring these changes, the molecular mechanisms of age-related memory disorders, such as Alzheimer's disease, may be elucidated.

316 STUDENT ROUNDTABLE ON EFFECTIVE PRESENTATIONS.

A. Wang. *Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA.*

The ability to present information to an audience in a clear, concise manner is a critical academic and career skill. An effective presentation conveys important knowledge, generally as a summarization of a larger body of data or ideas, often within a specified format or time frame, and provides a forum for a productive exchange of ideas. Typical formats include posters and oral presentations. These can be used in academic settings (e.g., an oral classroom presentation, a proposal defense, or a thesis/dissertation defense), at professional/scientific conferences (e.g., a poster presentation or a platform talk), or even in job interviews. This symposium will address some of the skills needed to deliver an effective presentation. Students will find the topics to be particularly useful and informative. The speakers will cover general concepts of communication, provide practical hints for organizing and conveying information in posters and oral presentations, and discuss the skills needed to effectively answer questions and comments from the audience.

317 PRESENTING AN EFFECTIVE POSTER.

S. C. Fitzpatrick. *Office of the Commissioner, USFDA, Rockville, MD.*

Presenting a poster is an exciting opportunity to showcase your research efforts to your scientific colleagues. However presenting an effective poster requires planning and thought. First, make sure your research is at a point where you have something valuable to present. Have you been able to draw scientifically valid conclusions from the work? Second, decide whether you really wish to present a poster or if you should be giving a platform presentation. Presenting a poster gives you the unique opportunity for one on one interaction with other scientists in your field. It enables you to get feedback and suggestions on your research. It may lead to collaborations with other scientists doing similar work. Third, decide who your audience will be and determine their level of understanding of the general field in which you work. Is this a specialty meeting where everyone is conversant in your field or is it more of a general science meeting? This will determine the level of background you must add to your poster to make it meaningful to those who read it. Think carefully about how you organize your poster. It must be attractive enough to draw readers to it in a room of many posters. The care and detail you put into putting together your poster is indicative of the overall care you put in doing your research. Make sure your print is big enough to read easily from about two feet away. Remember us senior scientists who may give you valuable feedback do not necessarily have the best eyesight anymore. Graphics that illustrate your research and are easily understood are one very positive and important element of good posters. Colors are more appealing than black and white. Think about what attracts you to a poster and incorporate some of these suggestions into your work. Finally relax and enjoy yourself. Speak up and show your excitement for your work. If your research is not interesting to you, it will not be to anyone else either. Ask questions of those who come to talk to you. This is an opportunity for you to get feedback to enhance your research- not simply a time to present your data.

318 PLATFORM PRESENTATIONS.

G. L. Kimmel. *Office of Research and Development, USEPA, Washington, DC.*
Sponsor: A. Wang.

A platform presentation provides the speaker with an opportunity to present information in an uninterrupted manner. It is important that the speaker prepare in a way that the audience will be able to understand to flow of thought, the conclusions drawn and the take home message. The current talk will focus on three considerations that are important to an effective platform presentation: the audience, the speaker, and the preparation. The audience is the group of people on the other side of the platform. Who are they? Academics, bureaucrats, lawyers, "the public?" They are there for a reason. What is it? Do they want to hear your latest research results? Do they expect an introduction to a series of talks that will follow? Or do they

expect a defense of a position that you or your organization has taken? They are there; but where is "there?" Are they physically in the same room or on video- or tele-conferencing? Each audience requires some consideration in preparing and giving a talk. The speaker is you. What type of speaker are you? Confident, nervous, loud, quiet, a reader, an ad liber? Regardless of what descriptor best fits, the important thing is to recognize it and use it to your advantage. Leaving your script can give the appearance that you are confident with your material. But, if this style of presentation is not you, it can be disastrous. With the audience and your speaking style in mind, the preparation can begin. What are the things that are most important? Give the audience a clear idea of what you are going to tell them. Generally, an outline at the beginning is very helpful for this. At the end, summarize for the audience what you've told them and reiterate the important points. Once the talk is drafted, practice! This will not only indicate whether you can get everything in within the allotted time period, but if the projector blows, you may be able to give the talk without missing a beat. And finally, when you have the talk within the assigned time period, cut it down. You will never have as much time as you think.

319 THINKING ON YOUR FEET.

B. A. Schwetz. *Office for Human Research Protections, Department of Health & Human Services, Rockville, MD.*

Answering questions from the audience after a presentation requires skills different from giving the talk itself. You can rehearse the talk until you are comfortable with its content; you can prepare visiuals that help you present your message. Once the questions start, you share control with the audience. How well you answer questions reflects how well you think under presssure and sometimes under unfriendly conditions. This presentation will offer advice on how to remain in control, how to buy time to think of a good answer, how to admit that you don't know an answer, and how to handle an unfriendly or hostile person in the audience. Answering questions well can enhance your credibility with peers and with the public.

320 GENE EXPRESSION INFLUENCES ON METAL IMMUNOMODULATION.

D. A. Lawrence[1]. [1]*Wadsworth Center, Albany, NY and* [2]*Wadsworth Center, Albany, NY.*

Metals can stimulate or modify immune responses by multiple modulatory mechanisms. The five topics in this symposium cover a wide range of different molecular means by which metals may directly or indirectly alter immunity, but all topics address differences that exist or can be better evaluated as a consequence of genetic differences or manipulations. The first presentation demonstrates how changes in the expression of metallothionein, whose exposure is modulated by certain metals, can alter immune responses at various cellular levels. The second talk discusses how genetic differences in one of the major histocompatibility complex antigens can affect T cell stimulation by beryllium. The third talk demonstrates how altered gene expression of select immune products such as cytokines can influence how lead modulates immunity. The fourth presentation will delve into the intracellular signaling networks to discuss genetic expressions associated with mercury-induced apoptosis of lymphocytes. The final talk will focus on the involvement of select genes in mercury exacerbation of autoimmune disease with emphasis on the various stages or check-points at which the development of autoimmune reactivities can be modified. In all, the presentations indicate that the metals can affect immune reactivities based on the genetics of the exposed cells as well as the influences of other environmental agents on altered gene expression.

321 INFLUENCE OF METALLOTHIONEIN GENE EXPRESSION ON STRESS-MEDIATED IMMUNOMODULATION.

M. A. Lynes[1], X. Yin[1], E. Canpolat[1], K. C. Crowthers[2], J. Youn[3] and N. Hadjout[1]. [1]*Molecular and Cell Biology, University of Connecticut, Storrs, CT,* [2]*Pathology, University of Massachusetts Medical Center, Worcester, MA and* [3]*Anatomy and Cell Biology, Hanyang University, Seoul, South Korea.*

Metallothionein is an unusual representative of the set of stress-response proteins that are produced by cells in response to changes in their environment. This small thiol-rich protein is usually associated with zinc and copper, and serves as an important reservoir of these essential metals. Metallothionein also acts as an important anti-oxidant, as a scavenger of toxic heavy metal cations, and as a regulator of specific transcription factor activity. In addition to these essential homeostatic roles, or perhaps because of them, metallothionein may serve as a critical regulator of immune functions in the context of toxicant exposures or oxidative stress. We have shown that metallothionein can be immunosuppressive, and that this suppression can be blocked with a monoclonal anti-metallothionein antibody. Moreover, we have shown that metallothionein-null animals display an enhanced response to antigen challenge. Our lab and others have shown that manipulation of metallothionein *in vivo* can result in changes to the progression of autoimmune disease. Targeted disruption of metallothionein in congenitally autoimmune animals accelerates the progression of disease, and injection of metallothionein can diminish the progression of induced models of autoimmune disease. The principal target appears to be the T cell: T dependent, but not T-independent, humoral responses are suppressed by metallothionein, and cytotoxic T cell function is also diminished in the presence of metallothionein. This suppression may reflect metallothionein interactions at the plasma membrane. Metallothionein binds to T cells in proximity to the T cell receptor, and can initiate T cell chemotactic responses. In view of the potential population diversity in the synthesis and release of metallothionein to the extracellular environment, this protein may represent an important susceptibility factor for toxicant-induced immunomodulation.

322 MHC GENETICS AND SENSITIVITY TO BERYLLIUM.

A. P. Fontenot. *University of Colorado Health Sciences Center, Denver, CO.* Sponsor: D. Lawrence.

Chronic beryllium disease (CBD) is a granulomatous disorder primarily affecting the lung that is caused by beryllium exposure in the workplace. Depending on the nature of the exposure and the genetic susceptibility of the individual, 2 to 16% of exposed workers eventually develop disease. CBD is characterized by a CD4+ T cell alveolitis, and evidence suggests that those T cells are critically involved in the immunopathogenesis of the disorder. Susceptibility to CBD is associated with the class II major histocompatibility complex (MHC) molecule HLA-DP, in particular, those HLA-DP molecules with a glutamate at the 69th position of the β-chain. The ability of certain HLA-DP molecules to bind and present beryllium to beryllium-specific CD4+ T cells provides a mechanism underlying this genetic susceptibility. For example, mAbs directed against HLA-DP abrogate beryllium-induced T cell proliferation and Th1-type cytokine production. These studies also pinpoint residues of the HLA-DP β-chain required for beryllium presentation. In addition, the presence of a defined antigen and an accessible target organ allow the performance of studies to further define the development of immunological memory. To date, it remains unknown how T cells recognize the beryllium-peptide-HLA-DP complex.

323 CYTOKINE GENE EXPRESSION MODIFIED BY LEAD.

D. A. Lawrence, Y. Heo, J. Kasten-Jolly and T. Mondal. *Wadsworth Center, Albany, NY.*

The environmental toxicant lead (Pb) has multiple effects on different components of the immune and nervous systems. Some of the immunomodulatory effects of Pb on both CNS and immune activities are posited to be partially due to alterations of cytokine expression in the brain and secondary immune organs. In general, Pb causes a preferential activation of type-2 immune responses by enhancing the expression of the type-2 cytokines, such as interleukin (IL)-4 and IL-10 and inhibiting the expression of the type-1 cytokine interferon-γ (IFNγ). However, these effects are dependent on the genetics of the exposed animal and the influences of other environmental exposures. For example, with wild-type BALB/c mice, Pb lowers the production of the type-1 antibodies (IgG2a) to the immunogen KLH, but with IFNγ-deficient BALB/c mice, Pb enhances production of these antibodies. STAT4 and STAT6 deficient mice also respond in an unexpected manner to immune modulation. Thus, knockout of some of the genes involved in signaling certain immune responses can alter the immunomodulatory effects of Pb. In addition, some of the peripheral immune modulations by Pb may relate to differential CNS effects, in that Pb alters cytokine expression in the brain and modulates the expression induced by a peripheral infection. Thus, there appear to be multiple mechanisms responsible for Pb effects on neuroimmune circuits and immune defenses.

324 MODULATION OF PROTEIN INTERACTIONS BY MERCURY: MOLECULAR ANALYSIS OF SIGNALLING PATHWAYS TO UNCOVER MECHANISMS OF Hg IMMUNOTOXICITY.

A. J. Rosenspire. *Biological Sciences, Wayne State University, Detroit, MI.*

While autoimmune disease can be understood as representing a breakdown in either central, or peripheral tolerance, or perhaps both, the specific etiology of most autoimmune diseases are unknown. The difficulties in elucidating these etiologies have generally been ascribed to the probability that in most instances the initiation

of autoimmune disease is likely multi factorial. In this regard a useful theoretical concept has been that autoimmune diseases can potentially be understood as arising as the result of a set of interactions between specific environmental inputs operating on susceptible genotypes. However while much progress has been made in recent years in elucidating the molecular biology and genetics of the normal mechanisms of peripheral and central tolerance, comparatively little is understood as to how environmental agents may interact with these mechanisms so as to initiate and/or perpetuate autoimmune disease. In fact, in most instances it has proven impossible even to conclusively link specific environmental agents with the onset of autoimmunity. One of the few exceptions is mercury (Hg), where animal studies have clearly established a connection between administration of the metal and systemic autoimmune disease in susceptible rodent strains. Recent work conducted in our laboratories has now established how non-cytotoxic concentrations of Hg interfere with the signal transduction pathways mediated by antigen receptors and CD95 in T and B lymphocytes. As antigen receptor mediated signal transduction has been shown to be crucial in the establishment of central tolerance, while CD95 mediated signaling is integral to the maintenance of peripheral tolerance, these findings suggest ways in which non-cytotoxic concentrations of Hg might deregulate immune system homeostasis so as to give rise to autoimmune disease. Supported by NIEHS grants ES011000, ES012403, and ES010351.

325 GENETIC CHECK POINTS IN HEAVY METAL INDUCED SYSTEMIC AUTOIMMUNITY.

K. M. Pollard. *Molecular & Experimental Medicine, Scripps Research Institute, La Jolla, CA.*

To investigate the role that specific genes play in systemic autoimmunity induced by heavy metals we have examined the disease profiles in mice with single gene deletions. In total 17 individual genes have been studied. Systemic autoimmunity was induced by subcutaneous injection of 40 ugs of mercuric chloride twice weekly for four weeks. Severity of disease was determined by measurement of hypergammaglobulinemia, autoantibodies, and tissue deposits of immune-complexes. Deletion of certain genes, such as CD40L, CD28, or Igh6 abrogated induction of autoimmunity. Other genes, including Igh5, IL-4, ICAM-1 or TNFRI and II, had little effect on the development of disease. Intermediate effects were observed in IL-6 deficient mice, while absence of β2microglobulin resulted in loss of hypergammaglobulinemia and IgG1 anti-fibrillarin autoantibodies but little change in anti-chromatin Abs levels or glomerular deposits. The most interesting observations were obtained with genes related to the expression or function of interferon-γ. Genes involved in upregulation of IFN-γ expression, such as IL-12, STAT-4, or ICE, did not significantly influence autoimmunity whereas absence of IFN-γ or IFN-γ receptor led to greatly reduced autoantibody responses and immunopathology. Absence of IRF-1, a gene expressed in response to IFN-γ, resulted in selective retention of anti-chromatin Abs but little glomerular pathology. As the results with the IRF-1 knockout demonstrate, specific defects in signaling pathways and gene expression subsequent to IFN-γ/IFN-γ receptor interaction may influence expression of specific parameters of autoimmunity elicited by mercury. In addition, IFN-γ influences the expression and function of other immunologically relevant genes such as IL-4, IL-6, and β2microglobulin. Thus the effects of these gene deletions on autoimmunity may result from the influence of downstream events following IFN-γ/IFN-γ receptor interaction.

326 OVERVIEW.

T. B. Knudsen[1] and W. Slikker[2]. *[1]Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA and [2]Neurotoxicology, NCTR/FDA, Jefferson, AR.*

Genomics and proteomics enable investigators to move from studies focused on single molecules, pathways and cells toward integrative function of the intact tissue and organism; however, new advances in bioinformatics and computational biology are needed to integrate these data and explain how processes work from a whole system perspective. This symposium will explore basic and applied concepts in systems biology as an emerging tool for computational methodologies, functional genomics, and molecular embryology to predict when, and understand how, molecular perturbations induced by drugs and chemicals might culminate in developmental toxicity. Basic concepts include the formal representation of cell signaling and gene regulatory networks in complex systems, how the flow of information within and between cells suggests a parody with similar phenomena in engineered systems, what efforts are needed to incorporate this kind of information into quantitative dose response models, and how to weave theoretical and empirical data into robust computational models that project to higher order functions in developmental processes and pathologies.

327 SYSTEMS BIOLOGY TOP DOWN.

E. Werner. *Cellnomica, Inc., Munich, Germany.* Sponsor: T. Knudsen.

Systems biology uses experimental, computational, theoretical methods to describe biological phenomena. In this talk, we give an introduction to the goals of systems biology as well as the methods used in systems biology to formalize what seem to be intractable and complex biological systems. In particular we focus on cell signaling and regulatory networks in multicellular genomes. Both cell signaling and genomic regulatory networks have become focal points for computational modeling and simulation efforts by engineers, computer scientists, mathematicians, and systems biologists. Computational biology methodologies require a precise description of the system under study, including key input and output variables and feedback control mechanisms, and use this description to simulate or model the described system. The formal representation of complex living systems is essential to the understanding of such systems as well as their pathologies. The use of in silico models to predict the dynamic behavior of a system in response to chemical agents may help in the processes of drug design, drug discovery and the development of therapeutic strategies. We will also discuss minimal multicellular organisms and their role in the future of systems biology and the health sciences. Minimal genomes for artificially constructed single cells and multicellular organisms will lead the way to integrating in silico simulations with *in vitro* and *in vivo* bioengineering methods. This will lead to the design of simple *in vitro* and *in vivo* multicellular systems making the science of systems biology realizable.

328 A GENE REGULATORY NETWORK FOR DEVELOPMENT.

G. Amore and E. H. Davidson. *Biology, Caltech, Pasedena, CA.* Sponsor: W. Slikker.

Large networks of regulatory genes control every aspect of the development and evolution of the body plan in animals. We have carried out an experimental exploration of the gene regulatory network that underlies the specification of the endoderm and the mesoderm of the sea urchin embryo. Genetic interactions between more than 40 genes have been reconstructed using data obtained from large-scale perturbation analyses, in combination with computational methodologies, genomic data, cis-regulatory analysis, and molecular embryology. Several differential screenings were performed to discover new regulators involved in the whole process. Whole mount in situ hybridization was extensively utilized to confirm these initial data. Perturbation of gene expression was attained by means of injection of morpholino antisense oligonucleotides or engrailed fusion mRNAs, using multiplex quantitative PCR analyses to reveal the regulatory interactions between each gene encoding a transcription factor and any other genes in the network responding to that factor. Many of the cis-regulatory regions that control the expression pattern of these genes are being studied actively. Recognizing these regions is now an efficient procedure, thanks to original computational approaches developed in our lab. Functional analysis of these regions is already illuminating many of the upstream relationships between these regulators. The translation of maternal inputs into a specific zygotic regulatory state, the segregation of different fates inside an initially homogeneous field, the transition from signal-dependent to self-sustained gene expression and the locking in of particular regulatory states, are now described at a gene circuitry level. Elements conveying robustness to the architecture of the system can be recognized at every level such as the extensive employment of feed-forward and feedback loops or redundancy. Interesting similarities as well as differences between evolution- and engineer-designed systems can be derived.

329 CHALLENGES AND OPPORTUNITIES IN UTILIZING SYSTEMS BIOLOGY APPROACHES FOR INFORMING DEVELOPMENTAL TOXICOLOGY.

E. M. Faustman. *Center for Child Environmental Health Risks, University of Washington, Seattle, WA.*

The "omic" revolution has provided us with not only unprecedented opportunities but also unforeseen challenges in unlocking it's riches for informing toxicology. In particular, for developmental toxicology, expected challenges of dose-response are complicated by the need for understanding dose and time responses across lifestages and within a three-dimensional context. Additional considerations for human development include the need for considering responses within the uterus where both genetic variations in response between the mother and conceptus can be significant. The purpose of this talk will be to provide some approaches for framing and modeling systems biology data for informing our understanding of mechanisms of developmental toxicology. The challenges and opportunities in utilizing systems biology information for understanding mechanisms of toxic action include understanding the importance of genomic conservation of cell signaling pathways,

the downstream diversification of cell systems, the three-dimensional characteristics of organ/system development, the concepts of dose and time response across multiple levels of biological organization and differences in environmental impacts on redundant versus unique cellular processes. Case examples using biologically based modeling approaches that build from physiologically based toxicokinetic and dynamic models will be presented. The use of both *in vitro* and *in vivo* data as well as model organism data will be discussed. Lessons learned as well as critical data needs will be highlighted. (Supported by the Center for Child Environmental Health Risks research through an EPA grant R826886 and NIEHS grants 1PO1ES09601, 32ESO7032, and ESO7033)

330 COMPUTATIONAL MODELING OF CELL SIGNALING PATHWAYS: A STEP ON THE ROAD TO IMPROVED CHARACTERIZATION OF DOSE- AND TIME-RESPONSE FOR THE ADVERSE EFFECTS OF TOXICANTS.

R. Conolly, Q. Zhang and M. Andersen. *Center for Computational Biology & Extrapolation Modeling, CIIT, Research Triangle Park, NC.*

Adverse reproductive and developmental outcomes arise when normal biological processes are disrupted by environmental stressors. Physiologically-based pharmacokinetic (PBPK) modeling is useful for characterizing the PK mechanisms that determine, in part, the dose-response and time to response relationships for these adverse effects. The greater challenge, however, is to understand the sequence of events that link the initial interactions of stressors at key sites in the target tissues with the final adverse outcomes. The relevant biology is so complex that intuition is not, by itself, an efficient tool. Computational models of biological processes, such as signal transduction, are just as capable of being wrong as is intuition. The models have the advantage, however, of a formal structure that can be iteratively refined through a process involving rigorous comparison of model behavior with data and revision of model structure. The model becomes increasingly accurate over time when linked with targeted laboratory studies. We are adapting computational models of signal transduction pathways (e.g., Science 297:1018-1023, 2002) with the longer-range goal of better understanding toxicological dose-response and time to response relationships. In this presentation we (1) exercise a computational model of the MAPK pathway to illustrate how this pathway can generate complex dose- and time-to response relationships and (2) consider how a better understanding of these relationships will impact human health risk assessment.

331 CURRENT STATUS AND FUTURE CONSIDERATIONS FOR THE DEVELOPMENT OF SKIN TOXICOLOGY ALTERNATIVE METHODS.

G. Gerberick[1] and I. Kimber[2]. *[1]Procter & Gamble Co., Cincinnati, OH and [2]Syngenta, Macclesfield, United Kingdom.*

The need for alternative approaches and *in vitro* test methods has never been greater than it is today. The continued development of such approaches and use of validated alternatives test methods is an integral part of toxicology in the 21st century. Collaboration of researchers and external scientific validation organizations such as ICVAAM and ECVAM that were established to provide a mechanism for alternatives test methods validation test methods spearheaded the way forward. Such efforts have led to significant progress in the replacement of animals, reduction in the number used and refinement of *in vivo* studies. In addition to scientific considerations, significant regulatory challenges lie ahead with the implementation of the 7th Amendment to the European Union Cosmetics Directive, The European Union Chemicals Policy and the United States program on High Production Volume Chemicals. This workshop will focus on the development of alternative approaches and *in vitro* methods for the evaluation of cutaneous toxicology. It will include detailed discussion on the use of *in vitro*/in silico and other alternatives methods/approaches used today for the evaluation of skin irritation skin sensitization and skin penetration. The workshop will be introduced by an overview on the use of alternatives in toxicology today and challenges for the future and will close with placing into context the scientific and regulatory challenges relative to societal expectations.

332 OVERVIEW OF ALTERNATIVES IN TOXICOLOGY: RECENT PROGRESS AND FUTURE OPPORTUNITIES.

W. S. Stokes. *DHHS/NIH, NIEHS, Research Triangle Park, NC.*

There has been significant progress in recent years in the use of alternative test methods and approaches for toxicological assessments. National and international authorities have adopted testing strategies that integrate the use of scientifically valid animal and non-animal alternatives, resulting in reduced animal use and reduced pain and distress. In some specific testing situations, hazard decisions can now be made without animals, whereas in others only a single animal or a small number of animals may be necessary. This progress has been aided by the establishment of processes and centers to facilitate the validation, scientific review, and regulatory acceptance of alternative methods. These include the Interagency Coordinating Committee on the Validation of Alternative Methods and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods in the US, and the European Center for the Validation of Alternative Methods in Europe. Despite current progress, recent events such as EU legislation imposing immediate and phased prohibitions on the use of animals for safety testing of cosmetic products and ingredients have created significant new scientific, regulatory, and ethical challenges. Focused research and application of new technologies will be essential to develop innovative alternative non-animal safety assessment methods that effectively address these new mandates and that continue to protect human, animal, and environmental health. This presentation will discuss the current status of alternative test methods, and challenges, opportunities, and strategies for future progress.

333 APPLICATION OF ALTERNATIVES IN THE EVALUATION OF SKIN IRRITATION.

D. A. Basketter. *SEAC, Unilever, Sharnbrook, United Kingdom.*

Considerable progress has been made over the past 10 years in the development of alternative methods for evaluation of potential skin irritants. Reliance on animal testing (e.g. the Draize rabbit skin irritation test) has been reduced significantly with the development of *in vitro* methods such as the use of skin equivalent models being used for assessing skin corrosion, as well as the deployment of these and other models for "in house" safety assessment. However, to date, there has been no success in the formal replacement of rabbit skin testing for regulatory assessment of skin irritation hazard. As a consequence, more basic work has been directed towards enhancing our fundamental understanding of the mechanism(s) of skin irritation. The aim ultimately is that new *in vitro* approaches can be developed which will be soundly based on this scientific knowledge. This presentation will consider in detail existing *in vitro* and in silico tools and ethical human testing strategies available as well as addressing the future opportunities for research in the area of skin irritation.

334 CHALLENGES IN DEVELOPMENT OF ALTERNATIVE METHODS TO EVALUATE SKIN SENSITIZATION.

G. Gerberick. *Procter & Gamble Co., Cincinnati, OH.*

There is considerable activity being directed at the development of alternatives test methods for evaluating skin allergens. Based on our increased understanding of the chemistry and biology of skin sensitization, effort is being directed at developing models which address the key hurdles of skin sensitization: penetration, protein reactivity and immune recognition. Techniques to address these areas such as QSAR modeling, peptide reactivity and molecular gene profiling will be discussed. This presentation will review scientific and regulatory progress made to date as well as focus on the challenges that lie ahead for the development of robust *in vitro*/in silico methods for reducing the need for animals for skin sensitization evaluations.

335 SKIN PENETRATION: CURRENT AND FUTURE DIRECTIONS IN THE USE OF ALTERNATIVE METHODS.

R. Bronaugh. *Office of Cosmetics and Colors, USFDA, Laurel, MD.*

A number of protocols have been developed in recent years for measuring skin penetration. A comparison of these protocols raises questions in the following major areas: (1) Should skin levels of absorbed chemical be included in measurements of systemic absorption? (2) What is the preferred diffusion cell receptor fluid? (3) Should human or animal skin be used? (4) Do you need *via*ble or non *via*ble skin? (5) What thickness of skin should be assembled in the diffusion cells? The OECD guideline on percutaneous absorption which was issued in 2003 attempted to reconcile some of these differences. The guideline states that skin absorption values at the end of a study must include skin levels as being systemically absorbed unless studies are conducted to demonstrate that these skin levels will not be systemically absorbed. It is also stated that the impact of metabolism of a test compound during skin absorption needs to be considered. The lack of a standard protocol has probably limited regulatory acceptance of this method. *In vitro* skin absorption studies are deemed appropriate for the evaluation of safety of cosmetics by the European Union. They have developed guidelines for conducting these studies. In the United States, *in vitro* measurements of skin penetration are routinely made by cosmetic companies. FDA expects the cosmetic industry to do appropriate safety testing and does not require specific tests for safety evaluations. For topical drug approval,

USFDA requires *in vivo* skin absorption data, in part, because of the need for a pharmacokinetic evaluation. *In vitro* studies may be useful in preclinical studies for a comparison of absorption from different formulations. Future efforts in developing this alternative method need to focus on the interlaboratory reproducibility of results from studies using a standard protocol. Also, the utility of pig skin as a suitable substitute for human skin needs to be more thoroughly addressed. The barrier properties of cultured human skin need to be improved. If not, its use in skin absorption evaluations may be limited to generating skin metabolism information.

336 SOCIETAL EXPECTATIONS ON THE USE OF ALTERNATIVES FOR THE PROTECTION OF ANIMALS AND HUMANS.

A. M. Goldberg. *CAAT, Johns Hopkins University, Baltimore, MD.*

Public concern over the use of animals in laboratory experiments has risen steadily over the last several decades. Questions about the morality, necessity and scientific validity of animal experiments have been raised. The publication in 1959 of "The Principles of Humane Experimental Technique, " by W. Russell and R. Burch, set the scene for the modern animal welfare movement dedicated to the 3Rs of alternatives: replacing animal procedures, reducing the numbers of animals, and refining procedures to decrease or eliminate pain in animal experiments. Russell and Burch indicated that humane science (incorporating the 3Rs of alternatives) was also the best science. Over the last four decades, the search for alternatives has become a movement of global proportions, and the philosophy of the Three Rs - replacement, reduction and refinement - has enabled researchers and animal protection advocates to find common ground, to meet societal expectations on the use of animals in research, and to demonstrate that humane science is the best science.

337 DIESEL EMISSIONS: NEW HORIZONS IN THE CHEMISTRY, HEALTH EFFECTS AND REGULATIONS.

I. Gilmour[1] and J. L. Mauderly[2]. *[1]NHEERL, USEPA, Research Triangle Park, NC and [2]Lovelace Respiratory Research Institute, Albuquerque, NM.*

Diesel exhaust is a complex aerosol comprised of carbonaceous particles and a mix of hydrocarbons, aldehydes and gases. The physical and chemical composition of the emission can vary dramatically depending upon the age and type of engine, fuel composition, load characteristics, presence and efficiency of control devices and climatic conditions. In 2002 the USEPA completed a health assessment of diesel exhaust, which concluded, that "long-term (i.e., chronic) inhalation exposure is likely to pose a lung cancer hazard to humans, as well as damage the lung in other ways depending on exposure." The assessment also indicated, that "evidence from numerous studies have shown that exposure to diesel exhaust increases lung cancer risk, and there is recent evidence to show that diesel may also promote the incidence and severity of allergic asthma." As these health data have emerged, regulations have also evolved to limit exposure through the promotion of new "cleaner" engines, and the development of control technologies which limit diesel emissions in terms of particle mass, CO and NO(x) output. While these changes in diesel exhaust have led to a general decrease of PM mass output per engine, the number of units has increased in the US and Europe. In addition, newer engines have significantly different emission profiles compared to older engines and there is virtually no comparative health data between diesel exhaust from light and heavy-duty engines of different ages. This symposium will contrast the historical understanding of diesel exposures with current knowledge of diesel exhaust chemistry and health effects; describe new and planned regulations; and identify future research needs. This abstract does not reflect EPA policy.

338 HEALTH EFFECTS OF DIESEL EMISSIONS: EVOLVING QUESTIONS FOR AN EVOLVING ISSUE.

J. L. Mauderly, J. D. McDonald and J. Seagrave. *National Environmental Respiratory Center, Lovelace Respiratory Research Institute, Albuquerque, NM.*

Historic concerns for heath hazards from diesel emissions centered on cancer risk from the mutagens and carcinogens in solvent extracts of elemental carbon-based soot particles. Long-term inhalation studies during the 1980s showed that extreme exposures increased lung tumors in rats, but not in Syrian hamsters or mice, and that the rat response had a threshold above human exposures. Although the rat results were not useful for human risk assessment, numerous epidemiological studies showed associations between diesel-associated occupations and increased lung cancer. Human cancer risk may be better resolved through studies better documenting actual exposures. Both the amount and nature of diesel emissions are changing in response to progressive emission standards, almost certainly reducing health hazards. Research issues are also evolving. There has recently been greater recognition of: 1) the scope of potential non-cancer hazards; 2) the importance of not only soot, but also other fractions of emissions; and 3) differences in toxicity between emissions from normal and high-emitting engines. Both particulate and non-particulate emissions have been shown to cause inflammatory responses and enhance allergic immune responses. Vapor-phase semi-volatile organics can be strongly inflammogenic. Environmental hot spot-level exposures may reduce resistance to viral infection. *In utero* (maternal) exposures may influence post-natal immune responses. Ultrafine particles, although historically present, may become relatively more important as soot emissions are reduced. Almost nothing is known about toxicity of the fraction of ultrafine particles consisting mostly of condensed organics. High-emitters may not only contribute more to exposure, but the toxicity may be greater per unit of mass. Overall, there has been little study of changes in health hazards as engines, fuels, and after-treatment technologies have evolved. As diesel emissions are strikingly reduced, comparisons of the residual hazards to those from alternate technologies are needed.

339 EPIDEMIOLOGY OF DIESEL: EXPOSURE ASSESSMENT AND HEALTH OUTCOMES.

E. Garshick[1, 2], F. Laden[2, 3] and T. J. Smith[3]. *[1]VA Boston Healthcare System, West Roxbury, MA, [2]Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA and [3]Department of Environmental Health, Harvard School of Public Health, Boston, MA.* Sponsor: I. Gilmour.

Exposure assessment in epidemiological studies has been based on surrogate exposure markers for diesel exhaust. These markers have been suitable for identifying diesel exhaust as a health hazard, but have not provided insight into a dose-response relationship. Relating specific exposure levels to health outcomes has been complicated because diesel exhaust is a complex mixture of particles and gases, the agents responsible for each health effect are not known, different agents may be important in causing different health effects, and these agents may not be unique to diesel. Despite these limitations, a 20% to 50% excess lung cancer risk has been observed in occupations where diesel exhaust exposure was likely. Ambient fine particulate matter (PM2.5) has been associated with increases in daily cardiovascular and respiratory mortality, hospital admissions, and lung cancer. Since diesel exhaust contributes to ambient PM2.5, particularly in settings related to traffic, there is concern that diesel contributes to these health effects. Studies mainly in children have noted an association between residence near a roadway with respiratory symptoms, asthma, and pulmonary function changes. Experimental studies suggest that diesel can act to enhance the response to allergens, and therefore there is the possibility of an association with allergic disease. An exposure database is being developed for a study of lung cancer and other health outcomes in the US trucking industry. Exposure scenarios include traveling on local roads in a truck, in long haul tractor-trailers on intercity highways, and upwind at urban and rural large and small truck terminals. Markers of exposure include elemental carbon (EC), organic carbon, and PM2.5, supplemented by molecular markers in particle-associated organics to estimate the source of EC. These data will provide exposure estimates in scenarios typical of general population exposures that have been previously unavailable.

340 CONTROLLED DIESEL EXPOSURES: INTER-PHASING HUMAN AND ANIMAL STUDIES AND THEIR USE IN THE RISK ASSESSMENT PROCESS.

M. C. Madden. *NHEERL\Human Studies Division, USEPA, Chapel Hill, NC.*

Particulate matter (PM) has been reported to be associated with health effects (e.g., premature deaths, hospitalizations, lung cancer), and gaseous components may influence the PM-induced effects. Diesel exhaust (DE) contributes to ambient PM and gaseous compounds, but the role that DE plays in PM-induced effects is not well established. Controlled exposure studies using human volunteers and animal models can confirm the role DE may play in PM toxicity; also the removal of a DE component (e.g., particles) may reveal whether the removed component is involved in the observed response(s). A limited number of acute, controlled exposure studies of human volunteers administered either whole DE or individual components by inhalation or instillation have been used to examine nasal and lung responses including indices of inflammation and allergy. More numerous acute exposures of nonhuman animal models have revealed similar pulmonary alterations, and have better linked lung responses to the composition of particles. Further acute exposure investigations with animal models have revealed DE-induced cardiovascular changes (hypertension, arrhythmias). Chronic exposure regimens have also been utilized with rodents to examine the carcinogenicity of DE. Exposures *in vitro* have been also utilized with cells from target organ systems in order to assess relative potencies of DE and DE constituents (e.g., organic solvent-extractable), potential mechanisms that operate *in vivo* (e.g., signal transduction), and other possible responses not widely examined *in vivo*. In combination with key human studies, animal and cell *in vitro* models have begun to elucidate the possible biological responses induced by DE exposure, determined bioactive component(s), better

established dose-response relationships, and identified possible susceptible populations. Findings from these studies assist in the assessment of risk from DE in different exposure scenarios, and ultimately in the management of health risks from DE exposure. [This abstract may not represent official EPA policy.]

341 FUTURE RESEARCH NEEDS FOR DIESEL EMISSIONS.

T. W. Hesterberg[1], W. B. Bunn[1], W. J. Slodowske[1] and C. A. Lapin[2].
[1]*International Truck and Engine Corporation, Warrenville, IL and* [2]*Lapin and Associates, Glendale, CA.*

Exposure of animals to diesel exhaust can produce lung and cardiovascular effects. However, most studies used exposure routes and dose levels that may not be useful for assessing the potential risks of diesel exhaust. Furthermore, many of these studies examined only the particulate matter fraction of the exhaust. Typically the diesel particulate matter (DPM) was collected on a filter and stored before being suspended in air or liquid for use in toxicity studies. The chemistry of DPM can change when concentrated and stored. When DPM is suspended in saline and instilled into animal lungs, the burdens can be very high and result in non-uniform distribution with local areas of even higher concentrations. *In vitro* studies have similar issues. Although a few animal studies have used concentrated airborne particles (CAPs) to increase the inhalation dose of DPM, there are also drawbacks to these studies. High exposure levels are often used and much of the gaseous components of the exhaust are removed. Removing the gaseous components not only could impact the overall toxicity of the exhaust, but may also alter the physico/chemistry of the concentrated DPM. Future studies should focus on exposing animals by inhalation to fresh whole diesel exhaust at multiple concentrations that are relevant to ambient and occupational levels. There are a number of animal inhalation models of lung and cardiovascular disease where whole diesel exhaust can be tested. In the last several years, to meet new EPA emission standards, the diesel industry has made significant improvements to engine technology that will result in near-zero emissions of particulate matter, hydrocarbons and other regulated chemicals. By 2010, USEPA regulations mandate that NOx emissions also be at near-zero levels. Toxicology data generated from past emissions profiles have limited relevance in evaluating health effects from exposure to current and future generation diesel engines. For assessing the newer technologies, emphasis should be on inhalation exposure to whole fresh engine exhaust tested at relevant exposure levels.

342 OVERVIEW- NUTRACEUTICALS AS A TOXICOLOGICAL DILEMMA.

J. J. Pestka. *Michigan State University, East Lansing, MI.*

Ehrlich introduced the concept of therapeutic index (TI) almost a century ago which is identified as the ratio of dose of a chemical required to produce a toxic effect and the dose needed for desired therapeutic effect. Bioactive dietary chemicals that are used as nutraceuticals are extraordinarily diverse with respect to chemical structures and biological activities. When used as foods or in supplements, these chemicals have the potential for prevention or retardation of chronic diseases. Paradoxically, some of these chemicals can also induce subtle toxic effects that exacerbate disease. Given the current regulatory environment created by the Dietary Health and Education Act of 1994 (DSHEA), providers of nutraceuticals are not subject to the degree of safety evaluation required for drugs and food additives. Thus, clear definitions of potential negative effects of nutraceuticals are not readily accessible. This is further complicated by factors such as disease, predisposing factors, age and genetic background of humans. This workshop focuses on the challenge of this double-edged sword by examining, from a mechanism-based perspective, examples of dietary chemicals that are being used or considered for disease prophylaxis relative to potential safety concerns and disease exacerbation.

343 PLEIOTROPIC EFFECTS OF CANCER PROTECTIVE DIETARY INDOLES.

L. Bjeldanes. *Department of Nutrition, University of California at Berkeley, Berkeley, CA.*

Indole-3-carbinol (I3C) is a product of Brassica plants that shows both cancer preventive and cancer promoting activities in different assays. I3C is converted to a series of self-condensation products that are responsible for the *in vivo* biological activities of this indole. One of the minor products, ICZ, is a potent ligand for the Ah receptor, and thus, behaves in some respects like the environmental toxin, TCDD. A major I3C product, CTr, is a potent ligand for the estrogen receptor and can induce the growth of estrogen responsive breast tumor cells. Studies of an additional major *in vivo* I3C product, DIM, show that this product can activate the estrogen receptor in a manner that does not require ligand binding. These activities may contribute to the cancer promoting effects of I3C. In addition, however, DIM exhibits several novel activities that may contribute to the cancer preventive effects of DIM and I3C. The antiproliferative effects of DIM in cultured endometrial cancer cells are mediated in part by DIM-induced expression of TGF-alpha. DIM treatment of endometrial cancer cells stimulated levels of secreted TGF-alpha protein by over 10 fold. The ectopic addition of TGF-alpha inhibited the growth of these cells, whereas incubation with an anti-TGF-alpha antibody partially reversed the growth inhibitory effects of DIM. In other studies with prostate tumor cells, we found that DIM is a potent antagonist for the androgen receptor that blocks androgen induced signaling and strongly inhibits the proliferation of androgen sensitive prostate tumor cells. These activities were not produced in androgen independent prostate tumor cells. Moreover, DIM inhibited transcription of the endogenous prostate tumor marker, PSA, and reduced intracellular and secreted PSA protein levels induced by androgen in androgen-dependent prostate tumor cells. These studies provide a mechanistic rationale for the desirable and the adverse effects of I3C, and suggest that the two types of effects might be separable.

344 BREAST CANCER THERAPIES AND DIETARY ESTROGEN (GENSITEIN) CONSUMPTION.

B. Helferich. *Food Science and Human Nutrition, University of Illinois, Urbana, IL.* Sponsor: J. Pestka.

Genistein, found in soy products, is a phytochemical with estrogenic activity. Our research has focused on the effects dietary genistein on growth of both estrogen (E)-dependent *in vivo*. Genistein enhances the proliferation of E-dependent human breast cancer tumor growth. Genistin, the glycoside form of genistein simulates growth similar to that of genistein and withdrawal of either genistin results in tumor regression. We have also demonstrated that soy protein isolates processed to contain low, medium and high amounts of isoflavones simulate tumor growth in a dose dependent manner. Expression of the estrogen-responsive gene, pS2 was also induced in response to treatment with dietary genistein. To evaluate whether dietary genistein interacts with current anti-estrogen breast cancer therapy such as tamoxifen (TAM). We implanted E-dependent tumors into ovariectomized athymic mice and administered estradiol, estradiol plus TAM, or estradiol, TAM + dietary genistein. In these studies dietary genistein was able to negate the inhibitory effect of TAM on E-stimulated tumor growth. In summary, genistein can act as an estrogen agonist resulting in proliferation of E-dependent human breast cancer cells *in vivo* and can negate the inhibitory effects of TAM on E-stimulated growth of MCF-7 cell tumors (*in vivo*) implanted into ovariectomized athymic mice.

345 ATTENUATION VERSUS POTENTIATION OF THE IMMUNE RESPONSE BY DIETARY OMEGA-3 POLYUNSATURATED FATTY ACIDS.

K. L. Fritsche. *Animal Sciences, University of Missouri, Columbia, MO.* Sponsor: J. Pestka.

Numerous studies have documented health benefits associated with increasing our intake of omega-3 polyunsaturated fatty acids (n-3 PUFA) found in fatty fish (e.g., tuna, herring, salmon, sardines). For example, consumption of 1-2 servings per week of such fatty fish has been shown to dramatically reduce the risk of dying from a heart attack. Greater amounts of n-3 PUFA can benefit people suffering from various inflammatory/auto-immune diseases. Concern arose, however, over the potential adverse effects of consuming large amounts of n-3 PUFA because these nutrients tend to have a suppressive effect on *in vivo* and *in vitro* immune responses. Evidence that n-3 PUFA affect, both positively and negatively, infectious disease resistance will be presented. The impact that n-3 PUFA have on host response to infectious agents is most likely a consequence their ability to reduce the biosynthesis of pro-inflammatory cytokines and eicosanoids. Novel findings by the authors suggest that n-3 PUFA may adversely affect host infectious disease resistance by reducing interferon-gamma receptor expression and signaling in macrophages. Direct examination of the effect of n-3 PUFA on human infectious disease resistance remains largely untested. We believe that the body of evidence suggests that such testing would be prudent.

346 PROMOTION VERSUS SUPPRESSION OF CHEMICAL CARCINOGENESIS BY CHLOROPHYLLS.

G. S. Bailey and R. Dashwood. *Linus Pauling Institute, Oregon State University, Corvallis, OR.*

Although ample epidemiology data indicate a protective role for green and leafy vegetables in human cancer risk, there is relatively little evidence for natural chlorophylls in cancer chemoprevention. However, a simple non-toxic water soluble de-

rivative of chlorophyll, chlorophyllin (CHL), has displayed potent anti-mutagenic and anti-carcinogenic activity in many model systems. Anti-carcinogenic activity was first demonstrated in the rainbow trout, where dietary CHL co-treatment provided dose-responsive inhibition of aflatoxin-DNA damage to an extent that precisely predicted inhibition of eventual tumor outcome. Subsequent experiments showed that CHL co-treatment could inhibit cancer initiation in several rodent models as well. With this background, a clinical intervention trial was undertaken in the Peoples Republic of China in 1997, in a population at high risk due to unavoidable aflatoxin exposure in the diet. The results of the trial showed that daily CHL supplementation was as effective in humans as it was in trout at reducing biomarkers of aflatoxin uptake and liver DNA damage. Recent experiments in the trout model show that CHL has comparable biomarker and cancer preventive efficacy against highly carcinogenic polyaromatic hydrocarbons. Although CHL thus shows great promise to diminish the effects of carcinogen exposure in certain high-risk populations, CHL may not be entirely risk-free. While there is no evidence for CHL toxicity in humans, post-carcinogen CHL treatment protocols in the rat have yielded mixed results. These include dose-dependent suppression of tumor progression in some organs but an inverse dose-dependency for elevated progression in others, principally the colon. Mechanism studies indicate that CHL can have differing effects on gene pathways regulating tissue homeostasis in the colon, and that this may be mediated through the selective progression of initiated cells containing a sub-set of beta-catenin mutations. Initial studies indicate that this enhancement is not seen with natural chlorophylls.

347 EFFECTS OF NATURAL AND SYNTHETIC FLAVONOIDS ON AROMATASE (CYP19) IN H295R HUMAN ADRENOCORTICAL CARCINOMA CELLS.

T. Sanderson[1], M. S. Denison[2], M. Springsteel[3], J. Hordijk[1], M. H. Nantz[3] and M. van den Berg[1]. *[1]Institute for Risk Assessment Sciences, University of Utrecht, Utrecht, Netherlands, [2]Department of Environmental Toxicology, University of California, Davis, CA and [3]Department of Chemistry, University of California, Davis, CA.*

Flavonoid structures have various (anti-)estrogenic effects. We investigated the effects of various naturally occurring and synthetically derived flavonoids on the catalytic activity and promoter-specific expression of aromatase (CYP19; which converts androgens to estrogens) in H295R cells. All exposures were for 24 h. The steroidal aromatase inhibitor 4-hydroxyandrostenedione (positive control) had an IC50 of about 20 nM. The flavones 7-OH-flavone, chrysin, and apigenin had IC50 values of about 6, 7, and 30 μM, respectively. The flavanones 7-OH flavanone and naringenin had IC50s of about 80-90 μM close to the highest tested concentration of 100 μM. In the case of apigenin and naringenin inhibition coincided with signs of cytotoxicity. The 7-methoxylated flavone and flavanone showed no aromatase inhibition, as was true for catechin and (-)-epicatechin. Several synthetic flavonoid and structurally related quinolin-4-one analogs were found to inhibit aromatase activity. The most potent inhibitor was 4′-tert-butyl-quinolin-4-one (IC50=2 μM) followed by several 2-pyridinyl-substituted α-naphthoflavones (IC50s between 5 and 30 μM). The 2-pyridinyl-substituted γ-naphthoflavones consistently produced biphasic concentration-response curves, causing about 1.5-fold aromatase induction below 1 μM and inhibition above 1 μM (IC50s between 10 and 100 μM). Various flavonoid structures with a bromine, fluorine or nitro-group at the 4′-position of the B-ring were inactive. The natural flavone quercetin and isoflavone genistein induced aromatase activity 4- and 2.5-fold induction, respectively at 10 μM. This coincided with increased levels of intracellular cAMP, and pII and to a lesser extent 1.3 promoter-specific aromatase transcript. These results shed light on the structure-activity relationships for aromatase inhibition and mechanism of induction in human H295R cells.

348 EXPRESSION, CHARACTERIZATION AND MUTATION OF RAT CYTOCHROME P450C24A1 (CYP24A1).

A. J. Annalora[1, 2], K. Bobrovnikova-Marjon[2], A. Pastuszyn[2], M. Chiu[3], C. Marcus[1] and J. L. Omdahl[2]. *[1]College of Pharmacy, University of New Mexico, Albuquerque, NM, [2]Department of Biochemistry and Molecular Biology, University of New Mexico, Albuquerque, NM and [3]Department of Structural Biology, Abbott Laboratories, Abbott Park, IL.*

The discovery of potent antiproliferative effects of 1, 25-dihydroxyvitamin-D3 on cancer cell lines has initiated a new era of pharmacological investigation focused on vitamin D. Cytochrome P450c24 (CYP24A1) is a multifunctional enzyme that mediates the side-chain cleavage and excretion of circulating vitamin D. Because CYP24A1 plays a central role in regulating vitamin D metabolism, identification of this enzyme's key residues and structural domains will be necessary to establish a biorational basis for future vitamin D-based drug development. A high level of functional recombinant rat CYP24A1 (40-50 mg/L) was obtained using an E. coli expression system. However, characterization of membrane-bound proteins, like CYP24A1, is problematic as these proteins aggregate in solution rather than forming the ordered crystals required for x-ray analysis. Therefore, we constructed a homology-based computer model of rat CYP24A1, using InsightII™, to help identify amino acid residues involved in substrate recognition and binding. Site-directed mutagenesis experiments were conducted to assess the role of these residues with respect to Km and Kd. It was determined that the 1a- and 25-hydroxyl groups on vitamin D3 metabolites were the major determinants for high-affinity binding to CYP24A1. Mutagenesis within the putative F-helix of CYP24A1 drastically altered substrate binding and metabolism. Most significant was the hydrophobic to polar mutant F249T that had a strong impact on lowering substrate-binding-affinity and catalysis of the final oxidation sequence from 24, 25, 26, 27-tetranor-1, 23-dihydroxyvitamin D3 to calcitroic acid. Two other hydrophobic 249 mutants (F249A and F249Y) also lowered substrate binding, but the associated metabolic defect occurred earlier in the pathway. Therefore, F249 was demonstrated to have a key role in properly aligning substrate in the CYP24A1 active site.

349 DEVELOPMENTAL EXPRESSION IN THE RAT PLACENTA OF ENZYMES INVOLVED IN FATTY ACID METABOLISM.

T. J. Cook, Y. Xu and G. T. Knipp. *Department of Pharmaceutics, Rutgers, The State University of New Jersey, Piscataway, NJ.* Sponsor: K. Reuhl.

The placenta regulates the delivery of nutrients and xenobiotics to the fetus, thus nourishing and protecting the fetus during development. Fatty acids (FA) are critical for normal fetal development. The two primary processes that govern FA supply from mother to fetus are metabolism and transport. Metabolism of FAs in the placenta may occur *via* several pathways. While these pathways are described in many other tissues, there is a dearth of information on the expression of FA metabolizing enzymes in the placenta. In our initial efforts to describe the functional FA metabolic pathways in the placenta, we have determined the gene expression of several enzymes involved in FA metabolism in the developing rat placenta: cytochrome P450 isoforms CYP4A1, 4A2, 4A3, and 4A8; lipoxygenases 12/15-and 5-LOX; cyclooxygenases COX-1 and -2; and uncoupling proteins UCP1, 2, 3, and 4. Placentas from rats on days 11, 13, 16, 19, and 21 of the gestation period were harvested. The junctional (JXN) and labyrinth (LAB) zones were separated under dissecting microscope. Gene expression was determined by RT-PCR using gene specific primers for each enzyme isoform. The relative expression of the CYP4A isoforms were: 4A1>4A2>4A3, 4A8, with greater expression in the JXN zone. For the UCPs, the relative expression were: UCP2>UCP4>UCP1>>UCP3. There was no clear preference for JXN vs LAB for UCP2 and UCP4. UCP1 expression shifted from LAB to JXN between day 13 and 16. 12/15-LOX was expressed throughout the gestation period with 5-LOX undetected. COX-2 exhibited a greater expression than COX-1 after day 11 of gestation with a shift in spatial expression from LAB to JXN as gestation progressed. The results demonstrate that the rat placenta expresses several enzymes involved in FA metabolism. The differential expression suggests that there may be changes in placental FA metabolism as a function of gestational age that can alter FA supply to the fetus. In addition, these results establish a template for studying the influence of xenobiotics on placental FA metabolism. Financial support from NAAR.

350 CYP1A2 PROTECTS AGAINST REACTIVE OXYGEN PRODUCTION IN MOUSE LIVER MICROSOMES.

H. G. Shertzer, C. D. Clay, M. Genter, S. N. Schneider, D. W. Nebert and T. P. Dalton. *Department of Environmental Health and Center for Environmental Genetics, University of Cincinnati Medical Center, Cincinnati, OH.*

H2O2 production was evaluated in liver microsomes prepared from Cyp1a1/1a2(+/+) wild-type and Cyp1a1(-/-) and Cyp1a2(-/-) knockout mice, pretreated with 5 μg TCDD/kg body weight or vehicle alone. NADPH-dependent H2O2 production in TCDD-induced microsomes from wild-type mice was approximately one-third of that in non-induced microsomes. In Cyp1a2(-/-) mice, H2O2 production was the same for induced and non-induced microsomes, with levels significantly higher than that in wild-type mice. Cyp1a1(-/-) microsomes displayed markedly lower levels of H2O2 production in both induced and non-induced microsomes, compared with that observed in wild-type and Cyp1a2(-/-) microsomes. The CYP1A2 inhibitor furafylline exacerbated *in vitro* microsomal H2O2 production proportional to the degree of CYP1A2 inhibition. In contrast, the CYP2E1 inhibitor diethyldithiocarbamate decreased H2O2 production proportional to the degree of CYP2E1 inhibition. Microsomal H2O2 production was strongly correlated to the NADPH-stimulated production of thiobarbituric acid-reactive substances, as well as to decreases in microsomal membrane polarization anisotropy, indicative of peroxidation of unsaturated membrane lipids. Our results suggest that, possibly acting as an electron sink, CYP1A2 might decrease CYP2E1- and CYP1A1-mediated H2O2 production and oxidative stress. In this regard,

CYP1A2 may be considered an antioxidant enzyme. (Supported by NIH grants R01 ES10133, R01 ES06321, R01 ES08147, R01 ES08799, T32 ES07250, and P30 ES06096)

351 QUANTIFICATION AND LOCALIZATION OF ROS PRODUCTION BY POLYCHLORINATED BIPHENYLS AND BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN.

J. Goldstone and J. J. Stegeman. *Biology, Woods Hole Oceanographic Institution, Woods Hole, MA.*

The mechanisms by which planar polyhalogenated aromatic hydrocarbons (PHAH), such as non-ortho-substituted polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins, exert toxicity are not well known but may involve oxidative stress. Our prior work has shown that cytochrome P450 1A (both fish CYP1A and human CYP1A1) catalytic cycles can be uncoupled by non-ortho PCBs resulting in the release of reactive oxygen species (ROS) and in the irreversibly inactivation of the enzyme. TCDD causes a similar uncoupling, resulting in a burst of ROS release (initially 24 nmole O_2^-/min/nmole CYP1A but rapidly declining to 8 nmol O_2^-/min/nmol CYP1A), and rapid inactivation of CYP1A. An increase in TCDD-induced mitochondrial production of ROS has been demonstrated in mice. To evaluate the relative contributions of CYP1A uncoupling and mitochondrial production of reactive oxygen in response to TCDD, we have examined the cellular localization of ROS production in cultured mouse and zebrafish hepatic cell lines (Hepa-1c1c and ZF-L) using multilaser confocal microscopy. The fluorometric ROS probe dihydroethidium was used to determine the production of superoxide (O_2^-). Localization of CYP1A (or CYP1A1) within individual cells was performed by examining the production of fluorescent resorufin following the CYP1A-mediated O-deethylation of ethoxyresorufin (EROD). The release of ROS resulting from the uncoupling of CYP 1As by pHAHs represents a possible mechanism of toxicity of these aryl hydrocarbon receptor agonists. (EPA R 827102-01-0, NIH P42-ES07381, NIH 1 F32 ES012794-01).

352 COAL DUST INCREASES BAX EXPRESSION, INCREASES APOPTOSIS, AND SUPPRESSES CYP1A1 INDUCTION IN A RAT MODEL OF MIXED EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS AND RESPIRABLE PARTICLES.

M. Ghanem[1, 2], L. Battelli[1, 2], R. R. Mercer[1, 2], M. L. Kashon[1], J. F. Scabilloni[1, 2], V. Castranova[1, 2], J. Nath[2], V. Vallyathan[1, 2] and A. F. Hubbs[1, 2]. *[1]NIOSH, CDC, Morgantown, WV and [2]West Virginia University, Morgantown, WV.*

Apoptosis has been described in lung following exposure to inflammatory agents, such as silica. The bax gene contains 2 Ah receptor response elements, suggesting a relationship between bax-mediated apoptosis and xenobiotic metabolism. Suppression of pulmonary CYP1A1 induction by coal dust (CD) exposure occurred in the rat lung. Therefore, we hypothesized that CD exposure in rat lung causes bax-mediated apoptosis and bax expression suppresses CYP1A1 induction. To explore this relationship, male Sprague-Dawley rats were intratracheally instilled with 2.5, 10, 20, and 40 mg CD/rat or vehicle (saline). On day 11, CYP1A1 was induced by intraperitoneal (IP) injection of 50 mg/kg beta-naphthoflavone (BNF). Rats were sacrificed on day 14, and lung sections were stained by immunofluorescence and morphometrically analyzed for CYP1A1, bax and an alveolar type II (AT-II) cell marker (cytokeratins 8/18). Bax expression was increased by CD in a dose-dependent manner, and CYP1A1 expression was inversely related to bax expression in AT-II cells. Since bax is a pre-apoptotic protein, we investigated the association of apoptosis with CYP1A1 suppression. Therefore, rats were injected IP with the caspase inhibitor, Q-VD-OPH or vehicle (DMSO) on days 0, 5, 9, 10, 11, 12, and 13 post CD-exposure. CYP1A1 was induced by BNF injection on day 11. Rats were sacrificed on day 14. CD exposure significantly suppressed CYP1A1 activity, increased bax expression and increased apoptosis in pulmonary cells. The injection of Q-VD-OPH significantly suppressed bax expression and apoptosis. However, the CD-mediated suppression of CYP1A1 induction was not significantly affected. These findings suggest that CD exposure suppresses CYP1A1 induction, but this suppression is not caused by bax expression or pulmonary cell apoptosis.

353 COMPARISON OF ACUTE AND CHRONIC EXPOSURE TO NONYLPHENOL REVEALS THAT CHRONIC EXPOSURE ATTENUATES P450 INDUCTION AND RXRα LEVELS.

W. Baldwin, R. Acevedo, L. M. Chapman and H. Villanueva. *Biological Sciences, University of Texas at El Paso, El Paso, TX.*

Nonylphenol (NP) is an environmental estrogen that also binds the pregnane X-receptor (PXR). Different laboratory feeding studies have shown both up-regulation and down-regulation of P450s by nonylphenol. The inconsistencies between laboratories for the differential effects of nonylphenol may be due to length of treatment. FVB/NJ mice were treated for 7 days with 0, 25, 50 and 75 mg/kg/day NP, and FVB/N-TgN(MMTVneu)Mul202 mice were treated for 32 weeks with 0, 30 and 45 mg/kg/day NP. The transgenic mice treated for 32 weeks at 45mg/kg/day demonstrated higher incidence of mammary cancer, indicating that the dose of NP provided was sufficient to cause estrogenic effects. After exposure, mice were euthanized, livers excised, and steroid hydroxylase assays and Western blots were performed for CYP 3A, CYP2B6 and CYP 2B10. Western blots revealed that CYP3A was not significantly induced by nonylphenol, but CYP 2B6 and CYP2B10 were induced 1.6X and 5X, respectively after the 7 day exposure. Estradiol and testosterone hydroxylation assays demonstrated an increase in the production of estriol and 16β-hydroxytestosterone, respectively, further demonstrating the induction of CYP2B enzymes. However, 32-week treatment showed no significant increase in testosterone or estradiol hydroxylation, nor significant induction of P450s. Clontech cDNA Atlas® arrays were performed to determine a potential mechanism for the lack of induction in mice treated for 32 weeks. Interestingly, RXRα, PXR's heterodimerization partner was reduced 1.9X. Q-PCR was performed to confirm down-regulation and indicated a 2.5X decrease in transcript levels in mice treated for 32 weeks with NP. Mice treated for 7 days showed no change in RXRα levels. Furthermore, Q-PCR of PXR, CAR and ER were not altered in the livers of mice from either NP treatment group. This data suggests that long-term exposure to nonylphenol can reduce RXRα levels, and attenuate P450 induction. This may ultimately reduce an organism's ability to adapt to toxicant exposure.

354 A NOVEL CLASS OF CYTOCHROME P_{450} REDUCTASE REDOX CYCLERS: CATIONIC MANGANOPORPHYRINS.

C. T. Kariya[1] and B. J. Day[1, 2]. *[1]Pharmaceutical Sciences, UCHSC, denver, CO and [2]Medicine, National Jewish Medical Research Center, Denver, CO.*

Manganoporphyrins are potent antioxidants and are currently being developed as novel therapeutic agents. The objective of this study was to investigate whether manganoporphyrins inhibit drug metabolism. *In vitro* studies were done to examine this issue. Microsomal cytochrome P_{450} activity was assessed using two different substrates, benzyloxyresorufin (BR) and methoxyresurofin (MR). The cationic manganoporphyrins AEOL 10113 and 10123 were found to be potent inhibitors of cytochrome P_{450} metabolism in both rat and human liver microsomes with IC_{50s} of 0.665 μM and 0.607 μM respectively. Since manganoporphyrins are redox active, we test the hypothesis that the mechanism of cytochrome P_{450} inhibition was due to redox cycling with cytochrome P_{450} reductase. We tested this hypothesis by examining their ability to stimulate NADPH and oxygen consumption in presence of purified human cytochrome P_{450} reductase. AEOL 10113 had Km = 4.3 μM, indicating that it was a potent redox cylcer. The manganoporphyrins AEOL 10113 and 10123 were nearly 2 orders of magnitude more potent than corresponding AEOL 10150 and 10201 in inhibiting cytochrome P_{450} metabolism and as redox cyclers with cytochrome P450 reductase. Structure activity relationships suggested that substitution of longer alkyl chains on the meso porphyrin position and decreased charged lessened the manganoporphyrin's ability to redox cycle with cytochrome P_{450} reductase and inhibit cytochrome P_{450} metabolism. To further examine the possible mechanism of cytochrome P_{450} inhibition, oxygen consumption studies were performed in the presence of a flavin domain inhibitor, diphenyleneiodinium (DPI). DPI blocked the consumption of oxygen in a concentration-dependent manner. These data are consistent with the hypothesis that manganoporphyrins redox cycle with the flavin domain of cytochrome P_{450} reductase and compete for electrons with cytochrome P_{450s}.

355 SF-1 FUNCTIONS SYNERGISTICALLY WITH CREB TO MEDIATE CAMP STIMULATION OF CYP1B1 VIA A FAR UPSTREAM ENHANCER (FUER).

W. Zheng and C. R. Jefcoate. *Pharmacology, University of Wisconsin-Madison, Madison, WI.*

Cyp1B1 plays a key role in the activation of polycyclic aromatic hydrocarbons in tissues like the adrenal ovary and testis that are hormonally regulated *via* cAMP. In primary adrenal cells Cyp1B1 is substantially induced by cAMP. Previously we used Cyp1B1-luciferase reporters to characterize a cAMP stimulation mechanism in Y-1 adrenal cell line, which depends on a far upstream enhancer region (FUER, -5298 to -5110). SF-1 and CREB commonly mediate cAMP responses. Here we show that SF-1 and CREB are equally essential for the cAMP activation of FUER. Four SF-1-binding sites in FUER can participate relatively equally and additively in support of an essential CREB interaction at the 3' end of the FUER. DAX-1, a suppressor of SF-1, also blocked FUER activity. An AP-1 binding site in proximal promoter region regulates basal activity of Cyp1B1 which is enhanced by FUER in Y-1 cells. The role of FUER has been tested by chromatin immunoprecipitation assay with anti-acetyl histone H3 anti-body in primary rat adrenal cells. Stimulation by

cAMP was associated with greatly increased histone H3 acetylation in FUER (6.6 fold) and proximal promoter region (3 fold) but not in AhR binding region, suggesting that FUER chromatin remodeling mediates cAMP induction.

356 METHOXYCHLOR-INDUCED ATRESIA WORKS THROUGH THE BCL-2 PATHWAY.

C. Borgeest[1], K. P. Miller[1], C. Greenfeld[2] and J. A. Flaws[1]. *[1]Program in Toxicology, University of Maryland, Baltimore, MD and [2]Department of Physiology, University of Maryland, Baltimore, MD.*

The organochlorine pesticide methoxychlor (MXC) has been shown to cause ovarian atresia (apoptosis of antral follicles). Bcl-2 (an anti-apoptotic protein) and Bax (a pro-apoptotic protein) are both important apoptotic factors in the ovary, and it is thought that the ratio of pro- to anti-apoptotic proteins is a determining step in whether follicles undergo atresia. Previous studies conducted in our laboratory have shown that MXC induces antral follicle atresia without changing serum levels of estradiol, follicle-stimulating hormone and luteinizing hormone. Since MXC induces antral follicle atresia without altering pituitary-gonadal hormones, our hypothesis is that MXC targets antral follicles directly through either Bcl-2 or Bax in the ovary. In order to test this hypothesis, CD-1 mice (39 days old) were treated with 64mg/kg MXC or sesame oil (vehicle), ovaries were collected during estrus and processed for immunohistochemical analysis of Bcl-2 or Bax proteins. Further, transgenic mice that overexpress Bcl-2 and mice deficient in Bax and their wild-type littermates were treated with either 64mg/kg MXC or vehicle, and their ovaries were collected during estrus and processed for morphological analysis of antral follicle atresia. Our results show that immunohistochemical staining for Bax was stronger in the MXC-treated mice, but Bcl-2 levels remain unchanged as compared to vehicle-treated mice. In addition, our results show that Bcl-2 overexpressing mice and Bax deficient mice were protected from MXC induced-atresia. There were significantly more healthy antral follicles in the MXC-treated Bcl-2 overexpressing mice than in the MXC-treated wild-type mice (Bcl-2 overexpressers = 903 ± 123, wild-type = 540 ± 72; n=7-9, p=0.03). MXC-treated Bax deficient mice had similar levels of atresia as the vehicle-treated Bax deficient mice (31% vs. 27%, respectively). Collectively, these data suggest that MXC induces antral follicle atresia *via* a mechanism that involves Bcl-2 and Bax. (Supported by NIH HD38955 and T32 ES07263-13).

357 *IN VITRO* FOLLICLE ASSAY ALLOWS GONADAL RISK ASSESSMENT FOR BENZODIAZEPINE.

R. Cortvrindt[1, 2], K. Van Wemmel[2], University. Eichenlaub-ritter[3] and J. Smitz[2]. *[1]EggCentris nv, Zellik, Belgium, [2]Follicle Biology Laboratory, Free University of Brussels, Brussels, Belgium and [3]Microbiology and Genetechnology, University of Bielefeld, Bielefeld, Germany.* Sponsor: M. Martens.

It is currently not clear whether chronic use of benzodiazepines can affect female fertility. *In vitro* studies suggest that acute high doses of diazepam (DZ) interfere with the meiotic process. Follicles exposed *in vitro* to chronic low doses of DZ had a reduced survival rate and poor oocyte quality. To investigate efficacy & specificity of the follicle culture system, 3 benzodiazepins with different receptor specificity were tested. Flumazenil (FL) binds to receptors in the CNS while 3-chlorodiazepam (CD)to peripheral receptors, diazepam (DZ) to both. Mouse preantral follicles were cultured singly for 12 days to the preovulatory stage, in α-MEM with FBS, FSH, LH. At day 12 an ovulatory stimulus (hCG) was added. Follicles were continuously exposed to FL, CD or DZ to 5, 10 or 15 µg/ml. Follicle growth, steroid secretion, oocyte quality were concurrently analysed. At all doses tested FL-exposure had no impact on folliculogenesis, steroid production or on oocyte maturation. CD acted similarly as DZ. Both drugs decreased follicle survival and altered steroid production dose dependently. At 15 µg/ml only 25 % of the follicles survived the 12-day culture period. CD blocked meiosis reinitiation while oocytes exposed to DZ started meiosis but progression was disturbed. Analysis of spindle formation revealed an increased aberration rate in the MII-oocytes exposed to CD and DZ but not to FL. At a dose of 5 µg/ml, cytogenetic analysis revealed a diploidy-rate of 15% and 29% for DZ and CD respectively. Five µg/ml FL had no effect on chromosome constitution. In accordance to their receptor binding specificity, benzodiazepines differ in their effects on ovarian function. FL was not cytotoxic during folliculogenesis while CD and DZ induced cytotoxicity and had an aneugenic effect on oocytes. The follicle culture system is a relevant *in vitro* model to study effects of NCE on the ovarian function and will be helpful to assess female fertility *in vitro.*

358 METABOLIC MECHANISMS OF METHOXYCHLOR TOXICITY IN MOUSE ANTRAL OVARIAN FOLLICLES.

K. P. Miller, C. Borgeest and J. A. Flaws. *Program in Toxicology, University of Maryland, Baltimore, MD.*

The organochlorine pesticide methoxychlor (MXC) is a reproductive toxicant that targets the mammalian ovary, causing reduced fertility and persistent estrus. Antral follicles are the primary targets of MXC toxicity, while less mature follicles are not affected by MXC exposure. Previous studies indicate that antral follicles from *in vivo*-exposed mice show increased atresia and altered expression of apoptotic proteins. Although MXC toxicity in non-ovarian tissues has been attributed to cytochrome P450 (CYP) metabolism to polar compounds including bis-hydroxy MXC (HPTE), little is known about ovarian mechanisms of MXC toxicity. Our hypothesis is that MXC metabolites are responsible for antral follicle-specific toxicity, and that CYP enzymes expressed in the ovary mediate MXC metabolism to cause antral follicle toxicity. Antral follicles were isolated from immature mouse ovaries and exposed *in vitro* to MXC (0.01-100µg/mL) or HPTE (0.01-1µg/mL) for 96hrs. Follicle diameters were measured to assess growth in response to MXC or HPTE. Cultured follicles were also processed for morphological analysis of atresia, as determined by numbers of pyknotic bodies in granulosa cells. Immunohistochemistry was performed on ovaries from *in vivo* MXC-exposed mice (sesame oil, 32mg/kg MXC, 20 days) to evaluate expression of MXC metabolizing enzymes CYP3A4 and CYP2C19. MXC significantly inhibited growth of antral follicles *in vitro* compared to controls at 10 and 100µg/mL (n=25 follicles/treatment; $p<0.01$), and increased atresia over controls at 100µg/mL. HPTE did not alter follicle growth, nor did it significantly induce atresia. Yet, both CYP3A4 and CYP2C19 were present in the ovary, and induced by MXC in corpora lutea, surface epithelium and antral/preovulatory follicles. These data suggest that metabolites other than HPTE may be responsible for suppression of antral follicle growth by MXC. Also, since CYPs are expressed in the ovary, metabolism of MXC by other ovarian components may subsequently cause antral follicle toxicity. (Supported by NIH HD38955, T32 ES07263-13 and a Colgate-Palmolive Fellowship).

359 ESSENTIAL ROLE OF NRF2 IN PROTECTION AGAINST OVARIAN FOLLICLE LOSS INDUCED BY 4-VINYCYCLOHEXENE AND 4-VINYLCYCLOHEXENE DIEPOXIDE IN MICE.

X. Hu[1], Y. Kan[2] and Q. Ma[1]. *[1]HELD/CDC, TMBB/NIOSH, Morgantown, WV and [2]Laboratory Medicine, Howard Hughes Medical Institute, University of California, San Francisco, CA.*

4-Vinylcyclohexene (VCH), an industrial chemical, and its metabolite 4-vinylcyclohexene diepoxide (VCD), represent a potential health hazard, because they selectively destroy oocytes in small pre-antral follicles leading to premature ovarian failure in animals. Previous studies suggest that metabolism of VCH and VCD by phase I and phase II drug-metabolism enzymes plays an important role in the ovotoxicity of these chemicals. Nrf2 is a member of the Cap ″N″ Colar bZip family of transcription factors that mediates the basal expression and induction of phase II enzymes such as NQO1. In this study, we examined the role of Nrf2-regulated gene expression in the ovotoxicity of VCH and VCD by using Nrf2 knockout mice. Immature (age, day 28) female wild-type and Nrf2-/- mice (both in B6 background) were treated with VCH or VCD using established protocols; 4 h following the final dose, ovaries were collected. Complete serial sections of ovaries were evaluated histologically for the presence of follicles. As expected, the primordial and primary follicle numbers in ovaries from wild type mice decreased significantly ($p<0.05$) following treatment with either VCH or VCD. However, the primordial and primary follicles in ovaries from Nrf2-/- mice exhibit much higher sensitive to the toxicity of VCH and VCD than those of wild type mice. Both VCH and VCD have no significant effects on growing or pre-antral follicles in either genotypes. Taken together, these results demonstrate that loss of Nrf2 function is associated with increased sensitivity to toxicity of VCH and VCD on ovary follicle development. The findings suggest that Nrf2-mediated expression of phase II genes plays an important role in detoxification of VCH and VCD, thereby protecting ovarian follicles from the ovotoxicity of the chemicals.

360 NEONATAL EXPOSURE TO GENISTEIN ALTERS OVARIAN DIFFERENTIATION RESULTING IN THE FORMATION OF MULTI-OOCYTE FOLLICLES.

W. Jefferson[1, 2], M. Pepling[3], E. Padilla-Banks[1] and R. Newbold[1]. *[1]Labortatory of Molecular Toxicology, NIEHS, Research Triangle Park, NC, [2]Department of Environmental and Molecular Toxicology, North Carolina State Univeristy, Raleigh, NC and [3]Syracuse University, Syracuse, NY.*

Genistein (Gen), the primary phytoestrogen in soy products, including soy-based infant formulas, was investigated for potential adverse effects on the developing ovary. We have previously shown that mice treated with Gen during the first week of life develop a dose dependent increase in the number of multi-oocyte follicles (MOF) at 19 days of age. During ovarian differentiation murine ovaries are composed of oocyte nests on day 1 which dissociate leaving predominantly single oocyte follicles by 5 days of age. To determine if Gen inhibits the breakdown of oocyte nests leading to MOF, we treated outbred CD-1 mice by subcutaneous injection on neonatal days 1-5 with Gen at 50 mg/kg/day and compared ovarian differentiation to age-matched untreated controls. Ovaries were collected on days 1-6

and fixed in 5.3% paraformadehyde. Ovaries were labeled with an oocyte marker, STAT3 and analyzed by confocal microscopy to observe oocyte nest breakdown. At 2 days of age, there was no apparent difference in the percent of single oocyte follicles (control 8%; Gen-treated 5 %). However, by 4 days of age, there was an apparent decrease in the % of single oocyte follicles in the Gen-treated ovaries compared to controls (Control 44%; Gen 21%). This difference was still apparent by 6 days of age (Control 57%; Gen 36%). To further determine the mechanism by which MOF are formed and since mice lacking GDF-9 and/or BMP-15 demonstrate the presence of MOF, we are currently investigating the role of these oocyte-secreted proteins in the Gen-treated ovaries compared to controls. Neonatal exposure to Gen alters the differentiating ovary by inhibiting oocyte nest breakdown leading to the development of MOF. This altered phenotype may play a role in the infertility seen in mice exposed developmentally to genistein. Whether other environmental estrogens cause similar effects is being investigated.

361 QUANTIFICATION OF TOXICANTS IN COMMERCIAL BRAND CIGARETTES AND CHARACTERIZATION OF THEIR EFFECTS ON OVIDUCTAL FUNCTIONING.

K. Riveles[1], R. Roza[1], D. Kwan[1], V. Tran[1], J. Arey[2] and P. Talbot[1]. *[1]Department Cell Biology & Neuroscience, UC Riverside, Riverside, CA and [2]Environmental Sciences, UC Riverside, Riverside, CA.*

Previously, mainstream (MS) and sidestream (SS) smoke solutions and individual components (pyridines, pyrazines, phenols, quinolines, and indoles) in University of KY 2R1 research cigarettes were shown to be toxic to mammalian oviducts in ciliary beat frequency, oocyte pickup rate, and smooth muscle contraction assays. The purpose of this project was to compare the concentrations of toxicants in MS and SS smoke solutions from a variety of commercial and research brand cigarettes: Omni, Advance, Marlboro, Camel, Kool, 2R1 & 1R4F, and to test these solutions in the oviductal assays. Although exact concentrations have not yet been calculated, preliminary analysis of peak size has shown that the above toxicants are more concentrated in SS than in MS smoke solutions. MS and SS smoke solutions made from several of the commercial brand cigarettes have been tested in the oviductal assays. For MS smoke solutions, the percentages of inhibition for ciliary beat frequency, oocyte pickup rate, and smooth muscle contraction for Camel brand cigarettes were: (27.12%±5.12), (36.20%±3.16), and (89.72%±0.39) respectively; for Kool were (17.44%±3.02), (49.14%±2.68), and (89.52%±1.31) respectively; and for Omni Light were (32.3%±9.77), (43.29%±3.16), and (5%±0.56) respectively. For SS smoke solutions, the percentages of inhibition for ciliary beat frequency, oocyte pickup rate, and smooth muscle contraction for Camel were (37.75%±3.97), (93.84%±0.23), and (99.59%±0.50) respectively; for Kool (99.5%±0.34), (94.64%±0.25), and (99.5%±0.55) respectively; and for Omni Light were (41.4%±4.67), (46.4%±0.38), and (78.23%±1.65) respectively. The results of this study show that sidestream smoke solutions were more inhibitory in the oviductal assays than mainstream solutions when puff equivalents were made equal. In addition, oocyte pickup rate and smooth muscle contraction were more affected than ciliary beat frequency.

362 GESTATION-AGE RELATED INCREASES AND ACTIVATION OF PHOSPHOLIPASE A_2 ENZYMES MEDIATE PCB 50 INDUCED STIMULATION OF RAT UTERINE FUNCTION.

K. A. Brant and R. Loch Caruso. *Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

We previously reported a gestation day sensitivity of rat uterine muscle to stimulation by the ortho substituted polychlorinated biphenyl (PCB) congener 2, 2′, 4, 6–tetrachlorobiphenyl (PCB 50). PCBs have been reported to activate phospholipase A_2 to release arachidonic acid (AA) from membrane phospholipids. We hypothesized that PCB 50 stimulates uterine function through activation of PLA_2 enzymes, with late gestation tissue being more sensitive to stimulation due to gestational increases in uterine PLA_2 expression. Western blot analysis on uterine tissue homogenates revealed a significant increase in calcium-independent and secretory PLA_2 expression from mid (gd10) compared with late (gd20) gestation ($p < 0.05$), with no changes in cytosolic PLA_2 expression. To examine the effects of PCB 50 on PLA_2 activity, primary cultures of gd20 rat uterine myocytes (rmc) were loaded with 0.5 μCi ^{3}[H]-AA prior to a 10, 20 or 30 min exposure to 1, 10, 15, 25, or 50 μM PCB 50, or 0.1% DMSO (control). PCB 50 stimulated the release of ^{3}[H]-AA from gd20 rmc in concentration- and time- dependent manners ($p < 0.05$). A significant release of ^{3}[H]-AA was first observed after 20 min in the 50 μM treatment group compared to control ($p < 0.05$). After 30 min, PCB 50 stimulated a concentration-dependent increase in ^{3}[H]-AA release at 15, 25, and 50 μM compared to control ($p < 0.05$), with values significantly greater than those observed at 10 and 20 min ($p < 0.05$). In the presence of 1 mM EGTA ^{3}[H]-AA release following stimulation was reduced, suggesting the release is, in part, calcium-dependent. PCB 50-stimulated ^{3}[H]-AA release was also reduced in the presence of secretory, cytosolic and calcium independent PLA_2 inhibitors ($p < 0.05$). These results indicate that PCB 50 stimulates ^{3}[H]-AA release *via* activation of multiple PLA_2s. Furthermore, the gestation age-related increases in secretory and calcium independent PLA_2 expression provide a potential mechanism for the gestation day sensitivity to rat uterine stimulation by PCB 50.

363 STIMULATORY EFFECTS OF A MICROBIALLY DECHLORINATED POLYCHLORINATED BIPHENYL (PCB) MIXTURE ON RAT UTERINE CONTRACTION *IN VITRO.*

R. Loch-Caruso[1], T. Tsuneta[1], M. Hanna[1], C. Grindatti[1], J. F. Quensen[2, 3] and S. A. Boyd[2, 3]. *[1]Environmental Health Sciences, University of Michigan, Ann Arbor, MI, [2]Crop and Soil Sciences, Michigan State University, East Lansing, MI and [3]Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.*

Previously, we reported that microbial dechlorination increases the ability of commercial PCB mixtures (Aroclor 1242, 1248 and 1254) to stimulate uterine contraction. In the present study, we analyzed the most abundant chlorobiphenyl (CB) congeners produced by microbial dechlorination of Aroclor 1260 in an attempt to identify stimulatory congeners. Aroclor 1260 was incubated under anaerobic conditions with bacteria isolated from Hudson River sediment to generate the partially dechlorinated mixture HR1260. A control mixture, Auto1260, was generated by incubating Aroclor 1260 with autoclaved Hudson River sediment microorganisms, and the congener composition of Auto1260 was identical to that of Aroclor 1260. To evaluate uterine contraction, uterine strips from midgestation rats were suspended in muscle baths and exposed *in vitro* to PCBs. HR1260 significantly increased contraction frequency to 718±134% at 70 μM, whereas Auto1260 and Aroclor 1260 had no significant effects on uterine contraction frequency. Compared with Auto1260, microbial dechlorination increased 2, 2’, 4, 4-CB in HR1260 to 24 mol %. Also, 2, 3’, 3, 5-CB, 2, 3’, 4, 5-CB, 2, 2’, 4, 4’, 6-CB and 2, 2’, 4, 5’, 6-CB co-eluted in a chromatography peak that increased 7.6 mol %. Of these congeners, only 2, 2’, 4, 5’, 6-CB and a mixture of 2, 2’, 4, 4 -CB and 2, 2’, 4, 5’, 6-CB significantly increased contraction frequency to 129±5.1% and 124±15% at 100 and 60 μM, respectively, but the magnitude of the increases were modest compared to the stimulatory effects of HR1260. These results show that anaerobic microbes isolated from river sediments dechlorinated the commercial PCB mixture Arochlor 1260 and, in doing so, generated a highly uterotonic PCB mixture. The most abundant PCB congeners in the dechlorinated mixture did not account for the uterotonic activity of HR1260, however.

364 DEVELOPMENTAL EFFECTS OF *IN UTERO* EXPOSURE TO BISPHENOL A ON THE UTERUS OF RAT OFFSPRING.

G. Schoenfelder[1], K. Friedrich[1], X. Wu[2, 1], M. Paul[1] and I. Chahoud[1]. *[1]Department of Toxicology, Campus Benjamin Franklin, Berlin, Berlin, Germany and [2]Department of Medicine, Thomas Jefferson University, Division of Endocrinology, Diabetes and Metabolic Diseases, Philadelphia, PA.* Sponsor: R. Stahlmann.

The origins of the endocrine disrupter hypothesis may be traced to reports on adolescent daughters born to women who had taken the synthetic oestrogen, diethylstilbestrol (DES), during pregnancy. Exposure to estrogenic compounds during critical periods of fetal development could result in adverse effects on the development of reproductive organs later in life. Bisphenol A (BPA) is widely discussed as a prime candidate for endocrine disruption. We examined the influence of BPA to address the question whether *in utero* exposure affects the uterus of the offspring. Gravid Sprague-Dawley dams were administered either 0.1 (low dose) or 50 mg/kg/d BPA or 0.2 mg/kg/d 17alpha-ethinyl estradiol (E2) by gavage on gestation days 6 through 21. Striking morphological changes, which are similar to DES-specific disruption patterns, were observed in the uterine epithelium of post-pubertal offspring during estrus of the *in utero* BPA-treated animals. Therefore, we examined uterine estrogen receptor (ERalpha) expression because BPA binds to the ERalpha which is important for growth of the uterine epithelium. It seems that within the 50 mg BPA dose-group the close epithelial-stromal tissue interaction is disturbed, because of the disorganization of ERalpha-immunostained stromal cells. Furthermore, in the E2- and 50 mg BPA-treated group the expression of ERalpha and intensity of the ERalpha-immunostaining increased at protein level when compared to the control and 0.1 mg BPA-treated uteri. Newly synthesized ERalpha could be demonstrated by significantly increased ERalpha-mRNA expression. In all, these results clearly indicate that *in utero* exposure (not neonatal) of rats to low-doses of BPA induces a permament developmental change and promotes uterine disruption.

365 METABONOMIC PROFILING OF URINE IN ANTIBIOTIC-INDUCED NEPHROTOXICITY IN FEMALE CYNOMOLGUS MONKEYS.

J. W. Davis[1], A. Buevich[2], F. M. Goodsaid[1], R. J. Smith[1], L. A. Obert[3], T. Chan[2] and I. Y. Rosenblum[1]. [1]*Molecular Toxicology, Schering-Plough, Lafayette, NJ,* [2]*Structural Chemistry, Schering-Plough, Kenilworth, NJ and* [3]*Pathology, Schering-Plough, Lafayette, NJ.*

Metabonomics is defined as the use of nuclear magnetic resonance spectroscopy to analyze urine for small molecule biomarkers of toxicity. Rodent models have been used to identify urinary markers of target organ toxicity. We developed a non-human primate model of nephrotoxicity in an effort to evaluate metabonomic results reported from rodent studies. Female cynomolgus monkeys were dosed with the aminoglycoside antibiotic gentamicin (10 mg/kg) and/or everninomicin (30 or 60 mg/kg), an experimental oligosaccharide antibiotic, for seven days. Urine was collected for 24 hr by cage run-off into beakers packed in ice prior to the start of dosing and on Days 1, 3, and 7 from all monkeys. Monkeys receiving both drugs showed proximal tubular injury as early as Day 1, consistent with a potentiation of toxicity resulting from co-administration of both test articles. By Day 7 monkeys dosed with 60 mg/kg everninomicin alone developed similar lesions, while the group exposed to both compounds had more extensive tubular damage. Principal components analysis (PCA) of the spectra obtained from all urine samples revealed a substantial shift in the first component as early as Day 1 in all groups receiving everninomicin, which became more pronounced on Days 3 and 7. The PCA from animals treated with gentamicin alone also shifted in the same direction, although only on Days 3 and 7. Further analysis indicated that decreased urinary hippurate was primarily responsible for the observed clusters. Decreased urinary hippurate had previously been identified in rodent models of nephrotoxicity using metabonomics. Gene expression analysis of kidneys from these animals revealed a decrease in abundance of organic anion transporter, kidney-1 (OATK-1) mRNA, the primary transport protein responsible for hippurate clearance. These results provide the first non-human primate data characterizing urinary hippurate as a potential biomarker of antibiotic-induced nephrotoxicity.

366 NMR-BASED METABONOMICS STUDY OF ETHANOL-FED RATS.

R. D. Beger[1], L. K. Schoenbachler[1], Y. P. Dragan[2], M. J. Ronis[3] and T. M. Badger[3]. [1]*Chemistry, National Center for Toxicological Research, Jefferson, AR,* [2]*Center for Hepatotoxcity, NCTR, Jefferson, AR and* [3]*Arkansas Children's Hospital and University of Arkansas for Medical Sciences, Little Rock, AR.*

Adult male Sprague-Dawley rats were fed by continuous intragastric infusion of a diet containing ethanol sufficient to provide 13 g/kg/day. This paradigm has previously been reported to result in urine ethanol concentrations (UECs) that cycle between 0 and 500 mg/dl over a 6 - 7 day period in rats infused with ethanol diets. Further, Class I alcohol dehydrogenase (ADH) expression is elevated at these high UECs and this results in greater ethanol metabolism that drives the UEC down. After 3 days, the mean UEC levels are reduced to near zero and the ADH expression was decreased. Here, the UECs were monitored by 1D 1H NMR and principal component (PC) analysis of the NMR spectra with the bins corresponding to the ethanol peaks removed revealed that the high and low UEC were in well-separated clusters. Further inspection of the PC loading map showed samples with a low UEC had a corresponding increase in the spectral bins at 3.26 ppm and 3.42 ppm matching to taurine, the bin at 5.38 ppm increased which corresponds to a fatty acid peak and the spectral bin at 3.02 ppm which correspond to a creatine peak. Spectral bins at 3.30 ppm, 3.50 ppm, and 3.70 ppm decreased with a decrease in UEC which correspond to carbohydrate sugar compounds. The taurine levels were increased by 110% in the samples with the lowest UEC when compared to samples with the highest UEC. Taurine and creatine has been previously reported as markers of hepatic dysfunction. Supported by 6251-51000-003-06S.

367 URINARY METABOLITE PROFILING OF RENAL INJURY USING NMR OR GC-MS.

C. E. Thomas[1], J. Colet[2], J. Eckstein[1], R. Julian[1], J. Sefton[1], J. Koers[1], M. Bollard[3] and B. Ackermann[1]. [1]*Toxicology & Drug Disp., Eli Lilly & Co., Indianapolis, IN,* [2]*Toxicology, Eli Lilly & Co., Mont-Saint-Guibert, Belgium and* [3]*Biol. Chem., Imperial College, London, United Kingdom.*

The study of metabolite fluxes in biological systems may be useful in assessing the efficacy and/or toxicity of drug candidates. Metabonomics has been used to describe the study of metabolites in living systems by NMR. Metabolomics generally refers to a more detailed elucidation of the metabolome, often in less complex systems, using mass spectrometry (MS). We compared urinary metabolite profiling by 1H-NMR and GC-MS in rats treated with the renal toxicant mercuric chloride ($HgCl_2$). Male, SD rats were treated with a single dose of $HgCl_2$ at 0.5 or 2 mg/kg and urine collected serially to 168 hrs post-dose. At 2 mg/kg, $HgCl_2$ induced marked tubular necrosis by 48 hr; the low dose resulted in more modest injury. Urinary metabolite changes, as determined by NMR, were analyzed using principle component analysis. At both doses, maximal effects were observed at 48 hr post-dose. Recovery of low dose animals was evident histologically and by the return of metabolites to near pre-dose values; the high dose group was still abnormal at 168 hrs. Separation of dose groups was attributable to a reduction in Krebs cycle intermediates including citrate, 2-oxoglutarate and succinate; and an increase in glutamate, leucine/valine and alanine. Urine from the 48 hr timepoint was derivatized, analyzed by GC-MS and peaks identified by comparison to authentic standards. Consistent with the NMR data, there was a dose-dependent decrease in 2-oxoglutarate, citrate, creatinine and hippurate. Conversely, a large increase in the excretion of urinary amino acids including glutamate, alanine and threonine was detected. A 'feature vector' method was developed to automatically identify significantly altered chromatographic peaks. Fifty two distinct peaks were identified; 23 were altered. Further metabolite identification and inclusion of more timepoints is ongoing. The results indicate that NMR and GC-MS data are largely in agreement and that MS can be used to identify key metabolites associated with target organ toxicity.

368 EVALUATION OF URINE METABONOMIC CHANGES IN ZDF RAT DURING THE DEVELOPMENT OF DIABETES AND TREATMENT WITH VANADYL ACETYLACETONATE.

J. A. Colet, A. Cauvin, K. Kramer and I. Smyej. *Toxicology, Eli Lilly and company, Mont-Saint-Guibert, Brabant, Belgium.* Sponsor: C. Thomas.

Introduction: New biomarkers of efficacy in animal models are needed for the development of anti-obesity drugs. Metabonomics is a new technology based on the analysis of metabolic changes in biofluids by proton Magnetic Resonance Spectroscopy (1H-NMR). Zucker diabetic fatty (ZDF) rat is a widely used model of Type 2 diabetes. The insulin-like potential of vanadium has been demonstrated *in vitro* and *in vivo* in rodents. Purpose: This study aimed at evaluating by 1H-NMR spectroscopy the urine metabolic changes occurring in ZDF rats during the development of the diabetic status, and during the subsequent treatment with vanadyl acetylacetonate (Vac). Methods: 5 ZDF (Gmi-fa/fa) rats and 5 lean rats (Gmi-lean +/+) were placed in metabolic cages at 6 week-old. Urine samples were collected daily for a period of 6 weeks for 1H-NMR analysis. Blood was collected once a week from tail vein for plasma glucose, fatty acids, triglycerides, cholesterol, and insulin measurements. At stabilized hyperglycemia, all animals received VAc in drinking water. The 1H-NMR spectra were acquired on a Bruker Avance 600 MHz spectrometer. Results: At 6 week-old, obese rats had higher plasma levels of insulin, glucose, and triglycerides than lean rats. At 9 week-old, the obese rats reached a stabilized hyperglycemia, and their metabonomic profile was clearly separated from the lean animals. This separation was mostly caused by higher urine levels of glucose, lactate, acetate, creatinine, TMAO/betaine, and methylamines. The treatment with Vac caused a progressive reduction in plasma levels of glucose in obese rats and an increase in cholesterol. Finally, a progression move from the obese metabonomic profile towards the lean profile was noticed in obese rats treated with Vac. Conclusion: This study has demonstrated the potential of metabonomics to follow the progressive metabolic changes in an animal disease model, as well as to evaluate the effect of a drug.

369 FOOTPRINT OF INFLAMMAGEN EXPOSURE IN THE RAT LIVER NUCLEAR PROTEOME AFTER LIPOPOLYSACHARIDE TREATMENT.

M. E. Bruno[1], J. E. Madenspacher[1], J. R. Dubin[2], J. F. Foley[1], K. B. Tomer[2] and B. A. Merrick[1]. [1]*Ntl Ctr Toxicogenomics, NIEHS, Research Triange Pk, NC and* [2]*LSB, NIEHS, Research Triange Pk, NC.*

Lipopolysacharide (LPS), induces acute systemic endotoxemia and a widespread inflammatory response after i.p. exposure with extensive release of cytokines, chemokines and bioactive peptides. Activation of Toll-like receptors and translocation of NF-κB from cytosol to nucleus precedes transcriptional activation for many target inflammatory genes. In liver, inflammatory effects and NF-κB activation usually subside after 24 hr with little residual histopathology. In this recovery state, we examined the hypothesis that LPS would leave a footprint upon the liver nuclear proteome reflected by continued changes in protein expression. Male, Sprague-Dawley rats were exposed a to 5 mg/kg i.p. dose of saline or LPS for 24 hrs. Rat nuclei were isolated, nuclear protein extraction performed and protein was separated by two dimensional (2D) gel electrophoresis followed by protein identification with mass spectrometry (MS). Image analysis revealed over 800 nuclear proteins, of which 41 proteins were altered at $p<.05$ significance level and an additional 17 proteins that were selected at a >2 fold change. Twenty six proteins were identified by MS using MALDI-MS and tandem MS. The proteins that underwent the greatest upregulation following LPS exposure were involved in inflammatory responses,

DNA binding and transcription-related pathways. LPS down-regulated proteins included splicing factor 3A, (SF3A) and formiminotransferase cyclodeaminase. Proteins not previously associated with LPS effects include coronin, nucleolin, ETI4A and SF3A. Additional western blot analysis validated the increased expression of HMG-1 and members of the ERM complex that were identified in LPS treated samples after separation by 2D gels electrophoresis. We conclude that a subset of nuclear protein changes persists, even after serum cytokines levels and liver NF-κB levels return to normal. Studies with additional inflammagens will solidify members of this nuclear protein footprint and further our understanding of inflammatory processes.

370 BIOMARKERS OF INFLAMMATION FROM RETENTATE CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS OF RAT SERUM AFTER ACUTE LIPOPOLYSACCHARIDE TREATMENT.

J. H. Madenspacher[1], L. Li[1], J. A. Taylor[1], M. E. Bruno[1], B. A. Wetmore[1], A. Xu[2] and B. A. Merrick[1]. *[1]National Ctr Toxicogenomics, NIEHS, Research Triange Pk, NC and [2]Ciphergen Biosystems Inc., Fremont, CA.*

Bacterial lipopolysaccharide (LPS) can induce septic shock by release of cytokines and vasoactive peptides. Serum from rats exposed to the model inflammagen, LPS, was analyzed by retentate chromatography mass spectrometry (RC-MS) to distinguish acute inflammatory response proteins over time. RC-MS is a type of proteomic analysis for biomarker discovery whereby serum proteins are selectively adsorbed to chromatographic surfaces and then read by a mass spectrometer (MS). Male Sprague-Dawley rats at 12 per group were treated with either saline or 5 mg/kg i.p. of LPS for 2 and 6 hr with biochemical characterization by TNF-alpha and IL6 serum ELISA. RC-MS analysis involved whole serum and multidimensional fractionation (MDF) of serum by strong anion exchange and step-wise pH elution. A map of differentially expressed proteins with peak changes at p⟨0.01 significance level showed 26 proteins were induced and 15 proteins were repressed overall with LPS exposure using MDF serum compared to only 6 protein changes detected from unfractionated serum. Most serum protein changes were detected after 2 hr LPS exposure compared to 6 hr. Additional bioinformatic analysis using the Genetic Algorithm/k-Nearest Neighbor technique (GA/KNN) for classification was used to screen combined data from entire mass spectrum scans for each animal for all analyzed multidimensional fractionated serums. GA/KNN analysis created a list of ten m/z peaks that were the most discriminative proteins in characterizing acute LPS exposure and these proteins are currently being identified. We concluded that RC-MS analysis of MDF serum is a more sensitive means of detecting LPS responsive biopeptides than whole serum alone and offers the opportunity for biomarker discovery. The differential expression profile in LPS treated animals at 2 and 6 hr also suggests RC-MS can accurately detect acute inflammatory responses in a temporal manner.

371 NOVEL *IN VITRO* SKIN IRRITATION MARKERS IDENTIFIED USING MICROARRAY TECHNOLOGY.

S. Fletcher, C. Duggan and D. Basketter. *SEAC - Safety and Environmental Assurance Center, Unilever, Sharnbrook, Bedfordshire, United Kingdom.*

To develop a relevant and sensitive *in vitro* testing strategy for the identification of mild to more substantially irritant compounds, mechanistic information on the skin irritation response is required. This study investigated the mechanisms of mild skin irritation for a number of compounds, identifying specific and general markers. EpiDerm™ (MatTek, USA), a reconstructed human skin model, was treated in triplicate with 0.1mg/ml benzalkonium chloride (BKC), 2.5mg/ml phenol, 0.1mg/ml sodium lauryl sulphate (SLS) or media control for 15min, 2, 4 or 24 hours. The doses used were non-cytotoxic, as determined by MTT assay and histology. Microarray analysis of 5 biological replicates was performed using Cy3 and Cy5 labelled samples hybridised to an in-house skin chip comprising of 2100 skin relevant genes in triplicate. For each irritant chemical around 400 genes were up-regulated. Known irritation markers found to be up-regulated, included IL-1α, IL-8, TNFα and EGF. 20% of genes were up-regulated with all three compounds and therefore could be classed as associated with general irritation, these included hsp27, integrin β3 and VCAM1 among others. In addition genes were found which were compound specific. IL-16, early growth response 1, caspase 8, caveolin 1, adenylate cyclase 8 are examples of genes up-regulated upon treatment with SLS. Treatment with BKC up-regulated, phospholipase A2, midkine, IL-12 receptor, retinoic acid receptor responder, transglutaminase 1, IL-6. Genes up-regulated in response to phenol included, fibronectin, integrin α6, calcitonin, serine protease inhibitor, transglutaminase 4 and thioredoxin. These results demonstrate that although compounds initially may use different mechanisms of action, there are nevertheless similarities and a number of genes could be investigated further as potential general markers of irritation.

372 THE ETAG MULTIPLEX ASSAY SYSTEM, A NOVEL ASSAY PLATFORM FOR ANALYZING GENOMIC AND PROTEOMIC ENDPOINTS DURING COMPOUND SAFETY AND TOXICITY ASSESSMENTS.

K. Steinmetz[2], T. Tian[1], L. Chen[1], Y. Badal[1], D. Ackley[3], K. Wehmeyer[3], J. Troutman[3], C. Virgo[1], S. Moore[1], V. Xiao[1], X. Jin[1] and S. Singh[1]. *[1]Aclara, Mt View, CA, [2]SRI Int., Menlo Park, CA and [3]P&G, Mason, OH.*

Routine evaluation of OMIC endpoints during safety assessments is limited by the sample quantity, cost, and time requirements. The eTag Multiplex Assay System (eTag Assay) permits rapid analysis of biomolecular targets from cultures and tissues using minimal sample processing. The eTag Assay utilizes electrophoretically distinct fluorescent molecules coupled to a broad range of biomolecular binding moieties, such as oligonucleotides, peptides, or proteins. The eTag molecules' unique physical properties allow simultaneous reactions and detection (multiplexing) within the same sample. eTag Assays have been developed for identifying a variety of biomolecular targets. Primary rat hepatocyte cultures were treated with compounds representing different nuclear receptor signaling pathways. Hepatocytes treated with Lovastatin (L) and Clofibrate (C) resulted in increased expression of mRNAs for CYP4A (L:3.5, C:10.3), CYP3A (L:6.2, C:21.3) and CYP2B (L:2.3, C:25.5) whereas 3-methylcholanthrene induced mRNA for CYP1A (25.8) exclusively. Differential induction of phase II enzymes such as Gsta2 and Ugt1a was also observed. A strong correlation was observed between mRNA expression and protein levels in the experiments. To demonstrate eTag Assay versatility, samples of rat tissues were evaluated for both mRNA levels and protein expression. Hepatic CYP1A1 (19.7) and 1A2 (17.5) were induced following *in vivo* treatment with a CYP1A inducer whereas there was no induction of other CYP isoforms. eTag Assay results were confirmed using TaqMan and Western blot analyses. The results from these experiments demonstrate the sensitivity, accuracy, and versatility of the eTag Multiplex Assay System. eTag Assays are an ideal technology to gather mechanistic information during routine toxicology and safety studies thereby allowing better selection of compounds early in the drug-development process.

373 PROTEOMIC CHARACTERIZATION OF THE EFFECTS OF CLOFIBRATE ON PROTEIN EXPRESSION IN RAT LIVER.

J. Leonard[1], C. Saulnier[1], M. Courcol[1], A. Charbonnier[1], E. Boitier[1], R. A. Roberts[1], M. Duchesne[2], F. Parker[2] and J. Gautier[1]. *[1]Drug Safety Evaluation, Aventis Pharmacology, Vitry-sur-Seine, France and [2]Functional Genomics, Aventis Pharmacology, Vitry-sur-Seine, France.*

Clofibrate is a peroxisome proliferator known to induce liver tumours in rats. A proteomics study was conducted in order to provide new insights into the molecular mechanisms of clofibrate-induced nongenotoxic hepatocarcinogenesis. Rats were treated with 250 mg/kg/day clofibrate orally and were sacrificed after 7 days. Proteins were extracted from liver and analyzed by 2D gel electrophoresis using differential gel electrophoresis technology. Protein identification of the up- and down-regulated spots was performed by mass spectrometry. Data showed that clofibrate induced up-regulation of 77 proteins and down-regulation of 27 proteins. The highest expression ratios corresponded to proteins involved in a series of biochemical pathways such as lipid metabolism, fatty acid metabolism, amino acid metabolism, protein metabolism, citric acid cycle, xenobiotic detoxification and oxidative stress. Proteins implicated in cell proliferation and apoptosis such as prohibitin, 10-formyl tetrahydrofolate dehydrogenase, senescence marker protein, pyridoxine 5′-phosphate oxidase and vimentin were also identified as being regulated. These latter results provide leads for ongoing investigation into the molecular mechanisms of liver tumors induced by clofibrate. In addition, mass spectrometry results showed that several proteins such as 10-formyl tetrahydrofolate dehydrogenase, carnitine-O-palmitoyl transferase II, ATP synthase beta chain subunit, long chain fatty acyl CoA ligase, aspartyl tRNA synthetase were detected as several spots corresponding to different isoelectric points. Differential effects on those pI variants could result from specific post-translational modifications and could be a specific molecular signature of the clofibrate-induced protein expression modulation in rat liver.

374 LEAD BINDING TO HUMAN SEMINAL PROTEINS AND ZINC EQUILIBRIUM.

C. Sarmiento-Mariscal, I. Hernandez-Ochoa and B. Quintanilla-Vega. *Toxicology Section, CINVESTAV-IPN, Mexico City, D.F., Mexico.*

Seminal liquid has a high content of zinc (Zn) from prostatic origin which plays important roles on spermatozoa function, such as chromatin condensation. Zinc redistribution from molecules of low molecular weight (LMWP) towards high affinity seminal proteins of high (HMWP) and intermediate (IMWP) molecular weight occurs at ejaculation. Lead (Pb) exposure has been associated with male reproductive effects, particularly on sperm chromatin condensation, probably by al-

tering Zn equilibrium in seminal liquid (Zn-bound/free Zn) favoring Zn entering the cell. Lead has affinity for thiol groups in Zn-depending proteins; thus in this study we evaluated Pb binding to seminal liquid proteins and displacement of Zn from its binding sites. Human seminal liquid (3.6mM endogenous Zn) from healthy donors were applied to a Sephadex G-75 column, and eluted with Tris 0.05M/NaCl 0.15M, pH 7.4 (0.3 mL/min flow rate). Proteins were monitored at 280 nm in 4.5 mL-fractions. Later, seminal liquid was incubated with 0.48µM of Pb (maximum concentration in occupationally-exposed men) at 37°C/1 h, applied to the Sephadex G-75 column and eluted as mentioned. Fractions were subjected to Zn and Pb determination performed by atomic absorption spectrophotometry (using flame and graphite furnace, respectively). Endogenous Zn profile (Zn-bound to proteins) showed a major peak corresponding to molecules of <3 kDa (LMWP), a second one to proteins >80 kDa (HMWP) and two minor peaks to proteins from 27-38 kDa and from 2-6 kDa. After Pb incubation, Pb was bound to proteins in factions where Zn content was decreased, corresponding to proteins of approximately 270, 110, 31, 6 and 5 kDa, suggesting a displacement of Zn. Also, a Zn re-distribution was observed mainly among HMWP. *In vitro* experiments are in progress to evaluate the effect of Pb-binding to seminal proteins on human sperm chromatin condensation as well as to complete the characterization of Pb binding to Zn-seminal proteins.

375 METALS IN INNER CITY AND SUBURBAN COMMUNITIES OF DETROIT AND NEW ORLEANS.

H. W. Mielke, C. Gonzales, A. Shah and E. Powell. *College of Pharmacy, Xavier University, New Orleans, LA.*

Nine metals (Pb, Zn, Cd, Mn, Ni, Cu, Cr, Co., and V) were analyzed in inner city and suburban census tracts of Detroit, Michigan and New Orleans, Louisiana. Detroit is located where glacial processes deposited tills during the Ice Ages. New Orleans is on allu*vial* soils deposited by flu*vial* processes of the Mississippi River Delta. Allu*vial* samples were taken from the Bonnet Carré Spillway after the flood of 1997. Soil samples from Detroit and New Orleans were collected from census tracts in the inner-city and suburbs of each city. Surface (2.5 cm) samples were collected according to a standard protocol (n=19). All samples were air-dried and sieved (2 mm screen). Soil metal extraction was done using a 5:1 ratio of 1 mol L-1 nitric acid (room temperature) to soil, shaken 2 hours, centrifuged (1000 x g - 15 min.) and filtered. Extraction pH and the temperature match the range of the human digestive tract. Metals were determined using inductively coupled plasma-atomic emission spectrometry. There are consistently smaller quantities of metals in the Bonnet Carré fresh alluvium compared to inner-city or suburban Census Tracts of New Orleans and Detroit. In both cities, the inner city soils always have larger metal quantities than the soils in suburban census tracts. Pb and Zn are exceptionally elevated in New Orleans compared with Detroit. The compactness of New Orleans compared to Detroit may explain this difference. Cd, Ni, Cr, Co and especially Mn are elevated in Detroit compared with New Orleans. Cu and V are about the same for both cities. In suburban census tracts Cd, Mn, Cr and Co are more elevated in Detroit and Zn is more elevated for New Orleans. Soil Mn and Zn probably reflect the geochemistry of glacial till compared to alluvium of the Mississippi River Basin. The Pb, Cu and V are similar in the suburban census tracts of these cities. Anthropogenic processes account for metals in each city, especially for Pb, Zn, Mn and Cu, and to a lesser extent Cd, Ni, Cr, Co., and V. Metal exposure probably influences human health in the inner cities.

376 MERCURY CONTAMINATION IN THE RED MEAT OF WHALES AND DOLPHINS MARKETED FOR HUMAN CONSUMPTION IN JAPAN.

T. Endo[1], K. Haraguchi[2], H. Yohei[1] and M. Sakata[1]. [1]*Clinical Toxicology and Metabolism, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido, Japan and* [2]*Health Science and Chemistry, Daiichi College of Pharmaceutical Sciences, Fukuoka, Fukuoka, Japan.*

We surveyed the total mercury (T-Hg) and methyl mercury (M-Hg) levels in odontocete red meat, the most popular cetacean products in Japan. We also analyzed the DNA of these to obtain information regarding species. According to the genetic analysis, the red meats originating from nine species of odontocete such as Dall's porpoise, pantropical spotted dolphin, Risso's dolphin, rough-toothed dolphin, bottlenose dolphin, false killer whale, pilot whale and Baird's beaked whale were sold in Japanese markets. T-Hg and M-Hg concentrations in all odontocete red meats (n=214) exceeded the provisional permitted levels of T-Hg (0.4 µg/wet g) and M-Hg (0.3 µg/wet g) in marine foods set by the Japanese government, respectively. The highest concentration of T-Hg was found in a false killer whale meat which contained 81.0 µg/wet g T-Hg and 13.4 µg/wet g M-Hg, exceeding the permitted levels by 200 and 45 times, respectively. The second highest concentration of T-Hg was found in a striped dolphin meat which contained 63.4 µg/wet g T-Hg and 26.2 µg/wet g M-Hg, exceeding the levels by 160 and 87 times, respectively. Chronic intoxication by Hg due to frequent consumption of odontocete red meats should occur. T-Hg versus the percentage of M-Hg/T-Hg in the red meat was well fitted by a logarithmic equation, suggesting the demethylation of M-Hg in the odontocetes. A high correlation was observed between T-Hg and selenium (Se) concentrations in the red meats, supporting the formation of Hg-Se complex. The formation of Hg-Se complex after the demethylation may contribute to the detoxification of M-Hg in odontocetes. The T-Hg concentration levels were slightly higher in Baird's beaked whales, pilot whales, bottlenose dolphins and striped dolphins caught off southern areas than those caught off northern areas, probably reflecting a higher Hg concentration in the seawater and/or their diet (squid and fish) in the southern area.

377 RENAL DYSFUNCTION IN CADMIUM EXPOSED HUMANS - RELATIONSHIP TO CHANGES IN BONE DENSITY AND METALLOTHIONEIN GENE EXPRESSION.

G. F. Nordberg[1] and M. Nordberg[2]. [1]*Environmental Medicine, Umea University, Umea, Sweden,* [2]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden,* [3]*Department of Occupational Health, School of Public Health, Fudan University, Shanghai, China,* [4]*Unit of Industrial Toxicology, Catholic University of Louvain, Brussels, Belgium and* [5]*Department of Bone Metabolism, Institute of Radiation Medicine, Fudan University, Shanghai, China.*

Nordberg G(1), Nordberg M(2), Jin T(3), Lu J(3), Bernard A(4), Ye T(3), Wang H(5). Long term exposure to cadmium (Cd) can give rise to both renal dysfunction and bone effects. Only few studies have reported both bone effects and renal dysfunction in the same persons and it is unclear whether bone effects occur at lower or higher internal doses of Cd than renal dysfunction. 734 persons living in a cadmium polluted area in China were studied by measurements of Bone Mineral Density (BMD)and urinary levels of retinol binding protein (RBP) and beta-2-microglobulin (B2M).Internal doses of Cd were indicated by levels of Cd in blood (CdB) in microgram/l or Cd in urine (CdU)in micrograms /g creatinine. An increased prevalence ($p<0.05$) of low BMD was found aomng persons with CdB>20 or CdU>20, but not among those with lower CdB or CdU. Increased prevalence of renal dysfunction (increased levels of RBP and B2M) was found ($p<0.05$)among persons with CdB>10 and CdU >5-10. These observations thus show that renal dysfunction occurs at lower internal doses of Cd than bone effects. With more sensitive renal dysfunction indicators, e g urinary N-acetyl-beta-D-glucosaminidase (UNAG), renal effects can be observed at even lower internal doses of Cd. In parallell studies on 59 Chinese Cd-workers, metallothionein gene expression (MT-GE) in peripheral lymphocytes (PL) was determined by RT-PCR and renal dysfunction was assessed by measuring UNAG. The level of MT-GE in PL was found to be inversely related to the level of UNAG. This finding indicates that MT-GE in PL may be a useful biomarker of suceptibility to renal Cd toxicity in humans. Supported by European Commission INCO-DC and Swedish Agency for Cooperation with developing countries (SIDA).

378 ANALYSIS OF THE FACTORS THAT INFLUENCE THE CHRONIC HEALTH EFFECTS IN RESIDENTS EXPOSED TO ARSENIC VIA THE DRINKING WATER IN INNER MONGOLIA, CHINA.

T. Yoshida[1], T. Ito[1], Y. Nakagi[1], H. Yamauchi[2], H. Aikawa[3], J. Pi[4] and G. Sun[4]. [1]*Health Science, Asahikawa Medical College, Asahikawa, Hokkaido, Japan,* [2]*St. Marianna Medical College, Kawasaki, Japan,* [3]*Tokai University Sch. of Med., Isehara, Japan and* [4]*China Medical College, Shengyang, China.*

The relationship between chronic arsenic (As) exposure and adverse health effects such as skin lesions were studied among the residents who drunk naturally As contaminated well water in Inner Mongolia, China. For the villagers with the informed consent, interview on subjective symptoms and life style, physical examinations especially on skin lesions, collecting biological material (blood, urine and hair) for evaluation of arsenic exposure and use for another analysis, were done. Individual As exposure was evaluated based on an interview of past history of drinking water source and to measure all of various water samples that subjects used. The odds ratio of either skin lesions, hyperkeratosis or dyspigmentation verses average As concentration of the drinking water for past 5 years showed a moderate, but significant, dose-response relationship. Previous 5 years As exposure, however, didn't show any significant results. Detailed analysis on the recent As exposure revealed that significant relationship was found in the As exposure during past 3-5 years. Male showed higher odds ratio than female did. The dose-response relationships of skin lesions, especially dyspigmentation, were confirmed. The significant increase in the prevalence of skin lesions even at 0.005-0.01 mg As/L in the drinking water, a level lower than the drinking water quality standard of WHO, was observed. Relationship between the prevalence ratio of skin lesions and the concentration of As in blood was also analyzed to confirm the influence of individual methylation

activity against As, by comparison of serum concentration of mono-methyl arsenic, di-methyl arsenic and inorganic As on the subjects who were exposed to As at stable level for recent 5 years in Inner Mongolia. Only MMA concentration showed significant relationship with the prevalence ratio.

379 EFFECT OF SEAFOOD CONSUMPTION ON URINARY ARSENIC SPECIATION.

J. D. Park[1], B. S. Choi[1], E. S. Park[2], K. S. Park[3], S. T. Kim[3] and Y. P. Hong[1]. *[1]Preventive Medicine, Chung-Ang University, Seoul, South Korea, [2]Pathology, Chung-Ang University, Seoul, South Korea and [3]Advanced Analysis Center, Korea Institute of Science and Technology, Seoul, South Korea.*

Arsenic(As) is widely distributed in environment and has been known as a human carcinogen. The main route of As in general population is an oral exposure through food and drinking water. Seafood is containing high level of As, which is much less harmful organoarsenic, such as arsenobetaine, arsenocholine and arsenosugar. However, it is important to understand whether seafood consumption affects the urinary level of inorganic As metabolites, arsenite, arsenate, monomethylarsonic acid(MMA) and dimethylarsinic acid(DMA), in Korean, who eat seafood much. In this study, we investigated effects on the urinary total arsenic and inorganic metabolites (inorganic As, MMA, DMA) after consumption of seafood by volunteers(7 males and 9 females). Urinary total As was analyzed by hydride generation method after microwave digestion, and arsenic speciation was performed by using HPLC with ICP-mass spectrometry. The study subjects ingested seafood daily for 6 days consecutively, and the first voided urine in the morning was collected before ingestion and at day 1, 2, 3, 4, 5, 6, 7, 10, and 14 after ingestion, respectively. They were refrained from eating seafood for 3 days prior to the urine collection and during urine collection. We purchased seafood from the market. The daily mean intake of total As was 6.97 mg, which was 4.69 mg of seaweed(67 %), 1.75 mg of flat fish(25 %) and 0.53 mg of conch(8 %). We observed substantial increases of urinary total As and DMA from day 1 and recovered to control level at day 10 after the consumption of seafood in volunteers. DMA is more harmful metabolite than organoarsenic. This result suggests that it is necessary to consider As metabolism in assessing the health effect of seafood consumption in Korean.

380 FLUORIDE EXPOSURE ALTERS THE METABOLISM AND EXCRETION OF ARSENITE IN MICE.

M. Espinosa, E. A. Garcia-Montalvo, O. L. Valenzuela and L. M. Del Razo. *Toxicology Section, Cinvestav-IPN, Mexico D.F, Mexico.*

Fluoride (F-) and arsenic (As) are ubiquitous compounds. Their co-exposure is frequent in several endemic areas due to the natural contamination of well waters supplies by F- and As-rich geological strata and/or domestic coal combustion, so many human populations are simultaneously exposed to high concentration to inorganic arsenic (iAs) and F-. The purpose of this study was to evaluate the excretion of F- and As species in three groups of female C57BL/6 mice dosed daily *via* gavage during 30 days with a) 3mgAs3+/kg of sodium arsenite, b) 10mg F-/kg of sodium fluoride, and c) both As and F-, 3mg/kg and 10mg/kg, respectively. Urine was recollected each week and the samples were analyzed for F- and As species including iAs monomethyl As (MMA) and dimethyl As (DMA). The putative more toxic trivalent arsenic species (iAsIII, MMAIII and DMAIII) were also speciated. The results indicated that subchronic arsenite exposure decreased (35-65%) the urinary concentration of F-, comparing with F- alone group. Similarly, the F- treatment altered the urinary concentration of As causing an increased of total As at first week with posterior decreased at 2nd, 3th and 4th weeks, comparing with As alone group. The main As specie that contributed to alteration of As excretion was DMAv. The present results clearly show that arsenite and fluoride may interact with each other. Studies to evaluate the potential toxicological consequences of simultaneous exposure to high levels to iAs and F-are warranted.

381 EFFECT OF DOSE ON THE EXCRETION AND METABOLISM OF MONOMETHYLARSONIC ACID IN THE MOUSE.

M. F. Hughes[1], V. Devesa[2], B. C. Edwards[1], C. T. Mitchell[1], E. M. Kenyon[1] and D. J. Thomas[1]. *[1]ORD/NHEERL, USEPA, Research Triangle Park, NC and [2]CEMALB, UNC-CH, Chapel Hill, NC.*

Monomethylarsonic acid (MMA(V)) is found in urine and tissues of mice exposed to inorganic arsenic. In addition, the sodium salts of MMA(V) are registered herbicides in the United States. The objective of this study was to determine whether administered dose affects the excretion and metabolism of this methylated arsenical. Adult female B6C3F1 mice were gavaged with MMA(V) (disodium salt) at a dose level of either 0.4 or 40 mg As/kg. Mice received either ^{14}C-MMA(V) (n=4/dose) or unlabeled MMA(V) (n=3/dose) and were housed in metabolism cages for collection of excreta. The 24-hr cumulative urinary excretion of chemical-derived ^{14}C was significantly lower ($p < 0.05$, t-test) in the low dose group (65.4 ± 9.5%) than in the high dose group (45.4 ± 11.5%). The 24-hr cumulative fecal excretion of chemical-derived ^{14}C was significantly lower ($p < 0.01$, t-test) in the low dose group (38.8 ± 8.8%) than in the high dose group (61.8 ± 8.6%). Analysis of urine of mice administered unlabeled MMA(V) by hydride generation atomic absorption spectrometry revealed the presence of monomethylarsonous acid (MMA(III)), dimethylarsinous acid (DMA(III)), trimethylarsine oxide (TMAO), MMA(V) and dimethylarsinic acid (DMA(V)). Administered dose did not affect the level of the arsenicals excreted. The rank order of excreted arsenicals was MMA(V) (88%), DMA(V) (7%), TMAO (2%), DMA(III) (2%), MMA(III) (1%). Dose affects the route of excretion of MMA(V) and metabolites but not its metabolism. (This abstract does not necessarily reflect EPA policy.)

382 COMPREHENSIVE ANALYSIS OF BIOLOGICALLY RELEVANT ARSENICALS BY PH-SELECTIVE HYDRIDE GENERATION-ATOMIC ABSORPTION SPECTROMETRY.

V. Devesa[1], L. Del Razo[4], S. Waters[2], Z. Drobna[3], M. Hughes[5], M. Styblo[1, 3] and D. Thomas[5]. *[1]CEMALB, University of North Carolina at Chapel Hill, Chapel Hill, NC, [2]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC, [3]Department of Pediatrics, University of North Carolina, Chapel Hill, NC, [4]CINEVSTAV, IPN, Mexico City, Mexico and [5]NHEERL, USEPA, Research Triangle Park, NC.*

A method based on pH-selective generation and separation of arsines is commonly used for analysis of inorganic, methylated, and dimethylated trivalent and pentavalent arsenicals by hydride generation-atomic absorption spectrometry (HG-AAS). We have optimized this method to permit quantification of trimethylated arsenicals. Increasing adsorbent density in the cold trap and optimizing the temperature gradient during release of arsines from the cold trap, improved the separatory power of this method. This optimized technique permits analysis of most common biologically relevant arsenic species, including arsenite (iAsIII), arsenate (iAsV), monomethylarsonic acid (MAsV), monomethylarsonous acid (MAsIII), dimethylarsinic acid (DMAsV), dimethylarsinous acid (DMAsIII), and trimethylarsine oxide (TMAO). Selective generation of arsines of trivalent species and TMAO at pH 6, and of all arsenicals (trivalents and pentavalents) at pH 1 permits quantification of each species. Calibration curves are linear for analyte concentrations of 0.5 to 100 ng and recoveries range from 85 and 124%. The variation of the method does not exceed 15%. The method is equally effective for analyses in aqueous solutions and in simple biological matrices. The optimized technique has been used to analyze arsenical metabolites in cultured human hepatocytes exposed to iAsIII and in urine of mice treated with MAsV or DMAsV. Methyl- and dimethylated arsenicals, but not TMAO, were detected in hepatocytes or culture medium. Considerable amounts of TMAO were found in urine of mice treated with MAsV or DMAsV. Hence, this optimized HG-AAS technique can be used for comprehensive analysis of arsenicals in biological matrices that do not require extraction before assay. (This abstract does not reflect USEPA policy).

383 INTERINDIVIDUAL VARIATION IN THE METABOLISM OF ARSENIC IN HUMAN HEPATOCYTES.

M. Styblo[1], F. F. Walton[1], Z. Drobna[1], S. B. Waters[3], E. L. LeCluyse[6] and D. J. Thomas[5]. *[1]Department of Pediatrics, UNC, Chapel Hill, NC, [2]Department of Pediatrics, UNC, Chapel Hill, NC, [3]Curriculum in Toxicology, UNC, Chapel Hill, NC, [4]Department Pediatrics, UNC, Chapel Hill, NC, [5]NHEERL, ORD, USEPA, Research Triangle Park, NC and [6]Division of Drug Delivery and Dispos., UNC, Chapel Hill, NC.*

The liver is the major site for the enzymatic methylation of inorganic arsenic (iAs) in humans. Primary cultures of normal human hepatocytes isolated from tissue obtained at surgery or from donor livers have been used to study interindividual variation in the capacity of liver to metabolize iAs. To minimize variability related to donor's health status and medication, hepatocytes were cultured under standard conditions for 5 or 6 days before use. This interval allowed cellular functions (redox status and activities of drug metabolizing enzymes) to stabilize and assured the subsequent studies of methylation capacity reflected genotypic and phenotypic variability. Using standardized culture conditions, we compared methylation patterns for iAs in hepatocytes prepared from 8 donors. Methylation rates and yields and distribution of metabolites were determined in cells exposed to arsenite (iAsIII) (0.3, 0.9, 3, 9 or 30 nmol/mg protein) for 24 hours. For most donors, high iAsIII concentrations inhibited methylation (especially DMAs production) and decreased the DMAs/MAs ratio. However, for some donors, methylation was not inhibited even at the highest iAsIII concentrations. Maximal methylation rates (usually in cells at 3 or 9 nmol iAsIII/mg protein) varied from 2.6 to 20 pmol CH3 transferred/mg protein/hour. iAs and MAs were major metabolites retained in cells; iAs and DMAs were the major metabolites in culture media at low iAsIII levels. Statistically significant positive correlations were found between the amounts of iAs and MAs retained in cells and the methylation rate. These data suggest that significant interindividual differences exist in capacities of human liver to methylate iAs. These differences may underlie interindividual variation in susceptibility to toxic and carcinogenic effects of iAs exposure. (This abstract does not reflect EPA policy.)

384 ARSENIC TRANSPORT BY THE HUMAN MULTIDRUG RESISTANCE PROTEIN 1 (MRP1/ABCC1): EVIDENCE THAT A TRI-GLUTATHIONE CONJUGATE IS REQUIRED.

E. M. Leslie[1], S. P. Cole[2] and M. P. Waalkes[1]. [1]*Inorganic Carcinogenesis Section, LCC, NCI at NIEHS, Research Triangle Park, NC and* [2]*Cancer Research Laboratories, Queen's University, Kingston, ON, Canada.*

Tolerance to arsenic (As) is frequently associated with a glutathione (GSH) dependent reduction in cellular accumulation of this metalloid. Cell lines chronically exposed to As often over-express glutathione *S*-transferase P1-1 (GSTP1-1) and certain ATP-binding cassette transporters including the multidrug resistance protein 1 (MRP1) which mediates active transport of GSH, sulfate and glucuronide conjugated organic anions. MRP1 also co-transports certain unmodified compounds with GSH. Although MRP1 confers resistance to As in association with GSH, the chemical nature of the transported As species has been difficult to elucidate. As-GSH conjugates have never been isolated in culture media, possibly due to their chemical instability at physiological pH. Alternatively, MRP1 could efflux As with free GSH through a co-transport mechanism. In the present study we investigated the form of As transported by MRP1 using $^{73}As^{3+}$ and inside-out membrane vesicles prepared from the small cell lung cancer cell line H69AR, which over-expresses MRP1. We found that MRP1 transports As but only in the presence of GSH. Transport of As did not occur in the presence of the GSH analogs γ-glutamyl-α-aminobutyryl-glycine and γ-glutamyl-methyl-Cys-Gly suggesting that the free thiol group of GSH is required. Transport of As in the presence of GSH was higher at pH 6.5 than at pH >7, consistent with the formation and transport of $AsGS_3$ which is more stable at acidic pH. The transport reaction was monitored by thin layer chromatography and $AsGS_3$formation was observed. Interestingly, under the transport assay conditions used, $AsGS_3$ was not formed in the absence of membrane vesicles. Immunoblot analysis revealed the unexpected presence of GSTP1-1 in association with the membrane vesicle fraction, an enzyme normally found in the cytosol. Our findings suggest that the formation of $AsGS_3$ is necessary for arsenic efflux by MRP1 and that GSTP1-1 could be important in this complex formation.

385 KINETICS OF ARSENIC METHYLATION BY FRESHLY ISOLATED MOUSE HEPATOCYTES.

G. L. Kedderis[1], K. A. Milne[2], A. R. Elmore[2], E. A. Crecelius[3], J. W. Yager[4] and T. L. Goldsworthy[2]. [1]*Independent Consultant, Chapel Hill, NC,* [2]*Integrated Laboratory Systems Inc., Research Triangle Park, NC,* [3] *Battelle, Sequim, WA and* [4] *EPRI, Palo Alto, CA.*

The toxic and carcinogenic effects of arsenic may be mediated by both inorganic and methylated arsenic species, but the kinetics of arsenic methylation have not been studied in detail. Therefore the kinetics of arsenic methylation were determined in freshly isolated hepatocytes from male B6C3F1 mice. Hepatocytes (>90% *via*bility) were isolated by collagenase perfusion and suspended in Williams Medium E with various concentrations of arsenic III (sodium m-arsenite). Aliquots of the cell suspension were lysed with 0.5% Triton X-100 and analyzed for arsenic species by ion pair chromatography-hydride generation-atomic absorption spectrometry. The formation of monomethylarsenic (MMA) from sodium arsenite (1 uM) was linear for >90 min. Dimethylarsenic formation did not become significant until 60 min. Metabolism of arsenic V was not observed, consistent with inhibition of arsenic V active uptake by phosphate in the medium. The formation of MMA III increased with increasing arsenic III concentrations up to approximately 2 uM and declined thereafter. The concentration dependence is consistent with a saturable methylation reaction accompanied by substrate inhibition of the reaction by arsenic III. Kinetic analysis of the data suggested an apparent KM of approximately 0.5 uM arsenic III and an apparent Vmax of approximately 3.2 mg MMA formed/L/hr/million cells. The kinetics of arsenic III methylation by isolated human hepatocytes will also be determined. These data will be compared with the mouse kinetic parameters and used as the basis for estimation of tissue exposure to MMA III following human exposure to arsenic. (Research supported by EPRI contract P10089/C5135).

386 RECOMBINANT RAT CYT19, AN ARSENIC METHYLTRANSFERASE, EFFICIENTLY GENERATES TRIMETHYLARSINE OXIDE IN THE ABSENCE OF GLUTATHIONE.

S. B. Waters[1], V. Devesa[2], Z. Drobna[3], M. Styblo[2, 3] and D. Thomas[4]. [1]*Curriculum in Toxicology, UNC-Chapel Hill, Chapel Hill, NC,* [2]*CEMALB, UNC-Chapel Hill, Chapel Hill, NC,* [3]*Department of Pediatrics, UNC-Chapel Hill, Chapel Hill, NC and* [4]*NHEERL, USEPA, Research Triangle PArk, NC.*

Chronic exposure to inorganic arsenic (iAs), a toxic metalloid sometimes present in drinking water, is associated with increased prevalences of various cancers and other disorders. Humans and many other species enzymatically convert iAs into methylated metabolites. The major metabolites are mono- and dimethylated arsenicals; however, trimethylated arsenicals have been detected in urine and expired breath following exposure to iAs. The As methyltransferase of rat liver is encoded by the cyt19 gene that produces cyt19, a 42kDa cytosolic protein. Recombinant rat cyt19 (rrcyt19) has been used to study the enzymology of As methylation. Mono- and dimethylated arsenicals generated in assays with rrcyt19 are usually resolved by thin layer chromatography using an acetone:water:acetate (3:1:1) solvent system. However, chromatography with an isopropanol:water:acetate (10:2.5:1) solvent system revealed an unidentified metabolite. This species comigrated with authentic trimethylarsine oxide (TMAO). This metabolite had identical properties with authentic TMAO when analyzed by hydride generation-atomic absorption spectrometry and was identified as TMAO by ICP-mass spectrometric analysis. Addition of glutathione (GSH) to reactions containing iAs and rrcyt19 increased the rate of formation of methylated and dimethylated arsenicals but suppressed formation of TMAO. Addition of GSH to reactions containing iAs and rrcyt19 also reduced the loss of a volatile arsenical that may be trimethlyarsine (TMA). Although TMAO is a not a toxic methylated arsenical, TMA has recently been shown as a potent DNA-damaging compound. Thus, generation of TMA in cyt19-catalyzed reactions may be a pathway for metabolic activation of iAs. (This abstract does not necessarily reflect USEPA policy.)

387 CHARACTERIZATION OF UROTSA/RCYT19, A CLONAL HUMAN URINARY BLADDER CELL LINE EXPRESSING RAT AS^{III}-METHYLTRANSFERASE.

Z. Drobna[1], S. B. Waters[2], F. S. Walton[1], V. Devesa[3], D. J. Thomas[4] and M. Styblo[1, 3]. [1]*Pediatrics, UNC, Chapel Hill, NC,* [2]*Curriculum in Toxicology, UNC, Chapel Hill, NC,* [3]*CEMALB, UNC, Chapel Hill, NC and* [4]*NHEERL, USEPA, Research Triangle Park, NC.*

In humans and rat, the methylation of arsenic (As) is catalyzed by an As^{III}-methyltransferase (Cyt19) and yields methylated pentavalent and trivalent arsenicals. Cyt19 activity and expression levels vary among tissues. For example, Cyt19 is expressed in human hepatocytes that are efficient methylators of As, but not in UROtsa cells, a human urothelial cell line that does not methylate As. Thus, UROtsa cells are an ideal null background to assess the role of As methylation in modulation of toxic and cancer promoting effects of this metalloid. A retroviral gene delivery system (pLEGFP-N1) was used to transduce UROtsa cells with a rat Cyt19 (rCyt19). The expression of rCyt19 in neomycin-selected UROtsa/rCyt19 clones was confirmed by RT-PCR analysis of Cyt19 mRNA and by immunoblot analysis, using rabbit anti-rCyt19 antiserum. The metabolism of As was then examined in the parent cell line and in the clonal cells using radiolabeled carrier-free arsenite as a substrate. During a 65-hour incubation, UROtsa/rCyt19 cells methylated between 20 to 25% of arsenite. Both methylated arsenic (MAs) and dimethylated arsenic (DMAs) were found in the culture. DMAs was the predominant metabolite in both cells and medium. After the incubation, the parent UROtsa cells, that did not methylate arsenite, retained more As than methylating UROtsa/rCyt19 cells. The results of preliminary experiments show the parental cells are more susceptible to the cytotoxicity of arsenite and of methylarsine oxide than are UROtsa/rCyt19 cells. In contrast, iododimethylarsine is more cytotoxic in the clonal cells. These data suggest that the capacity to methylate As modifies the response of cells to acute cytotoxic effects of this metalloid. Future studies will provide information about the role of methylation in modulation of effects associated with chronic exposures to As. (This abstract does not reflect USEPA policy.)

388 EFFECTS OF ARSENITE AND MONOMETHYL ARSONOUS ACID ON UROTSA CELLS: LOW-LEVEL EXPOSURE CAUSES ACCUMULATION OF UBIQUITINATED PROTEINS WHICH IS ENHANCED BY REDUCTION IN CELLULAR GLUTATHIONE LEVELS.

T. G. Bredfeldt[1], M. J. Kopplin[1], E. A. Mash[2] and A. Gandolfi[1]. [1]*Pharmacology and Toxicology, University of Arizona, Tucson, AZ and* [2]*Chemistry, University of Arizona, Tucson, AZ.*

Arsenic is a ubiquitous environmental toxicant. Chronic arsenic exposure increases risk for the development of diabetes, vascular disease, and cancers of the skin, lung, kidney, and bladder. This study investigates the effects of arsenite [As(III)] and monomethyl arsonous acid [MMA(III)], a product of As(III) biotransformation, on human urothelial cells (UROtsa). As(III) toxicity was determined by exposing confluent UROtsa cells to As(III) (0.5-200 uM). Depleting cellular glutathione levels with BSO (butathionine sulfoximine) potentiated the toxicity of As(III). Cell *via*bility was assessed with the MTT [(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay. The ability of UROtsa cells to biotransform As(III) was determined by exposure to environmentally-relevant concentrations of As(III) followed by HPLC/ICP-MS analysis of cell media and lysate. UROtsa cells were found to oxidize and methylate As(III). Both pentavalent and trivalent monomethylated products were detected. Although cytotoxicity was observed at high

concentrations of As(III) (~100 uM), perturbations of a variety of molecular processes occurred at much lower concentrations. Exposure to low-level As(III) (0.5-25 uM) causes an accumulation of ubiquitin (Ub)-conjugated proteins. This effect is greatly enhanced when cellular glutathione levels have been reduced with BSO treatment. Ongoing studies investigating the effects of MMA(III) on UROtsa cells, suggest that this metabolite is more toxic than As(III) to UROtsa cells. Since As(III) and MMA(III) have many effects on UROtsa cells, a greater understanding of how these arsenicals affect cellular proteins in a target tissue will lead to a better understanding of the mechanism of toxicity and pathogenesis for low-level exposure to arsenic (NIEHS 04940, 07091, 06694).

389 INHIBITION OF LUMINAL CYSTINE TRANSPORT BY THE MERCURIC CONJUGATE CYS-S-HG-S-CYS IN ISOLATED-PERFUSED S2 SEGMENTS OF THE RABBIT RENAL PROXIMAL TUBULE.

D. W. Barfuss[1] and R. K. Zalups[2]. *[1]Biology, Georgia State University, Atlanta, GA and [2]Mercer University School of Medicine, Macon, GA.*

Lumen-to-cell transport and cellular uptake of S^{35}-L-cystine across the luminal membrane was studied in isolated perfused S2 (cortical pars recta) segments of the rabbit proximal tubule in the absence and presence of various concentrations of the mercuric conjugate Cys-S-Hg-S-Cys. In the absence of luminal Cys-S-Hg-S-Cys, isolated tubules perfused with a range of S^{35}-L-cystine concentrations (20 to 500 µM) transported the S^{35}-L-cystine from the luminal fluid in a concentration dependent manor (curvilinear pattern), which was consistent with a single transport system having Michaelis-Menten kinetics. The calculated transport maximum for L-cystine was 800 fmol min^{-1} (mm tubular length)$^{-1}$, while the affinity (Km) of the L-cystine was approximately 50 µM. The presence of mercuric conjugate Cys-S-Hg-S-Cys in the luminal fluid inhibited the lumen-to-cell transport of S^{35}-L-cystine in a concentration-dependent manor. Specifically, 100 µM Cys-S-Hg-S-Cys inhibited the lumen-to-cell transport of luminal S^{35}-L-cystine (at 100 µM) by approximately 75%, from about 528 to 128 fmol min^{-1} (mm tubular length)$^{-1}$. In addition, the cell-to-lumen ratio of S35-L-cystine was reduced by 90% (30 to 2.1). We conclude from these experiments that the mercuric conjugate Cys-S-Hg-S-Cys is transported by transporters of L-cystine localized in the luminal membrane of the cortical pars recta of the rabbit nephron, perhaps systems $b^{0,+}$ and/or $B^{0,+}$. We also conclude the affinity of the luminal L-cystine transporter(s) for Cys-S-Hg-S-Cys is greater than that for L-cystine.

390 COPROPORPHYRINOGEN OXIDASE (CPOX) POLYMORPHISM ALTERS THE EFFECT OF MERCURY (Hg) ON PORPHYRIN EXCRETION IN HUMANS.

J. S. Woods[1, 2], D. Echeverria[2, 1], N. J. Heyer[2], A. C. Bittner[2] and F. M. Farin[1]. *[1]Environmental Health, University of Washington, Seattle, WA and [2]Battelle Centers for Public Health Research and Evaluation, Seattle, WA.*

Previous studies have documented the efficacy of urinary porphyrin profile changes as a biomarker of Hg body burden in humans and have identified the etiology of this effect as Hg-mediated alteration of CPOX activity in the kidney. This study focused on the genetic etiology of an atypical porphyrinogenic response (APR) seen among 12-16% of Hg-exposed subjects, characterized by substantially excess urinary excretion of CPOX precursors, particularly the atypical porphyrin, most likely keto-isocoproporphyrin (KICP), than is predicted solely on the basis of Hg body burden. Automated DNA sequencing-based assays were developed to examine the 7 exons and flanking intron-exon boundaries of the CPOX gene from 389 subjects with low-level Hg exposure (mean urinary Hg = 1.8(1.7) ug/L). Genetic analysis revealed an A814C polymorphism in exon 4 encoding a N272H substitution that was predominant among subjects with the APR. Overall population frequencies were 73% WT, 26% Het, 2% Mut. Multiple regression analyses suggested that this CPOX polymorphism (β=.08**) preferentially enhanced the specific effect of Hg on KICP excretion, when controlling for gender (β=.03**), the CPOX substrate, 5-carboxylporphyrin (5-CP) (?=.80**), urinary Hg (β=.11**), and the interaction of 5-CP and Hg (β=.09**), where ** = p<.05. These data substantiate previous findings of a possible genetic predisposition to an altered biological response to Hg that could affect Hg health risks. The APR, particularly KICP excretion, might therefore serve as a biomarker of both Hg exposure and susceptibility to Hg toxicity. Supported by ES04696 and ES07033

391 UPTAKE OF BIOLOGICALLY RELEVANT FORM(S) OF INORGANIC MERCURY BY HUMAN ORGANIC ANION TRANSPORTER 1 (hOAT1).

S. Ahmad and R. K. Zalups. *School of Medicine, Basic Science, Mercer University, Macon, GA.*

The kidney is the primary target affected adversely by inorganic mercury (Hg^{++}). Within the kidneys, Hg^{++} accumulates mainly along the three segments of the proximal tubule. Recent findings have implicated indirectly the organic anion transport systems in the basolateral uptake of thiol-conjugates of Hg^{++} in proximal tubular epithelial cells. In the present study, we used hOAT1-transfected and non-transfected Madin-Darby Canine Kidney II (MDCK II) cell lines to define more precisely the role of the organic anion transporter 1 (hOAT1) protein in the uptake of thiol-conjugates of Hg^{++}. Significant levels of uptake of Hg^{++} occurred in the hOAT1-transfected cells when the cells were exposed to mercuric conjugates of Homocysteine (hCys-Hg-hCys; 9.97±0.18 pmol x mg $protein^{-1}$ x min^{-1}), Cysteine (Cys-Hg-Cys; 5.65±0.08 pmol x mg $protein^{-1}$ x min^{-1}) or *N*-Acetylcysteine (NAC-Hg-NAC; 3.42±0.08 pmol x mg $protein^{-1}$ x min^{-1}). By contrast, no significant uptake of Hg^{++} occurred in the control, non-transfected MDCK II cells when they were exposed to these thiol-conjugates. Intrestingly, uptake of Hg^{++}, in the form of mercuric conjugates of Cysteinylglycine (gly-cys-Hg-cys-gly) or Glutathione (GSH-Hg-GSH) was negligible in both hOAT1-transfected and non-transfected MDCK II cells. The uptake of hCys-Hg-hCys, Cys-Hg-Cys and NAC-Hg-NAC in hOAT1-transfected MDCK II cells was inhibited in a dose dependent manner by *p*-aminohippurate or the dicarboxylic acids (adipate or glutrate). Transport of hCys-Hg-hCys was time-dependent and temperature-sensitive. Overall, the current transport data indicate that hCys-Hg-hCys has a higher affinity for hOAT1 than Cys-Hg-Cys or NAC-Hg-NAC. Moreover, the findings from the present investigation indicate that OAT1 is likely the primary transporter responsible for basolateral uptake of thiol-conjugates of Hg^{++} along the proximal tubul *in vivo*.

392 ALUMINUM TRANSPORT AND UPTAKE IN CACO-2 CELLS.

Y. Zhou[1] and R. A. Yokel[2, 1]. *[1]Graduate Center for Toxicology, University of Kentucky Medical Center, Lexington, KY and [2]College of Pharmacy, University of Kentucky Medical Center, Lexington, KY.*

Aluminum (Al) has been suggested to be a contributor to dialysis encephalopathy, a low-turnover osteomalacia and Alzheimer Disease. However the mechanism by which it is absorbed from the GI tract is uncertain. In the present work, confluent Caco-2 cell monolayers grown 1) on porous membranes which were mounted in vertical diffusion chambers and 2) in 35 mm diameter dishes were used to study apparent Al permeability and uptake kinetics, respectively. For the permeability study, Al citrate or Al maltolate was added to the medium on the apical side of the Caco-2 monolayer and the amount of Al on the basal side measured. The permeability of lucifer yellow and [3H]-propranolol, which pass through the paracellular and transcellular pathways, respectively, were concurrently measured. For the uptake study, Caco-2 cells were incubated with Al citrate or Al maltolate for 5, 10, 30, 60, 120 or 240 min. They were washed in the absence or presence of 2 mM EDTA to determine Al associated with the cell monolayer. The apparent permeabilities of Al citrate and Al maltolate were ~ 30% of, and correlated highly with, the apparent permeability of lucifer yellow (r=0.98, r=0.99). In contrast, Al and propranolol permeabilities did not correlate. The high correlation between Al and lucifer yellow permeation as well as greater Al permeation than uptake suggest Al citrate and Al maltolate apical to basal flux across the Caco-2 cell monolayer is mostly *via* the paracellular pathway. EDTA washout reduced Al associated with Caco-2 cells after Al citrate but not Al maltolate exposure, suggesting 2 pools of Al citrate and 1 of Al maltolate uptake. Intracellular Al concentration after 240 min of Al citrate and Al maltolate exposure was 0.7- and 2-fold the medium Al concentration, respectively. Al citrate, but not Al maltolate, uptake rate decreased over time. Al citrate and Al maltolate similarly diffuse through the paracellular pathway but enter cells by different rates/processes. Funded by EPA STAR Grant 829783.

393 MANGANESE CONCENTRATIONS IN THE AIR OF THE MONTREAL (CANADA) SUBWAY IN RELATION TO SURFACE AUTOMOBILE TRAFFIC DENSITY.

N. Boudia[1], R. Halley[2], G. Kennedy[3], L. Gareau[1] and J. Zayed[1]. *[1]Environmental and occupational Health, University of Montreal, Montreal, QC, Canada, [2]Transport Montreal Society, Montreal, QC, Canada and [3]Department of Engineering Physics, Ecole Polytechnique de Montreal, Montreal, QC, Canada.*

Since its introduction in Canada in 1976, the use of MMT in gasoline increased substantially until it completely replaced tetraethyl lead in 1990. A correlation has been established several times by our research group between atmospheric Mn concentration and automobile traffic density, suggesting that methylcyclopentadienyl manganese tricarbonyl (MMT) in gasoline could play a significant role. This study aims to measure manganese (Mn) concentrations in the air of the underground subway in Montreal (Canada) and to examine the relation with nearby surface automobile traffic density and, by extension, with the use of MMT in gasoline. Three subway stations were chosen for their location in different microenvironments with different traffic densities. Respirable (MnR < 5 µm) and total Mn (MnT) were sampled in the stations about 25 m below ground level, over two weeks, 5 days/week, 12 h/d from 6:00 h to 18:00 h. For the station located in the lower traffic density area, relatively low levels of MnR and MnT were found, with averages of

0.022 μg/m3 and 0.035 μg/m3 respectively. For the other two stations, the concentrations were more than 7 times higher and far exceeded the USEPA reference concentration of 0.05 μg/m3. Although there may be several sources of Mn from the different components of the subway structure and vehicles, no correlation was found between these and the observed Mn concentrations in air. However, a significant correlation ($p \leq 0.05$) was found between atmospheric Mn concentrations and nearby automobile traffic density. Since the air in the metro is pumped directly from outside without filtration, our findings suggest that the combustion of MMT in automobiles may be an important factor.

394 POTENTIAL NEUROLOGICAL EFFECTS OF MANGANESE EXPOSURE DURING WELDING: A "STATE-OF-THE-SCIENCE" REVIEW.

A. Santamaria[1], A. Li[2], F. Mowat[3], C. Cushing[4] and B. Finley[5]. *[1]Exponent, Houston, TX, [2]Exponent, Oakland, CA, [3]Exponent, San Francisco, CA, [4]Exponent, Boulder, CO and [5]Exponent, Santa Rosa, CA.*

Some recent studies have suggested that exposure to manganese (Mn), an essential component of welding electrodes, can cause neurotoxicity in welders ("manganism" a pattern of neurological effects that bears some similarity to Parkinson's Disease and related syndromes). This review presents a critical analysis of the published data regarding Mn exposure during the welding process and the human health studies evaluating neurotoxicity in welders. Our review indicates that the Mn composition of welding fumes is dependent on the composition of the metal being welded, welding process, and electrodes used. In addition, welders often perform a variety of different tasks with varying degrees of duration and ventilation, and, hence, in order to accurately assess manganese exposures that have occurred in different occupational settings, some specific information on the welder's historical work patterns is desirable. Currently, there is limited information relating airborne Mn levels or Mn exposure biomarkers with specific welding activities, but the data that do exist suggest that Mn exposures in welders are far below those associated with the increased incidence of manganism that has been documented in miners and smelter workers. We believe the available data might support the development of reasonable "worst-case" exposure estimates for most welding activities, and suggest that exposure simulation studies (re-creating a welder's activities and collecting airborne Mn data) would significantly refine such estimates. We also found that, while manganism has been documented in highly exposed populations, there are no epidemiological studies of manganism incidence in welders; the literature to date consists of case reports. Clearly, a well-designed epidemiological evaluation of manganism in welder's would be useful. Such a study (in addition to exposure simulation) could also evaluate the confounding exposures to other potentially neurotoxic compounds that occur during welding.

395 PHARMACOKINETIC ANALYSES OF THE EFFICIENCY OF UPTAKE OF INHALED MANGANESE FROM THE OLFACTORY MUCOSA INTO THE CENTRAL NERVOUS SYSTEM IN RATS.

D. B. Rao, D. C. Dorman and M. E. Andersen. *CIIT Centers for Health REsearch, Research Triangle Park, NC.*

Exposures to high atmospheric concentrations of manganese (Mn) such as in poorly controlled work environments cause neurodegenerative disorders, producing increased concentrations of Mn in specific brain regions including globus pallidum and striatum. Inhaled or instilled Mn can be directly transported from the nasal olfactory mucosa (OM) to the brain. Brain regions showing increased Mn concentrations include the olfactory bulb (OB), the olfactory tract and tubercle (OTT) and to a lesser degree, the striatum (ST). Here, we analyze published time course data from our laboratory for two different forms of Mn; MnCl2 and the less soluble MnHPO4. The time-dependent distribution of Mn in the brain following inhalation in rats was evaluated with a 4-compartment pharmacokinetic (PK) model. The model had deposition of Mn on the OM (compartment 1). Subsequent compartments include the OB (compartment 2), OTT (compartment 3) and striatum, ST (compartment 4). The volume of each compartment was characterized by measured weight. Movement among these 4 well-mixed compartments was described as first-order. Optimization in Berkeley Madonna provided estimates for fitted parameters, namely, the rate constants for intercompartmental transfer. Intercompartmental transfer rates were 0.01, 0.03 and 0.07 per hour for k12, k23 and k34, respectively. A proportion of Mn from one compartment moving to the deeper compartments within the CNS (from OB-OTT-ST) was required. All Mn transferred from mucosa through the OTT; however, less than 5% of the OTT Mn moved through to the striatum. PK model structures with deep tissue stores for Mn in the OTT provided a better description of the overall datasets than the single well-mixed OTT compartment. Less Mn was deposited into the olfactory mucosa following MnHPO4 inhalation, although the subsequent disposition was similar to that as MnCl2. This simple PK model will be useful in examining the importance of direct nerve transport as a factor in elevating brain Mn following inhalation in rats and other species.

396 DOPAMINE TRANSPORTER LEVELS ARE TRANSIENTLY INCREASED IN THE NON-HUMAN PRIMATE STRIATUM FOLLOWING ACUTE MANGANESE EXPOSURE: PRELIMINARY FINDINGS USING *IN VIVO* BRAIN IMAGING.

M. K. Chen[1], J. S. Lee[2], J. L. McGlothan[1], R. J. Adams[3], M. Alexander[2], D. F. Wong[2] and T. R. Guilarte[1]. *[1]Environmental Health Sciences, Johns Hopkins University, Baltimore, MD, [2]Radiology, Johns Hopkins University, Baltimore, MD and [3]Comparitive medicine, Johns Hopkins University, Baltimore, MD.*

Exposure to high levels of manganese (Mn) causes a Parkinsonian-like syndrome in humans and non-human primates. The exact molecular mechanisms and anatomical loci of Mn-induced dopaminergic dysfunction are not clearly elucidated. We used Positron Emission Tomography (PET) to non-invasively measure the effect of acute exposure to manganese sulfate (MnSO4) on dopamine transporter (DAT) levels in the corpus striatum. Baboons (papio anubis) received [11C]-WIN 35, 428 PET scans to measure dopamine transporter (DAT) levels before and after MnSO4 administration. Binding potential (BP), a measure of DAT binding site availability in the striatum was generated from each PET study using a 2-tissue compartment model with the cerebellum as reference region. In one animal, we measured a 46% increase in DAT BP from a baseline of 6.19 to 9.04 at 1 week after two Mn injections (50 mg/kg S.C. one week apart). DAT levels returned to baseline values at 4 months (BP= 6.75) and remained constant at 10 months (BP= 6.87) after treatment. A subsequent single MnSO4 injection (10 mg/kg I.V.) to the same animal also resulted in an increase in DAT BP from 6.87 to 10.83, two days after administration. In a second animal, a 76% increase in DAT BP was measured from a baseline value of 3.89 to 6.83 at 3 days after Mn injection (20 mg/kg I.V.). In this animal, the BP returned to baseline levels (BP= 4.38) after 1 month. These findings suggest that acute Mn exposure may cause an up-regulation of DAT protein in the non-human primate striatum. An alternative explanation may be that Mn decreases dopamine levels at the synapse increasing the availability of ligand binding to DAT. These preliminary findings provide helpful insights on potential mechanisms of Mn-induced neurotoxicity and indicate that the striatum is a target for Mn in the primate brain [Supported by NIEHS ES07062 to TRG and NIDA K24DA00412 to DFW]

397 BRAIN REGIONAL DIFFERENCE IN IRON TRANSPORT AND THE EFFECT OF MANGANESE EXPOSURE.

R. Deane[1] and W. Zheng[2]. *[1]Center for Aging Research, University of Rochester, Rochester, NY and [2]School of Health Sciences, Purdue University, West Lafayette, IN.*

Regulation of brain iron (Fe) homeostasis is still incompletely understood. Using an in situ rat brain perfusion model, we studied the transport Fe, either transferrin (Tf)-bound or free, at the blood-brain/CSF barrier. Data showed that brain capillary endothelia avidly took up circulating ^{59}Fe (1 nM), at rates (Kin) of 136+/-26 (Tf-bound) and 182+/-23 (free) ml/g/min x10^3, respectively. However, the rates of ^{59}Fe entry into brain parenchyma were much reduced, i.e., 1.52+/-0.48 (Tf-bound) and 1.68+/-0.56 (free) ml/g/min x10^3, respectively, in comparison to the capillary uptake. The pattern of brain Fe uptake shows a regional difference. In ^{59}Fe-only perfusions, the Kin exhibits in a descending order, cerebellum (CB) > striatum (ST) > brain stem (BS) > hippocampus (HP) > frontal cortex (FC), while with ^{59}Fe-Tf, the order is ST > HP > CB> FC > BS. We further studied regional differences in Tf receptor (TfR) activity on brain capillaries. The Bmax (receptor density) and KD (affinity constant) of Tf binding were significantly higher in capillaries from the HP and ST as compared to FC (n=4, $P<0.05$), suggesting an uneven distribution of TfR in brain regional capillaries. Patterns of TfR distribution appear to correlate with the observed regional differences in rates of transport of Tf-bound 59Fe. In the absence of perfusate Tf, Mn exposure in rats (6 mg Mn/kg, ip, once daily for 2 wks) significantly increased the influx of ^{59}Fe to CP, but not the influx to the rest regions examined. In the presence of Tf, however, Mn exposure decreased the Fe uptake to most brain regions, except for a 3-fold increase into CSF compared to controls. Our results suggest that brain endothelia are capable of accumulating Fe and thus limiting the entry of Fe into brain parenchyma. There is a regional difference in Fe transport to brain. The difference in regional endothelial TfR activity may partly underlie the regional differences in Fe transport to brain. (US-NIEHS ES08146 and UK-Burroughs Wellcome Foundation #021366 and #1001670)

398 COMPARATIVE NEUROTOXIC EFFECTS OF MN(II) VERSUS MN(III) IN A RODENT MODEL.

S. H. Reaney[1, 2], G. Bench[3] and D. R. Smith[2]. *[1]Chemistry and Biochemistry Department, UCSC, Santa Cruz, CA, [2]Department of Environment Toxicology., UCSC, Santa Cruz, CA and [3]LLNL, Livermore, CA.*

Chronic elevated manganese (Mn) exposure has been associated with neurological disorders. Since chronic elevated exposures to Mn may result from the combustion of MMT, and MMT combustion products contain Mn-oxides and phosphates of

various Mn oxidation (ox.) states (Mn(II), Mn(III) and Mn(IV)), there is a need to investigate how the ox. state of Mn exposure mediates toxicity. Recent work from our lab (Reaney and Smith, SOT abst, 2003) substantiates this need. This study evaluated how exposures to Mn as Mn(II) or Mn(III) affected the regional distribution and neurotoxic effects of Mn in the frontal cortex (FC), striatum (STR), globus pallidus (GP), and thalamus (Th) regions of the basil ganglia in Long Evans rats. Rats were exposed (IP) to Mn(II)-chloride or Mn(III)-pyrophosphate over a 5 week period at total cumulative doses of 0, 30 or 90 mg Mn/kg body weight. Results indicate that Mn(III) exposures produced significantly higher blood Mn levels than exposures to Mn(II). Liver Mn levels also increased with dose, but there was no effect of Mn ox state. In the brain, FC Mn levels increased in a dose dependent manner ($p<0.05$) with high Mn(III) exposures producing significantly higher (~45%) levels than exposures to high Mn(II). Analyses of Mn in other brain regions (STR, GP, Th) are on-going. GABA levels were significantly increased in the GP ($p<0.05$) and trended to increase in the Th and STR ($p>0.05$) following exposures to either Mn(II) or Mn(III). In contrast, levels of DA showed no measurable change. TfR expression increased in the GP with Mn dose ($p<0.05$), and trended up ($p>0.05$) in other brain regions as well. These data suggest that the oxidation state of Mn exposures mediate acquired blood Mn levels, which in part drives brain Mn uptake, and presumably resultant neurotoxicity.

399 MORPHOLOGICAL EVIDENCE OF DIFFERENTIAL CYTOTOXICITY OF MN(II) AND MN(III) IN HUMAN DOPAMINERGIC SH-SY5Y CELLS *IN VITRO*.

C. Zhang[1], J. Li[2, 3], C. Zhou[1] and W. Zheng[3]. *[1]Xinjiang University of Med. Sciences, Urumqi, China, [2]Capital University of Med. Sciences, Beijing, China and [3]School of Health Sciences, Purdue University, West Lafayette, IN.*

Results from this and other laboratories have shown that Mn(III) species appear to be more toxic than Mn(II) in cell killing and cellular biochemical alterations. This study aimed to use morphological approaches to verify the differential toxicity between Mn(II) and Mn(III) in human neuroblastoma dopaminergic SH-SY5Y cells. Cultured cells were exposed to 0.5 mM of either Mn(II) as $MnCl_2$ or Mn(III) as Mn acetate for 24 or 72 hrs. Changes in cell structure and alterations in cellular apoptotic markers were examined using light microscopy (LM), transmission electron microscopy (TEM), or immunocytochemistry. Following Mn exposure, both Mn species caused cell detachment from culture dishes and shrinkage in cell bodies and nuclei. TEM revealed that Mn exposure resulted in cytoplasmic vacuolation, dense nuclei, chromosome condensation, formation of granules surrounding the nucleus, and decrease in mitochondrial numbers. These morphological damages were evidently much severer in Mn(III)-treated cells than in Mn(II)-exposed cells. In particular, Mn(III) treatment exhibited more profound toxic effect than Mn(II) on cytoplasmic apparatus. To further investigate Mn-induced apoptosis, the expression of caspase-3, a key mediator of apoptosis of mammalian cells, and the activity of nitric oxide synthase (NOS) were studied. Immunocytochemistry showed that incubation with either Mn species equally increased the expression of caspase-3. While Mn treatment did not change the status of neuronal nNOS, it increased the expression of inducible iNOS. Co-incubation with an NO inhibitor (N-monomethyl-L-arginine) significantly reduced either Mn species-elicited cell killing by a flow cytometry. These data suggest that Mn(III) species causes severer morphological damage than Mn(II). In addition, Mn-mediated apoptosis may be associated with the activity of NOS/NO in cytoplasm and mitochondria. (Supported in part by PRC-NSF #3000140, Beijing Science Committee #9558102800, and US-NIEHS ES08146)

400 MOTOR AND NEUROCHEMICAL EFFECTS OF SUB-CHRONIC LOW MANGANESE EXPOSURES IN A RODENT MODEL.

R. Gwiazda, C. Kern and D. Smith. *Environmental Toxicology, University of California, Santa Cruz, Santa Cruz, CA.*

The mechanisms and functional outcomes of exposures to Mn from environmental (e.g., from MMT combustion) and more elevated occupational (e.g., welding) sources have sparked interest in elucidating the physiological basis of the neurological effects due to cumulatively low levels of Mn exposure. Particular concern exists for individuals in the early stage of neurological disease who could be further impaired by Mn neurotoxicity. As a first step in evaluating this question 36 Long-Evans female rats were divided in three treatment groups: control, low and high dose, and received 0, 1.6 or 4.8 mg Mn/kg, three times a week for 5 weeks, respectively. The outcomes evaluated at the completion of exposures were spontaneous motor activity using a digitized Video system, latency to fall from an accelerating rotating rod, and the ability to cross a suspended balance beam. Neurotransmitter levels (dopamine, GABA, aspartate, glutamate) in striatum, globus pallidus and motor regions of the thalamus were also evaluated. Animals exposed to the high Mn dose regimen had significantly higher levels of spontaneous motor activity (they traveled farther and spent less time at rest), and remained on the accelerating rotorod longer before falling compared to animals from either the control or the low Mn dose groups. Animals from the low dose group were significantly impaired in crossing the balance beam compared to animals from the control and high dose groups. Levels of the neurotransmitters dopamine, aspartate, glutamate and GABA were not measurably affected by manganese treatments in striatum and globus pallidus, but the low dose treatment significantly raised aspartate and GABA levels in the motor regions of the thalamus. These effects were elicited at the lowest Mn dose ever administered in a rodent study. Collectively, these results substantiate previous findings that low exposure to Mn induces adverse motor effects and alters aminoacid neurotransmitter levels in brain regions within the basal ganglia without having an effect on striatal dopamine.

401 LOCOMOTOR ACTIVITY IN UBIQUITIN MUTANT MICE AFTER NOSE-ONLY INHALATION OF MANGANESE.

J. Karlsson[1], R. H. Gwiazda[4], O. Myers[1], W. Barrington[1], G. A. Douglas[5], E. Barr[2], S. R. Donald[4], G. Bench[3] and J. Lewis[1]. *[1]Community Environmental Health Program, University of New Mexico, Albuquerque, NM, [2]Lovelace Respiratory Research Institute, Albuquerque, NM, [3]Lawrence Livermore National Laboratory, Livermore, CA, [4]Environmental Toxicology, University of California, Santa Cruz, CA and [5]Centre for Cancer Therapeutics, Ottawa Regional Cancer Centre, Ottawa, ON, Canada.*

Manganese (Mn) toxicity has been linked to the Parkinsons disease-like syndrome manganism. Mn can be directly transported from the nose into the CNS by the olfactory pathway, and can also be further transported trans-synaptically to deeper brains structures. Ubiquitin plays an important role in tagging damaged proteins for proteosomal degradation. The ubiquitin pathway is implicated in pathogenesis of neurodegenerative diseases such as Parkinsons, Huntingtons, Alzheiemers disease and amyotrophic lateral sclerosis. The aim of the present study was to investigate the role of ubiquitin in behavioral and neuropathological response to inhaled low-dose Mn. Transgenic mice expressing dominant negative mutant (K48R) or wild-type human ubiquitin, as well as FVB/N non-transgenic strain control mice were used as experimental animals. Mice were exposed to air or MnCl2 aerosol (dose of 2.02±0.26 mg/m3, particle size 1.76±0.33 ?m) by nose-only inhalation for 2 weeks (5 days/week, 6h/day to mimic occupational exposures). 18 months after exposure, mouse spontaneous activity, velocity and time spent resting or moving were evaluated over 20 min sessions using a computerized global tracking system. Ubiquitin mutant mice traveled farther, spent less time at rest, spent less time going slow, spent more time going fast, and had higher average velocity when moving than the other genotypes. Activity did not differ between ubiquitin control and FVB/N control mice. When controlled for genotype, Mn-exposed mice traveled less far, spent more time at rest, spent less time going fast, and had a slower velocity when moving compared to air-exposed animals. Ongoing neuropathological evaluation of the brains will shed further light on underlying mechanisms for the altered behavior.

402 MANGANESE EXPOSURE ARRESTS CELL PROLIFERATION AND ALTERS SIGNAL TRANSDUCTION ON MAPK'S CASCADES IN PC12 CELLS.

J. Chen[1], W. Xu[1], W. Luo[1] and W. Zheng[2]. *[1]Occupational & Environmental Health Sciences, Fourth Military Medical University, Xian, Shanxi, China and [2]School of Health Sciences, Purdue University, West Lafayette, IN.*

Our previous studies suggest that manganese (Mn) exposure leads to cell death by damage to mtDNA and initiation of oxidative stress in cultured PC12 cells (Chen et al., Toxicol Appl Pharmacol 175:160, 2001). This study aimed to test the hypothesis that Mn-induced cellular stress altered the highly conserved MAPK (mitogen-activated protein kinase) signal transduction, particularly on ERK (extracellular signal regulated protein kinase) and p38 pathways, leading to apoptosis. PC12 cells were exposed to Mn at 0, 200, 400, 600, 800 uM as $MnCl_2$ for up to 4 days. Flow cytometry revealed that the cell proliferation cycle was arrested at the S phase; the cell inhibitory ratio exceeded 50% of the control. Transmission electron microscopy (TEM) and DNA fragment assay confirmed the apoptosis on day 4 following Mn exposure (600 uM). Western-blot showed that Mn treatment decreased phosphorylation of ERK2 in a time-dependent manner, possibly due to a down-regulation of ERK1/2 activity *via* the action on MEK1/2, as attested by studies using PD98059, a specific inhibitor of MEK1/2. Mn exposure, however, increased the phosphorylation of p38 during the same time period. By using SB203580, a specific inhibitor of p38, it was found that the up-regulated p38 activity by Mn treatment may pertain to Mn action on MEK3/6. These data suggest that the apoptosis may underline Mn-induced arrest in cell proliferation. An alteration in intracellular signal transduction appears to occur in a time sequence preceding Mn-in-

duced morphological damage. Further, we demonstrate that Mn acts on MAPK signal transduction cascade by down-regulating ERK2 and up-regulating p38; the combination of both effects may lead to apoptotic cell death in Mn-exposed PC12 cells. (Supported by China National Science Foundation Grant#30100148 and US NIH/NIEHS ES08146)

403 PROTEOLYTIC ACTIVATION OF PROAPOPTOTIC KINASE PKCδ CONTRIBUTES TO MANGANESE-INDUCED APOPTOTIC CELL DEATH IN DOPAMINERGIC NEURONAL CELLS.

C. Latchoumycandane, M. Kitazawa, V. Anantharam and A. Kanthasamy. *Department of Biomedical Sciences, Iowa State University, Ames, IA.*

The utilization of methylcyclopentadienyl manganese tricarbonyl (MMT) as a gasoline additive has raised health concerns and renewed interest in understanding the neurotoxic effects of manganese in humans (Science 2003, 300:926-928). Chronic exposure to manganese causes Manganism, a neurological disorder similar to Parkinson's disease. However, the cellular mechanism by which manganese induces cell death in dopaminergic neurons remains unclear. We previously reported that manganese induces oxidative stress-dependent apoptotic cell death in the non-neuronal pheochromocytoma (PC12) dopaminergic cell model. In the present study, we investigated apoptotic cell signaling events following chronic exposure to manganese in mesencephalic dopaminergic neuronal (N27) cells. Manganese chloride (300 μM for 24 hr) induced time-dependent cell death in N27 cells, as determined by Sytox green nuclear staining. The cell death was accompanied by sequential activation of mitochondrial-dependent proapoptotic events including mitochondrial membrane depolarization, cytochrome c release, caspase-3 activation and DNA fragmentation, indicating that the mitochondrial-dependent apoptotic cascade primarily triggers the manganese-induced apoptotic cell death. Furthermore, manganese treatment proteolytically activated a member of a novel class of protein kinases, namely protein kinase Cδ (PKCδ). The caspase-3 specific inhibitor zDEVD-FMK significantly blocked PKCδ cleavage, indicating that caspase-3 mediates the cleavage. Pretreatment with the PKCδ inhibitor rottlerin or the caspase-3 inhibitor zDEVD-FMK almost completely blocked manganese-induced DNA fragmentation. Additionally, N27 cells expressing a catalytically inactive PKCδK376R protein (a PKCδ dominant negative mutant) were resistant to manganese-induced apoptosis. Collectively, the results suggest that the mitochondrial-dependent apoptotic cascade mediates apoptosis *via* proteolytic activation of PKCδ in manganese-induced dopaminergic toxicity [NIH grant ES 10586]

404 ASTROGLIAL-MEDIATED NEURONAL APOPTOSIS FOLLOWING EXPOSURE TO MANGANESE AND CYTOKINES REQUIRES NF-KAPPA B-DEPENDENT PRODUCTION OF NO.

X. Liu, R. Mouneimne and R. Tjalkens. *Toxicology Program, Department of Integrative Biosciences, Texas A&M University, College Station, TX.*

Neuronal injury in Parkinsons disease is accompanied by regional increases in inflammatory mediators and activation of astroglia, with subsequent overproduction of nitric oxide (NO). The present studies postulate that astroglial-derived NO mediates neuronal apoptosis induced by manganese (Mn) and pro-inflammatory cytokines. PC12 cells were differentiated with nerve growth factor for 5 days and incubated with primary astrocytes grown on permeable cell culture inserts. Following exposure to Mn and interferon-γ (IFN-γ)/tumor necrosis factor-α (TNF-α), neuronal caspase activity was determined by live-cell fluorescence imaging and the number of apoptotic cells determined by terminal deoxynucleotide transferase-mediated dUTP-biotin nick-end labeling (TUNEL) and morphologic analysis of condensed nuclei. Mn synergistically enhanced both production of NO and induction of NOS2 mRNA in primary cortical astrocytes. In co-cultured neurons, apoptosis following exposure to Mn and TNF-α/IFN-γ was only observed in the presence of astrocytes; neurons exposed to Mn and TNF- in the absence of astrocytes did not undergo apoptosis. Overexpression of mutant IkBα (S32/36A) in astrocytes prevented all indices of apoptosis in co-cultured neurons. Inhibition of NOS2 with (±)-2-Amino-5, 6-dihydro-6-methyl-4H-1, 3-thiazine (AMT) significantly reduced neuronal apoptosis. In neurons exposed to Mn and TNF-α/IFN-γ in the absence of astrocytes, the apoptotic phenotype was recovered through addition of low concentrations of the NO donor, S-nitroso-N-acetyl penacillamine. It is concluded that Mn- and cytokine-dependent apoptosis in PC12 neurons requires astroglial-derived NO and that selective overexpression of mtIkBα in astrocytes prevents apoptosis in co-cultured neurons. Thus, therapeutic strategies that target the molecular signaling pathways regulating NOS2 expression in astroglia may be effective in mitigating neuronal injury in parkinsonian syndromes.

405 MANGANESE NEUROTOXICITY CORRELATES WITH INCREASES IN OXIDATIVE DAMAGE TO DNA IN THE NIGROSTRIATAL PATHWAY BUT NOT WITH CHANGES IN ANTIOXIDANTS.

D. Cox, C. Bolin and F. Cardozo-Pelaez. *Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT.* Sponsor: A. Holian.

Exposure to Mn can occur from industrial settings such as mining and from drinking water contamination. Environmental levels could increase as more countries use MMT as an additive to gasoline. Chronic exposure to Mn has been associated with the onset of neuropathological changes similar to Parkinsons disease (PD) with effects localized to the basal ganglia. It is believed that Mn neurotoxicity occurs *via* free radical damage. Antioxidant defenses protect against free radical damage; however exposure to Mn has been shown to deplete antioxidants and lead to increased damage to major cellular macromolecules. Elevated levels of 8-hydroxy-2-deoxyguanosine (oxo^8dG), oxidative damage to DNA, have been found in brain regions associated with major neurodegenerative disease in humans, and in brain of animals challenged with various neurotoxicants. However, it is unknown whether DNA damage is responsible for neuronal loss or if it is mere epiphenomena of neurodegeneration. This study has made use of a mouse model genetically engineered to be deficient for the DNA repair enzyme 8-oxoguanine glycosylase (OGG1) that is primarily responsible for the removal of oxo^8dG. Wild type (WT) and knockout OGG1 (KO) mice were exposed to $MnCl_2$ in drinking water from gestation to post-natal (PND) 31. Dopamine was measured in caudate putamen (CP) along with oxo^8dG levels in midbrain (MB) and CP. A correlation between a rise in oxo^8dG in MB, and a drop in DA in CP was seen in the KO mouse. Although Mn produced changes in endogenous antioxidant systems in both WT and KO mice, DA loss in CP was only associated with increases in oxo^8dG levels in the nigrostriatal system. DNA repair activity was assessed in the WT showing a decrease repair capacity in MB and CP as a consequence of Mn exposure. This study has produced some accepted biomarkers for Mn neurotoxicity and it has revealed Mn-induced changes in DNA damage and repair that could enlighten molecular pathways involved in the neuropathological changes seen in Mn neurotoxicity.

406 OXIDATIVE STRESS AND GLOBAL RAT GENE EXPRESSION PROFILES IN PC12 CELLS AFTER MANGANESE TREATMENT.

R. R. Reams[1] and E. Taka[1]. *[1]College of Pharmacy, Florida A & M University, Tallahassee, FL and [2]College of Pharmacy, Florida A & M University, Tallahassee, FL.*

A Clontech Rat oxidative stress micro array was used to examine manganese chloride induced alteration of gene expression in rat pheochromocytoma cells (PC12 cells). Experimental samples included PC12 cells, which were exposed for 24 hours under control conditions (no added MnCl2) and treated conditions (300mM MnCl2). Two genes, heme oxygenase 1 (HMOX1; HO1) and ribosomal protein S30 40S subunit (RPS30) were upregulated as much as four-fold and three-fold respectively. Five genes, Cu-Zn Superoxide Dismutase, DNA-binding protein inhibitor 1 (ID1), heat shock 27-kDa protein (HSP27), Prothymosin-alpha (PTMA), and Heterogeneous nuclear ribonucleoprotein K (HNRNPK) were partially upregulated, in that they only met one of the two conditions for up regulation. We have confirmed the altered expression of two candidate genes, heme oxygenase 1 and ribosomal protein S30 40S subunit (RPS30) also known as FAU by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and western blotting analysis, respectively. This is the first report on the direct role of manganese in the global gene expression profile of oxidative stress in PC12 cells. Our findings suggests that 24 hour exposure of PC12 cells to manganese chloride resulted in the upregulation of genes involved in protection against oxidative stress and genes involved in protein synthesis/turnover. In a parallel micro array study, the effect of manganese on global gene expression was examined using Agilents rat global cDNA micro array . The molecular signature after Mn treatment included 405 signature genes. Genes with greater than 4-fold change in gene expression included HOX-1, Diaphorase (NADH/NADPH), rattus norvegicus dithiolethinone-inducible gene-1 mRNA and tartrate-resitant acid phosphatase type 5 mRNA.(Supported by ATS/DR and MHPF #U50/ATU398948-10)

407 ACTIVATION OF NITRIX OXIDE SYNTHASE DURING MODULATION WITH MANGANESE TOXICITY INVOLVES EARLY SIGNALING TRANSCRIPTION FACTOR, NF-KB: IMPLICATION FOR CELL DEATH.

P. G. Gunasekar[1], K. Prabhakaran[2] and D. Ghosh[1]. *[1]Biological Sciences, Texas Southern University, Houston, TX and [2]Medicinal Chemistry and Mole. Pharmacology, Purdue University, West Lafayette, IN.*

In the treatment of Parkinson's disease (PD), dopamine (DA) modulation has been implicated in the degeneration of dopaminergic neurons. Previously, we reported that modulation of DA enhanced manganese (Mn)-induced cell death which was

mediated by oxidative stress following NMDA receptor activation and involved nitric oxide synthase (NOS). In the present study, mesencephalic cells were used to investigate the early signaling events responsible for the induction of NOS and mediated apoptosis in Mn-induced cell death in the presence and absence of DA. Mn exposure to mesencephalic cells for 24 hr induced minimal apoptotic cell death. However, 2 hr exposure to DA in cell culture potentiated Mn-induced apoptotic cell death significantly in which Nuclear factor Kappa B (NF-KB) was involved. The ability of Mn-induced apoptotic cell death to DA exposure was determined by cell *via*bility assay and Apoptag TUNEL staining. These studies support our hypothesis that greater level of DA in the cellular system interacts with Mn, and may cause cells to be more susceptible. In addition, co-incubation of NF-KB inhibitor (SN50) with Mn and DA protected significantly from cell death especially apoptosis dose dependently, which indicates the involvement of early signaling factor NF-KB during Mn and DA interaction induced dopaminergic toxicity. In order to confirm the role of NF-KB in NOS induction during DA potentiated Mn toxicity, Western blot study was carried out. Mn in the presence of DA (2 hr) markedly induced NOS activation in mesencephalic cells, which was reduced significantly in SN50 pre-treated cells. Taken together, these results indicate that DA modulation at the cellular level strongly interacts with Mn and thereby potentiates NOS mediated dopaminergic cell death. The activation of NF-KB signaling is an upstream event in Mn and DA interaction-induced cell death mechanism (Supported by American Parkinson Disease Association, Inc.).

408 MANGANESE POTENTIATES LIPOPOLYSACCHARIDE-INDUCED EXPRESSION OF NOS2 IN C6 GLIOMA CELLS THROUGH MITOCHONDRIAL-DEPENDENT ACTIVATION OF NUCLEAR FACTOR KAPPA B.

R. Mounemne, J. Faske, X. Liu and R. Tjalkens. *Toxicology Program, Department of Integrative Biosciences, Texas A&M University, College Station, TX.*

Excessive accumulation of manganese (Mn) causes a neurodegenerative condition in humans that shares neurologic and pathologic features with idiopathic Parkinsons disease. It has been proposed that increased production of nitric oxide (NO) by inducible nitric oxide synthase (NOS2) in activated astroglia exacerbates neuronal injury. Manganese enhances the effects of proinflammatory cytokines on expression of NOS2 but the molecular basis for this effect is unclear. It was postulated in the present studies that Mn enhances expression of NOS2 through the cis-acting factor, nuclear factor kappaB (NF-κB). Exposure of C6 glioma cells to lipopopolysaccharide resulted in increased expression of NOS2 and production of NO that was dramatically potentiated by Mn and was blocked through overexpression of mutant IκBα (S32/36A). LPS-induced DNA binding of p65/p50 was similarly enhanced by Mn and was decreased by mutant IkBa. Phosphorylation of IκBα was synergistically increased by Mn and LPS and was not blocked by U0126, a selective inhibitor of ERK1/2. Mn decreased mitochondrial membrane potential and increased matrix calcium, associated with a rise in intracellular reactive oxygen species (ROS) that was attenuated by a mitochondrial-specific antioxidant. Blocking mitochondrial ROS also attenuated the enhancing effect of Mn on LPS-induced phosphorylation of IκBα and expression of NOS2, suggesting a link between Mn-induced mitochondrial dysfunction and activation of NF-κB. Overexpression of a dominant-negative mutant of the NF-κB-interacting kinase prevented enhancement of LPS-induced phosphorylation of IκBα by Mn. These data indicate that Mn augments LPS-induced expression of NOS2 in C6 cells by increasing mitochondrial ROS and activation of NF-κB.

409 COMPARISON OF DIFFERENTIATED AND NON-DIFFERENTIATED PC CELLS RESPONSE TO MNCL2, MPP+, AND ROTENONE.

L. Russell IV. *University of Maryland, Baltimore, MD.* Sponsor: K. Squibb.

There is an increasing belief that differentiated cells have greater susceptibility than non-differentiated cells following exposure to certain neurotoxicants. Excessive exposure to MnCl2, MPP+, and Rotenone has been shown to produce neurotoxic impairments in dopaminergic neurons of mammalian brains that lead to the development of a Parkinson-like Syndrome. The present study was undertaken to test the hypothesis that differentiated PC12 cells are more sensitive to MnCl2, MPP+, and rotenone than non-differentiated PC12 cells. Non-differentiated cells were differentiated by five-day exposure to 100 ng/mL of nerve growth factor. Differentiated and non-differentiated PC12 cells were evaluated for their response following 24-hour exposure to MnCl2, MPP+, and rotenone. The Alamar Blue cell *via*bility assay was used to measure the metabolic activity of PC12 cells. Differentiated PC12 cells are more metabolically active than non-differentiated PC12 cells. In dose dependent studies MnCl2 decreases the cell *via*bility in a concentration dependent manner in non-differentiated PC12 cells whereas MPP+ and rotenone have no effect. Only MPP+ was able to decrease cell *via*bility in differentiated PC12 cells. In the interaction studies the MPP+ and rotenone interaction in differentiated cells caused the greatest decrease in cell *via*bility as compared to all other interactions studied in differentiated and non-differentiated PC12 cells. These results suggest that differences in differentiated and non-differentiated sensitivity may involve differences in the expression of genes involved in protection from oxidative damage and dopamine receptor and transporter proteins.

410 MANGANESE AND LEAD DISPLAY DIFFERENT PATTERNS OF NEUROTOXICITY IN HUMAN SY5Y NEUROBLASTOMA CELLS.

Y. Qian, Y. Zheng and E. Tiffany-Castiglioni. *Texas A&M University, College Station, TX.*

Epidemiologic studies suggest that manganese (Mn) and lead (Pb) contribute to the onset of Parkinson's disease (PD). The α-synuclein-positive Lewy body is a pathological hallmark of PD and its aggregation in dopaminergic neurons may be stimulated by environmental insults that generate the production of reactive oxygen species (ROS). In this report, we used the human SY5Y neuroblastoma cells as a dopaminergic neuronal model to profile effects of Mn and Pb on ROS levels, ROS-related enzymes and α-synuclein aggregation. In cells exposed to MnCl2 (0-250 μM) for 3 days, both cytotoxicity and ROS levels increased with metal concentration. In contrast, ROS levels decreased while cytotoxicity increased relative to Pb concentration in cells exposed to Pb acetate (0-10 μM). In cells exposed to a mixture of 100 μM Mn and 3 μM Pb, Pb cytotoxicity was partially attenuated by Mn while ROS levels dramatically increased. However, Pb had no effect on Mn cytotoxicity and Mn-induced ROS generation. Mn induced Cu/Zn-SOD expression, as detected by Western blot analysis, but had no effect on Mn-SOD and catalase expression. Whereas Pb did not change SOD expression, Pb and Mn mixture induced CuZn-SOD but not Mn-SOD expression, similar to Mn alone. Effects of Mn, Pb and their mixture on α-synuclein aggregation were profiled by fluorescence imaging of cells transfected with three chimeric cDNAs containing the transcript for EGFP: WP, A30P or A53T. EGFP was used as a control. In EGFP-, WP-EGFP- and A30P-EGFP- transfected cells, no change of protein distribution or aggregation was observed after metal treatment. However, in A53T-EGFP-transfected cells, A53T-EGFP aggregation by Mn, Pb and mixture was observed. These data suggest that Mn, not Pb, cytotoxicity is dependent on ROS generation and Mn can reduce Pb cytotoxicity. Mn-induced ROS generation occurred in the cytosol but not in mitochondria. Therefore, Mn and Pb may have different mechanisms for modifying α-synuclein distribution and aggregation.

411 HISTOPATHOLOGY, IMMUNOHISTOCHEMISTRY AND ELECTRON MICROSCOPY OF SPONTANEOUS UVEAL MELANOMA IN TWO HAN WISTAR RATS.

C. Barton and A. Moran. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: D. Everett.

Uveal melanomas in rats are unilateral neuroectodermal tumours derived from uveal melanocytes. They occur in numerous species of animals including laboratory animals and are most frequently reported in cats where they are the most common intraocular tumuor. Here we describe the histopathologic, immunohistochemical and electron microscopic characteristics of uveal melanomas in two Han Wistar rats. The tumours occurred in two, one year old Han Wistar rats that were involved in a long term drug study. Fresh tissues were routinely fixed and processed. Paraffin embedded sections were stained with haematoxylin and eosin. Additional sections were immunohistochemically examined for reactivity to Vimentin, S100, Neuron Specific Enolase (NSE), Melan A, Ki-67 and Proliferating Cell Nuclear Antigen (PCNA). In addition sections were also routinely processed for Transmission Electron Microscopy. Histologic examination of the eye in each case revealed an unencapsulated infiltrative densely cellular mass composed of sheets and nests of tumour cells supported by a fine fibrovascular stroma. The tumour cells were round to spindloid with variably distinct cell borders. Occasional cells contained a dark brown granular pigment within their cytoplasm. The tumour cells were positive for Vimentin, S100, NSE and Melan A. Cell proliferation was apparent with Ki-67 and PCNA.

412 BACKGROUND CHANGES FOLLOWING DAILY INTRAVITREAL INJECTION FOR THREE CONSECUTIVE DAYS IN BEAGLE DOGS AND DUTCH-BELTED RABBITS.

M. Vezina, A. Patel and C. Copeman. *CTBR, Senneville, QC, Canada.*

Intravitreal injection is becoming more common as a route of administration in the prevention and treatment of diseases in the eye. Previously, multiple intravitreal injections were performed no more than weekly for reasons not only related to the test article, but also to the concern for cumulative trauma caused by the injection procedure. As the injection procedure is becoming more common in the clinic and

clinical ophthalmologists more comfortable with the procedures, the concept of daily injections to maximize exposure and increase efficacy has been investigated. The background data presented is from 12 dogs and 6 rabbits. Intravitreal injections were performed bilaterally once daily for 3 consecutive days in both species using a 27G needle for the dogs and a 29G needle for the rabbits. Ophthalmology was performed predose, on each day of dosing, the day following dosing and 1 and 2 (dogs only) weeks after the first dose. In addition, ERG and tonometry were performed on the dogs the day following Dose 3 and on Days 7 and 14. In both species, background changes were mild and transient, and consisted of subconjunctival hemorrhage in all eyes, with occasional conjunctival hyperemia, chemosis and/or slight aqueous flare, which were associated with manipulation of the eye and the injection. These changes mostly resolved by Day 7 and were completely resolved by Day 14. There were no ERG or tonometry changes in the dogs. It was concluded that a daily intravitreal injection performed over 3 consecutive days was well tolerated in beagles and Dutch-belted rabbits and is a suitable method for multiple intravitreal injection toxicity studies.

413 A 14-DAY SYSTEMIC AND OCULAR TOXICITY STUDY IN THE DOG OF INTRAVENOUSLY ADMINISTERED HEAT SENSITIVE LIPOSOMES CONTAINING CARBOXYFLUORESCEIN.

R. E. Rush[1], S. A. D'Anna[2] and R. C. Zeimer[2]. *[1]Charles River Laboratories- Ohio Division, Spencerville, OH and [2]The Wilmer Ophthalmological Institute, Johns Hopkins University, Baltimore, MD.*

Vascular diseases, such as age related macular degeneration, are the leading cause of severe vision loss. Local delivery of drugs to the retinal vasculature could allow for interventions not currently feasible. Laser targeted drug delivery (LTD) has been developed to provide a minimally invasive access to this vasculature. Drugs that are encapsulated in heat-sensitive liposomes and injected intravenously can be released by warming the liposome with a laser pulse directed through the pupil. Previous studies in rodents have demonstrated the potential benefits of LTD. In order to further evaluate LTD, a dog toxicology study was performed to explore the potential systemic effects of heat sensitive liposomes containing carboxyfluorescein (CF) and potential ocular toxicity of retinal exposure to blue light (used in angiography) in the presence of CF. Heat sensitive liposomes containing CF were intravenously injected into eight dogs at a dose of 10 ml/kg (10x the intended clinical dose). In each eye, two 20 degree areas of the fundus were exposed to blue (490 nm) light at the intended power density. In one eye, the procedure was performed once with 3x the maximal intended duration (2 sec). In the opposite eye, blue light exposure was performed 10 times at 2x the intended duration (0.2 sec). Four other dogs were administered CF liposomes and returned to their cages without light exposure. A separate group of 8 dogs was similarly treated except that phosphate buffered saline was injected. All animals were observed for signs of systemic toxicity and received ophthalmologic and histopathologic evaluation. The results of this study indicate that intravenous injection of heat sensitive liposomes containing CF at 10x the intended clinical dose along with 490 nm blue light exposure produced no systemic or local toxicity.

414 PHOTOBIOMODULATION ATTENUATES METHANOL-INDUCED RETINAL TOXICITY.

J. T. Eells[1, 3], M. M. Henry[2], P. Summerfelt[2], M. T. Wong-Riley[2], E. V. Buchmann[3], M. Kane[3] and H. T. Whelan[3]. *[1]Health Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI, [2]Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI and [3]Neurology, Medical College of Wisconsin, Milwaukee, WI.*

Our laboratory has developed a rodent model of acquired mitochondrial dysfunction produced by the mitochondrial toxin, formic acid, generated during methanol intoxication. Methanol intoxication produces ocular toxicity and blindness. Formic acid inhibits the essential mitochondrial enzyme, cytochrome oxidase. Low energy photon irradiation (photobiomodulation) by light in the red to near infrared spectral range (630-1000 nm) has been demonstrated to enhance mitochondrial activity and promote cell survival *in vitro* by stimulation of cytochrome oxidase activity. The present studies were undertaken to test the hypothesis that exposure to 670 nm radiation from light-emitting diode (LED) arrays would attenuate the retinotoxic actions of methanol-derived formic acid in this animal model. Using the electroretinogram as a sensitive indicator of retinal function, we demonstrated that that 3 brief (2 min. 24 sec.) 670 nm LED treatments (4 J/cm2), delivered at 5, 25 and 50 h of methanol intoxication, attenuated the retinotoxic effects of methanol-derived formate assessed at 72 h of intoxication. The same treatment protocol administered during the 72 h intoxication period improved the recovery of retinal function assessed following a 72 h recovery period. Our studies document a significant improvement of rod and cone-mediated function in LED-treated methanol-intoxicated rats during intoxication and a complete recovery of rod and cone-mediated function following recovery. We further show that LED treatment protected the retina from formate-induced histopathology. These findings provide a link between the actions of 670 nm light on mitochondrial oxidative metabolism *in vitro* and retinoprotection *in vivo*. Based on these findings, we suggest that photobiomodulation may represent a novel therapeutic approach for the treatment of retinal diseases including age-related macular degeneration in which mitochondrial dysfunction has been proposed to play a role.

415 GENE REGULATION BY THE α-SECRETASE CLEAVED AMYLOID PRECURSOR PROTEIN.

J. A. Johnson[1, 2, 3] and T. D. Stein[1]. *[1]Neuroscience Training Program, University of Wisconsin, Madison, WI, [2]Environmental Toxicology Center, University of Wisconsin, Madison, WI, [3]School of Pharmacy, University of Wisconsin, Madison, WI and [4]Waisman, University of Wisconsin, Madison, WI.*

Mice engineered to overexpress disease-causing mutant amyloid precursor proteins (APP) display plaque deposition, but lack the hyperphosphorylated tau and massive neuronal loss characteristic of Alzheimer's disease (AD). The Tg2576 mice overexpressing a mutant APP (APPSw) demonstrated increased expression of the amyloid sequestration protein transthyretin (TTR) and the neuroprotective insulin-like growth factor 2 (IGF-2). Both the γ-secretase cleaved COOH-terminal fragment (CTFγ) and the α-secretase cleaved NH2-terminal of APP (sAPPα) can regulate gene expression. While β-amyloid (Aβ) and CTFγ can lead to toxicity and cell death, sAPPα promotes neurite outgrowth, enhances memory, and protects against a variety of insults, including Aβ toxicity. In AD, Aβ levels increase while sAPPα levels decrease. In contrast, sAPPα levels are dramatically increased in the hippocampus of APPSw mice. In mouse hippocampal slice cultures sAPPα treatment completely protects against Aβ toxicity. Furthermore, sAPPα leads to increased expression of a number of neuroprotective genes, including TTR and IGF-2. Blockade of TTR or IGF-2 with either antibodies or siRNAs prevents the protective action of sAPPα, indicating that increased expression of these proteins is necessary for sAPPα protection against Aβ toxicity. Chronic injection of an antibody against transthyretin into the hippocampus leads to increased Aβ, tau phosphorylation, and neuronal death in the CA1 neuronal field of mice overexpressing APPSw. Therefore, elevated expression of transthyretin by sAPPα protects APPSw mice from developing the full neuropathologies observed in AD. Supported by grants from the National Institutes of Environmental Health Sciences (ES08089, ES10042).

416 BREVETOXIN-INDUCED COINCIDENT ACTIVATION OF SRC KINASE AND VOLTAGE-GATED SODIUM CHANNELS ENHANCES NMDA RECEPTOR SIGNALING IN NEOCORTICAL NEURONS.

T. F. Murray[1], D. G. Baden[2] and S. M. Dravid[1]. *[1]Physiology and Pharmacology, University of Georgia, Athens, GA and [2]Center for Marine Science Research, University of North Carolina at Wilmington, Wilmington, NC.*

Brevetoxins (PbTx) are potent allosteric enhancers of voltage-gated sodium channel (VGSC) function and are associated with periodic Red Tide blooms. These neurotoxins produce neuronal injury and death in cerebellar granule cells (CGC) following acute exposure. In murine neocortical neurons brevetoxin induces calcium influx that is mediated through both glutamatergic and non-glutamatergic pathways. Inasmuch as Src kinase is capable of upregulating the NMDA subtype of glutamate receptors, we determined whether Src kinase participated in PbTx-2-induced calcium influx. Inhibition of Src kinase blocked PbTx-2 induced calcium influx. PbTx-2 treatment moreover increased tyrosine phosphorylation of the NR2B subunit. A rise in intracellular [Na+] and phosphorylation of NMDA receptors by Src kinase is known to increase NMDA receptor activity. We therefore explored the influence of brevetoxin on NMDA receptor function. We found that PbTx-2 augments NMDA receptor mediated calcium influx in both spontaneously oscillating mature neurons and in non-oscillatory immature neurons. PbTx-2 also enhanced the effect of bath applied NMDA on extracellular signal-regulated kinase 2 (ERK2) activation. These results suggest that brevetoxin augments NMDA receptor signaling in neocortical neurons, and this upregulation may be mediated by coincident elevation in intracellular [Na+] and Src kinase activation.

417 CYCLOOXYGENASE-2-CATALYZED OXIDATION OF 6-HYDROXYDOPAMINE IN PC12 PHEOCHROMOCYTOMA CELLS. IMPLICATION FOR PARKINSON'S DESIASE.

A. A. Kapralov[1], Y. Y. Tyurina[1], G. G. Borisenko[1], N. F. Schor[4, 6], S. H. Graham[3] and V. E. Kagan[1, 2, 5]. *[1]EOH, University of Pittsburgh, Pittsburgh, PA, [2]Pharmacology, University of Pittsburgh, Pittsburgh, PA, [3]Neurology, University of Pittsburgh, Pittsburgh, PA, [4]Pediatrics, University of Pittsburgh, Pittsburgh, PA, [5]Cancer Institute, University of Pittsburgh, Pittsburgh, PA and [6]PCN, CHP, University of Pittsburgh, Pittsburgh, PA.*

Parkinson's disease is characterized by a progressive loss of dopaminergic neurons likely associated with dysregulation of dopamine oxidation pathways. It has been suggested that cyclooxygenase-2 (COX-2) is involved in pathogenesis of the disease

although specific pathways have not been identified. We hypothesized that COX-2 enhances the dopamine-induced toxicity by catalyzing its oxidation. To experimentally address the hypothesis in physiologically relevant environments, we used a dopamine analogue - 6-hydroxydopamine (6-OHDA) and COX-2-transfected PC12 pheochromocytoma cells. Using arachidonic acid as a substrate we confirmed that recombinant COX-2 increased the rate of oxidation of 6-OHDA in cell-free systems. COX-2-induced oxidation of 6-OHDA was suppressed by NS-398 (a COX-2 inhibitor) and superoxide dismutase (SOD). In cell homogenates prepared from COX-2-transfected cells, oxidation of 6-OHDA proceeded at a greater rate as compared to homogenates prepared from moc-transfected cells. Addition of NS-398 to homogenates from COX-2 transfected cells resulted in an inhibition of 6-OHDA oxidation. Next we performed cell *via*bility experiments using Trypan blue test. Cells were incubated with 6-OHDA in the presence of SOD to avoid extracellular oxidation of 6-OHDA. Under these conditions, we found that 6-OHDA was toxic to both COX-2-transfected and moc-transfected cells in a concentration dependent manner; COX-2-transfected cells were significantly more sensitive to 6-OHDA than moc-transfected cells. These results suggest that COX-2 contributes to 6-OHDA oxidation. Consequently, inhibitors blocking COX-2-dependent component of 6-OHDA oxidation may be promising for development of new therapeutic approaches. Supported by NIH NS41297.

418 STRESS AND COMBINED EXPOSURE TO LOW DOSES OF PYRIDOSTIGMINE BROMIDE, DEET, AND PERMETHRIN PRODUCE NEUROCHEMICAL AND NEUROPATHOLOGICAL ALTERATIONS IN CEREBRAL CORTEX, HIPPOCAMPUS, AND CEREBELLUM.

E. M. El-Masry, A. Abdel-Rahman, S. M. Abou-Donia, A. K. Shetty and M. B. Abou-Donia. *Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.*

Exposure to a combination of stress and low doses of pyridostigmine bromide, (PB), DEET and permethrin in adult rats, a model of Gulf war exposure, produces blood-brain barrier (BBB) disruption and neuronal cell death in the cingulated cortex, dentate gyrus, thalamus and the hypothalamus. In the present study, neuropathological alterations in other areas of the brain where no apparent BBB disruption was observed was studied following the above exposure. Animals exposed to both stress and chemicals exhibited decreased acetylcholinesterase (AChE) activity in the midbrain, brainstem and the cerebellum and a decreased m2 muscarininc ACh receptor ligand binding in the midbrain and cerebellum. These alterations were associated with significant neuronal cell death, reduced MAP-2 expression, and increased GFAP expression in the cerebral cortex, and the hippocampal subfields CA1 and CA3. In the cerebellum, the neurochemical alterations were associated with Purkinje cell loss and increased GFAP immunoreactivity in the white matter. However, animals subjected to either stress or chemicals alone did not show any of the above changes in comparison to vehicle treated controls. Collectively, these results suggest that prolonged exposure to a combination of stress and PB, DEET and permethrin can produce significant damage to the cerebral cortex, hippocampus and the cerebellum, even in the absence of apparent BBB damage. The above alterations could lead to many physiological, pharmacological, and behavioral abnormalities, particularly motor deficits and learning and memory dysfunction. Supported, in part by the US Army Medical and Materiel Command under contract project order DAMD 17-99-1-9020.

419 NEURONAL DEGENERATION AND NEUROBEHAVIORAL DEFICITS FOLLOWING DERMAL EXPOSURE WITH MALATHION, DEET, AND PERMETHRIN, ALONE AND IN COMBINATION IN RATS.

A. Abdel-Rahman, A. M. Dechkovskaia, L. B. Goldstein, S. L. Bullman, W. A. Khan, E. M. El-Masry and M. B. Abou-donia. *Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.*

In the present study, the neurotoxic effects of malathion, DEET and permethrin, alone or in combination, on the sensorimototor behavior, central cholinergic system and histopathological alterations were studied in male Sprague-Dawley rats following daily dermal dose of 44.4 mg/kg malathion, 40mg/kg DEET and 0.13 mg/kg permethrin for 30 days. Neurobehavioral evaluations for sensorimotor functions included beam-walk score, beam walk time, incline plane and grip time response assessments. Twenty-four hrs after the last treatment with each chemical alone or in combination all behavioral measures were impaired. The combination of DEET and permethrin, malathion and permethrin or the three chemicals together resulted in greater impairments in inclined performance than permethrin alone. Only animals treated with a combination of DEET and and malathion, or DEET and permethrin exhibited significant increases in plasma butyrylcholinesterase (BChE) activity. Treatment with DEET or permethrin alon, malathion and permethrin, or DEET and permethrin produced significant increases in cortical acetylcholinesterase (AChE) activity. Combinations of malathion and permethrin or DEET and permethrin produced significant decreases in midbrain AChE activity. Animals treated with DEET alone exhibited a significant increase in cortical m2 muscarinic ACh receptor ligand binding. Quantification of neuron density in the dentate gyrus, CA1 and CA3 subfields of the hippocampus, midbrain, brainstem, and cerebellum revealed significant reduction in the density of surviving neurons with various treatments. These results suggest that exposure to real-life doses of malathion, DEET, and permethrin, alone or in combination, produce no overt signs of neurotoxicity, but induce significant neurobehavioral deficits and neuronal degeneration in brain.

420 LOCOMOTOR PERFORMANCE DEFICITS AND DIFFERENTIAL EFFECTS ON BRAIN REGIONAL ACETYLCHOLINESTERASE ACTIVITY FOLLOWING CO-EXPOSURE TO VARIOUS DOSES OF PYRIDOSTIGMINE BROMIDE WITH DEET, AND PERMETHRIN IN RATS.

A. M. Dechkovskaia, L. B. Goldstein, A. Abdel-Rahman, S. L. Bullman, W. A. Khan and M. B. Abou-Donia. *Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.*

Male Sprague-Dawley rats were treated with pyridostigmine bromide (PB) alone or in combination with DEET (4, 40, 400 mg/kg, dermal) and permethrin (0.013, 0.13 and 1.3 mg/kg, dermal) daily for 60 days. PB (0.13, 1.3, and 13 mg/kg, oral by gavage) was given during the last 15 days. Neurobehavioral performance was assessed on day 60 following the beginning of the treatment with DEET and permethrin. Treatment with PB alone, in combination with DEET, or in combination with DEET and permethrin resulted in deficit in beam-walk score as well as longer beam-walk times as compared with controls. PB alone, in combination with DEET, in combination with permethrin or in combination with DEET and permethrin resulted in impairments in incline plane performance and forepaw grip strength. Treatment with PB alone at all the doses resulted in a slight inhibition of plasma BChE activity, whereas combination of PB with DEET or permethrin resulted in an increase in the activity. AChE activity in brainstem showed a significant increase following treatment with a combination of either DEET or permethrin at all doses. Treatment with PB alone or in combination with DEET and permethrin at all doses resulted in an increase in ligand binding for m2 muscarinic acetylcholine receptors in the cortex. Co-exposure with PB and DEET together or a combination of PB, DEET and permethrin resulted in a significant increase in the ligand binding for nicotinic acetylcholine receptors. These results suggest that exposure to various doses of PB, alone and in combination with DEET and permethrin, leads to neurobehavioral deficits and differential alterations of the cholinergic system in the CNS. Supported, in part by the US Army Medical and Materiel Command under contract project order DAMD 17-99-1-9020.

421 DISTINCT GENE REGULATION EVENTS ACCOMPANY ENHANCED VULNERABILITY IN HIPPOCAMPUS AFTER REPEATED EXPOSURES TO LOW-LEVEL SOMAN.

E. Caba, S. F. Caskurlu, D. M. Boschetto and B. A. Bahr. *Pharmacology Sciences./Center of Drug Discovery, Univerisity of Connecticut, Storrs, CT.*

The organophosphate compound soman (GD) is an irreversible anticholinesterase that produces convulsions, memory impairment, and cell loss in the brain, especially in the hippocampus. The nerve agent is also known to promote release of glutamate that can potentially lower the threshold for excitotoxic damage. Interestingly, we previously found that after repeated exposures to sub-toxic GD levels, hippocampal slice cultures from rat exhibit enhanced vulnerability to brief excitotoxic episodes of glutamate receptor over-stimulation. Here, we assessed such enhanced vulnerability with the Affymetrix neuro-specific microarray. Single daily applications of 150 nM GD were used to produce transient exposure of the slice cultures before rapid GD hydrolysis. The intentionally subtle treatment will help identify gene regulation events that may explain how asymptomatic GD contact increases vulnerability to injury. After 6 days, total RNA was isolated from 2 control and 2 treated slice groups for cDNA and biotin-labeled cRNA synthesis, which was used to inoculate the arrays. Gene induction or repression was tabulated when the change was ±50% with statistical significance confirmed by the Affymetrix software. A group of 11 genes exhibited consistent changes by GD. BDNF and heme oxygenase (HO-1) exhibited 50-60% declines in expression, which could lead to neuronal vulnerability. BDNF reduction, in fact, is one of the pathogenic changes associated with acute excitotoxicity *via* 20-min NMDA exposure, and neuroprotec-

tants that increase BDNF expression offset excitotoxic damage (Bahr et al. Exp. Neurol. 174:37, 2002). Also of interest is that distinct compensatory events occur in response to the different toxic exposures. NMDA exposure leads to increases in HSP27 and HO-1, whereas these genes are reduced in the GD-slices. Low-level GD causes a 11-fold increase in IL-15, while it is reduced in the NMDA response. Perhaps compensatory pathways identified with microarrays can provide strategies with which to offset GD-induced vulnerability.

422 SIGNALING PATHWAYS ASSOCIATED WITH GLIOSIS CAN BE STUDIED USING A BRAIN SLICE PREPARATION.

C. L. Damiani and J. P. O'Callaghan. *NIOSH, Morgantown, WV.*

Damage to the CNS is characterized by activation of microglia and astroglia, a phenomenon termed reactive gliosis. These cellular reactions have been implicated in both regeneration and degeneration of the CNS. Damage-induced production of cytokines and chemokines has been linked to the initiation of gliosis using several injury models, including exposure to known neurotoxicants. The Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway is a downstream effector of cytokines and activation of this pathway is associated with gliosis. In particular, the transcription factor STAT3 is critical for astroglial response to CNS damage. Here, we used brain slices from mice treated with the dopaminergic neurotoxicant, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) to examine astroglial STAT3 activation responses. 12 hours post MPTP (12.5 mg/kg, s.c.) or saline treatment, C57Bl/6 female mice were sacrificed by decapitation and coronal slices of brain were prepared. Striatum was dissected and assayed for activated STAT-3 (Tyr705 phosphorylation). Phospho-STAT3 was present in slices from MPTP treated mice immediately following sacrifice while absent in control mice, recapitulating *in vivo* observations. These data suggest that activated STAT3 is preserved in brain slices. Striatal slices were then exposed to phosphate free oxygenated buffer for various times. After 45 minutes both MPTP treated and control striata expressed p-STAT3, indicating that MPTP induced phosphorylation is retained *ex vivo* and that STAT3 activation can also result from slice injury. Production of cytokines upstream of STAT3 was examined by RT-PCR. Messenger RNA for CNTF and IL-6 was not altered while mRNA for MCP-1 and LIF was increased. Finally we determined that AG490, a JAK inhibitor, and lavendustin A, a tyrosine kinase inhibitor, could diminish STAT3 phosphorylation in slices. These data suggest that activation of STAT3 is an early event in both toxicant and slice-induced glial activation. In addition, this investigation establishes the brain slice preparation method as a reliable model to examine reactive gliosis.

423 HEXANEDIONE (HD)-INDUCED CHANGES IN THE POLYMERIC AND MONOMERIC STATE OF RAT SPINAL CORD CYTOSKELETAL PROTEINS.

M. L. Reid and R. M. LoPachin. *Anesthesiology, Albert Einstein College of Medicine, Bronx, NY.*

Accumulating evidence now suggests that axon atrophy in PNS and CNS is the hallmark morphological feature of gamma-diketone neuropathy (e.g., Toxicology. Appl. Pharmacology 135: 58-66, 1995; 165: 127-140, 2000; in press, 2003). Adult axon caliber is maintained by interaction of the cytoskeletal polymer with a mobile pool of exchangeable NF proteins. This interaction can be studied by triton-extraction of nervous tissue followed by differential fractionation. We used this experimental approach to determine how HD intoxication affected NF subunit content in the triton-insoluble cytoskeletal polymer and the soluble monomeric fraction. Lumbar spinal cords from moderately affected HD-intoxicated rats (175 mg/kg/d x 101 days; 400 mg/kg/d x 26 days) or their age-matched controls were homogenized in triton buffer and subjected to differential centrifugation. The low-speed (15, 000g) triton-insoluble pellet (P1 or highly polymerized cytoskeleton), and the high-speed (100, 000g) triton-soluble pellet (P2, or limited NF polymer) and corresponding supernatant (S2, or triton-soluble NF monomer/oligomer) were retained for immunoblot analyses. Results show that, regardless of HD dose-rate, cytoskeletal proteins (tubulin, NF-L, -M and –H) in the P1 fraction were not significantly altered, whereas the NF subunit contents of the P2 and S2 fractions were substantially depleted. Studies with antibodies directed against phosphorylated (RT97) and nonphosphorylated (SMI32) epitopes on NF-H and measurements of corresponding subunit isoelectric point suggest that HD-induced changes in phosphorylation were not involved. These results are consistent with previous published findings regarding NF content in nervous tissue of HD-exposed rats and suggest that atrophy is related to depletion of exchangeable monomeric subunit in the S2 fraction with subsequent impairment of NF turnover. Supported by NIEHS grant ESO7912-7.

424 SUPRAPHYSIOLOGICAL LEVELS OF THE STRESS HORMONE CORTICOSTERONE ATTENUATE BLOOD-BRAIN BARRIER DISRUPTION AND MICROGLIAL ACTIVATION IN HIPPOCAMPUS OF C57BL/6J MICE TREATED WITH KAINIC ACID.

S. A. Benkovic, J. P. O'Callaghan and D. B. Miller. *TMBB, CDC-NIOSH, Morgantown, WV.*

Kainic acid intoxication in C57BL/6J mice causes neuronal damage and the activation of glial cells. Neurotoxicants can alter blood-brain barrier integrity, and the influx of blood-borne factors may contribute to the total toxicity profile. We evaluated the consequences of kainate treatment on the blood-brain barrier and microglial activation, and the ability of high levels of corticosterone to modulate pathology. Male mice were implanted with a corticosterone pellet (192 mg/kg/d) to mimic the sustained activation of the HPA axis associated with chronic stress, and allowed to recover for seven days. Control and implanted mice were injected intraperitoneally with saline or 25 mg/kg kainic acid, and sacrificed at 1, 3, 6, and 12 hours posttreatment. Kainate-induced seizures were scored at Stage 1 (Racine scale), and corticosterone pretreatment did not alter seizure activity. Analysis of hippocampal IgG levels by Western blotting revealed a kainate-induced breach of the blood-brain barrier, and subsequent influx of plasma-derived IgG by one hour, which continued to increase and achieved significant elevation at six hours posttreatment. Corticosterone pretreatment attenuated the kainate-induced influx of IgG at all time points. Immunohistochemical localization of IgG in hippocampal parenchyma paralleled blot data. Microglial activation following kainate treatment was evaluated by silver staining and revealed activated cells at one hour posttreatment. Staining with Isolectin B4 revealed numerous microglial cells throughout the hippocampal parenchyma at 12 hours posttreatment, and an attenuation in the quantity of microglia by corticosterone pretreatment. The interactions of chronic stress and chemical intoxication, and subsequent effects on neuroanatomy and physiology are complicated, and though many literature reports describe exacerbation of neurotoxicity by stress, our data suggest steroid treatment can be protective.

425 USE OF MAGNETIC RESONANCE IMAGING (MRI) TO EXAMINE MORPHINE-INDUCED INTRATHECAL GRANULOMAS.

T. L. Yaksh[1], J. W. Allen[1], R. F. Mattrey[2], J. Corbeil[2], K. Horais[1] and N. Tozier[1]. *[1]Anesthesiology, University CA-San Diego, La Jolla, CA and [2]Radiology, University CA-San Diego, La Jolla, CA.*

Continuous intrathecal (IT) delivery of morphine sulfate (MS) for 28 days can produce aseptic subdural extramedullary inflammatory masses (granulomas) localized at the catheter tip. To develop a non-invasive method of assessing the dynamics of granuloma formation and progression we investigated the use of MRI with gadolinium (Gd) enhancement. Beagle dogs were implanted with chronic intrathecal lumbar catheters and received MS infusions *via* vest mounted pumps for 28 days at a concentration known to produce granuloma formation (12.5 mg/ml, 0.96 ml/day). Animals were assessed for behavior and motor function twice daily. Prior to initiation of morphine infusion, baseline T1 and T2 weighted MRI scans were obtained with a Siemens Symphony 1.5 Tesla system while dogs were anesthetized with propofol. At 7-day intervals dogs underwent repeat MRI scans. If pathology was noted, additional imaging studies were conducted pre and post intravenous gadoversetamide (Optimark), 0.2 mmol/kg. On the day of the final scan, images were made pre and post IT injection of 1 ml of gadopentetate diglumine (Magnavist) 1:400 in saline prior to the intravenous Gd studies. Animals underwent necropsy within 24 hours of the final scan. Animals displaying severe side-effects prior to 28-days were sacrificed to prevent undue suffering. Repeat MRI scans with propofol anesthesia were well tolerated in dogs. There were no apparent abnormalities attributable to the MRI, propofol or Gd exposure. Serial saggittal and axial MRI imaging clearly displayed progression of a mass localized in the area of the catheter tip that was confirmed at necropsy. Intravenous Gd greatly enhanced differentiation of masses from spinal parenchyma. IT Gd displayed irregular distribution indicating probable alterations in cerebral spinal fluid flow patterns and thus morphine distribution. Physical signs were well correlated with the progression and localization of IT masses. MRI with Gd enhancement is a *via*ble method for evaluation of intrathecal mass development on dogs. Supported in part by DA-15353.

426 MOLECULAR ACTIONS OF ACRYLAMIDE (ACR) AT THE NERVE TERMINAL.

R. M. LoPachin[1] and D. S. Barber[2]. *[1]Anesthesiology, Albert Einstein College of Medicine, Bronx, NY and [2]CEHT, University of Florida, Gainsville, FL.*

We have provided evidence that nerve terminals in the CNS and PNS are primary sites of ACR action and that compromise of neurotransmission might mediate neurotoxicity (Neurotoxicology 23: 43-59, 2003; Toxicology. Appl. Pharmacology

188: 135-153, 2003). To investigate molecular mechanisms, we used GC-mass spectroscopy to measure levels of the ACR-cysteine adduct, S-carboxyethylcysteine (SEC), in isolated brain synaptosomes exposed to ACR (1mM – 1 M x 60 mins incubation). Previous *in vitro* studies showed that this concentration range produced graded inhibition of synaptosomal neurotransmitter release that was correlated to a reduction in free sulfhydryl groups (Neurotoxicology, in press, 2003). Results of the present study show that *in vitro* ACR exposure produced concentration-dependent increases in SEC (range 0.10 ±0.03 – 5.6±1.2 ng CEC/μg synaptosomal protein), which suggests that ACR impaired transmission by adduction of functionally important cysteine groups on nerve terminal proteins. To identify putative targets, we exposed synaptosomes to ^{14}C-ACR (7 μCi) and then separated proteins by 2-D gel electrophoresis. Labeled proteins were detected by phosphoimaging and subsequently identified by overlay immunoblot analysis. Results show that ACR adducted numerous presynaptic proteins including N-ethylmaleimide sensitive factor (NSF) and SNAP-25, but not synaptobrevin. Inhibition of NSF activity by sulfhydryl alkylation has been shown to impair neurotransmission by preventing disassembly of the 7S SNARE complex. In our studies, *in vitro* exposure of synaptosomes to ACR (175-700 mM) significantly increased 7S complex levels as determined by immunoblot analysis (mean fold increases = 1.8 ±0.22 – 5.5 ±0.81). These results are consistent with the possibility that ACR disrupts neurotransmission by adducting sulfhydryl groups on cysteine residues of presynaptic proteins such as NSF or SNAP-25 which inhibits corresponding function. Supported by NIEHS grant ESO3830-17.

427 DEGRANULATION OF DURAL MAST CELLS BY *IN VIVO* AND *EX VIVO* OPIATE EXPOSURE.

J. W. Allen, W. Zielinska, D. Cizkova and T. L. Yaksh. *Anesthesiology, University CA-San Diego, La Jolla, CA.*

Opiates produce degranulation of some, but not all populations of mast cells (e.g. cutaneous vs mucosal). Recent work showing that continuous intrathecal delivery of high concentration morphine sulfate (MS) leads to inflammatory mass (granuloma) formation localized at the catheter tip in humans and animals led us to focus on the effects of opiates on dural mast cells. We examined dural mast cell degranulation following MS exposure *in vivo* and *ex vivo*. For *in vivo* studies, beagle dogs were implanted with chronic intrathecal lumbar catheters and received MS infusions for 28 days at a granuloma inducing concentration (12.5 mg/ml, 0.96 ml/day). At necropsy dura was harvested from lumbar and cervical regions and stained for biogenic amines with acidic Alcian blue (pH 1.4). For *ex vivo* studies dura was harvested from opiate naive dogs. Dura was stripped of pia-arachnoid and dissected into 10 mg sections. Sections were incubated in Kreb's-bicarbonate (95%:5% O2:CO2) for 30 minutes in duplicate. The ability of MS or hydromorphone to induce histamine release was examined using Compound 48/80 (0.1 mg/ml) as a positive control. Histamine release was quantified by EIA. Dura samples were then stained with acidic Alcian blue to examine degranulation. *In vivo*, intrathecal infusions of MS produced degranulation of mast cells in the lumbar, but not cervical regions in dogs receiving 12.5 mg/ml MS. *Ex vivo*, MS (0.1 mM, approximating concentrations in dog CSF following 12.5 mg/ml infusion) more than doubled histamine release over control treatment. This represented over half of the 48/80 sensitive histamine pool. Hydromorphone (0.01 and 0.1 mM) produced an increase of approximately 75% in histamine release. *Ex vivo* histochemistry displayed mast cell degranulation following MS and hydromorphone incubation. These data suggest opiates produce degranulation of dural mast cells both *in vivo* and *ex vivo*. Dural mast cells may be involved in formation of granulomas due to chronic intrathecal opiate infusions and may represent a target for therapeutic intervention to prevent granuloma formation. Supported by DA-15353

428 DYSREGULATION OF DOPAMINE HOMEOSTASIS AND OXIDATIVE STRESS IN PCB-EXPOSED NEURONAL CELLS.

D. W. Lee and L. A. Opanashuk. *Department of Environmental Medicine, University of Rochester SMD, Rochester, NY.*

PCB exposure has been associated with neurological and cognitive impairments. These adverse health effects are thought to arise from disrupted neurotransmission. Previous studies have demonstrated that PCBs can impair dopaminergic (DAergic) systems by inhibiting DA uptake into synaptic vesicles and DA reuptake into presynaptic terminals. This study tests the hypothesis that subtoxic PCB concentrations alter DA homeostasis, increase oxidative stress, and render DAergic neurons vulnerable to subsequent insult. The direct toxicity of PCB treatment was evaluated in MN9D cells, a mesencephalic-derived DAergic cell line. Cultures were exposed to 0-20ppm Aroclor 1254 for 3-48h then assayed for cell *via*bility by measuring LDH release and WST-1 response. Cell *via*bility did not differ significantly between control and treated cells except in response to 20ppm, where approximately 60% cells survived. Additional experiments analyzed whether PCB treatment modulates DA levels, which could lead to reactive oxygen species (ROS) production and subsequent activation of antioxidant defense systems. Neurochemical studies demonstrated that subtoxic PCB treatments produced a concentration-dependent reduction in intracellular DA levels, which were accompanied by an increase in DA turnover. Furthermore, PCB treatment induced a time- and concentration-dependent elevation in ROS production, as detected by increased dichlorofluorescein staining. Immunoblot analyses revealed that PCB-induced ROS production were accompanied by coincident alterations in antioxidant defense enzyme expression. While MnSOD protein levels exhibited a concentration-dependent decrease with PCB treatment, CuZnSOD protein levels remained unchanged. Additionally, the upregulation of heme oxygenase-1 levels provides supporting evidence that PCBs stimulate an oxidative stress response in dopamine neurons. Although the intracellular source of ROS remains unknown, these results suggest that PCBs activate an oxidative stress-related pathway potentially by disrupting DAergic neuron function. Supported by NIH ES00375 and ES01247.

429 OVEREXPRESSION OF BCL-XL ALTERS THE SUSCEPTIBILITY OF PRIMARY RAT ASTROCYTES TO 1, 3-DINITROBENZENE.

A. D. Phelka, M. M. Sadoff, B. P. Martin and M. A. Philbert. *Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

Exposure to 1, 3-dinitrobenzene (DNB) produces an edematous, glio-vascular lesion that is initially confined to brainstem nuclei with high energy requirements. Selective vulnerability of brainstem astrocytes to DNB is mediated by a ten-fold lower threshold for opening of the mitochondrial permeability transition pore (mtPTP). Concentrations of 100 μM DNB induce complete loss of mitochondrial membrane potential by 10 min in brainstem astrocytes. Cortical astrocytes remain unaffected by 100 μM DNB for at least 1 hour. The BCL family of proteins is known to modulate the open probability state of the mtPTP. It is hypothesized that the balance in expression of BCL-2 agonist and antagonist proteins dictates regional astrocytic susceptibility to DNB. Western blot analyses demonstrate that *in vivo* exposure to 30 or 50 mg/kg 1, 3-DNB does not significantly alter the constitutive expression of BCL-XL, BAX, or BCL-2 in either the brainstem (sensitive region) or cortex (non-sensitive region) of F344 rats or C57/Bl6 mice relative to that observed in the vehicle control (DMSO) animals. *In vivo* exposure to DNB also fails to induce subcellular translocation of BAX or BCL-XL from the cytosol to the mitochondria, where the proteins are known to interact with one another and the mtPTP. However, overexpression of BCL-XL in primary rat astrocytes significantly reduces DNB-induced loss in cell *via*bility when evaluated by an MTT assay following a 24-hour exposure up to 1 mM DNB. Therefore, while DNB exposure alone is not sufficient to alter the ratio of death promoting to death repressing members of the BCL-2 family, genetic manipulation of this ratio alters the susceptibility of sensitive astrocytes to DNB. Supported by R01 ES08846.

430 1, 3-DINITROBENZENE INHIBITS THE PYRUVATE DEHYDROGENASE COMPLEX.

J. A. Miller and M. A. Philbert. *Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

Prolonged or repeated exposure to 1, 3-Dinitrobenzene (DNB) produces topographically comparable lesions to those observed in various pathological conditions of energy deprivation including thiamine deficiency, exposure to misonidazole, metronidazole, and inborn errors of metabolism. A key enzyme complex involved in energy metabolism, pyruvate dehydrogenase complex (PDC), acts as the central gateway between glycolysis and the more efficient energy-producing pathway, TCA cycle, through production of acetyl-CoA. A disturbance in PDC activity compromises the brains ability to maintain sufficient ATP levels needed for proper cerebral function. The present study investigated the potential inhibitory effects of DNB on PDC. Purified porcine heart PDC was used to determine the effect of DNB on activity. Kinetic analysis of NADH absorbance showed a rapid dose-dependent inhibition of PDC by DNB (31.25uM-1mM). Approximately 45% inhibition of activity occurred at the lowest dose and greater then 90% inhibition was observed at the highest dose. Inhibition of activity was isomer specific. The para isomer of DNB did not inhibit PDC activity significantly at 250, or 500uM. Consistent with the kinetic analysis, Acetyl-CoA production was also inhibited in a dose-dependent manner by DNB as revealed by HPLC analysis. C6 Gliomas were used to investigate DNB toxicity as a function of energy metabolite levels. Cell morphology, LDH release and lactate production were used as indices of cell damage. Treatment of C6 gliomas with 1mM DNB resulted in cytoplasmic swelling, extensive vacuolation, increased LDH release and nearly a 45% increase in lactate production, relative to controls. Co-treatment with 5mM acetoacetate, which is converted to acetyl-CoA, diminished lactate production by approx. 20% and resulted in complete inhibition of LDH release compared to DNB alone. Morphologically, no

swelling or vacuolation was observed in acetoacetate protected cells. The results suggest a potential role for PDC inhibition in the mechanism of DNB induced energy deprivation. Supported by RO1-ES088446.

431 N, N-DIETHYLDITHIOCARBAMATE PRODUCES COPPER ACCUMULATION, LIPID PEROXIDATION AND DEMYELINATION IN PERIPHERAL NERVE.

W. Valentine[1], E. G. Tonkin[1], H. L. Valentine[1], D. M. Milatovic[2], K. Amarnath[1] and V. Amarnath[1]. [1]*Pathology and Center in Molecular Toxicology, Vanderbilt University Medical Center, Nashville, TN and* [2]*Pathology, University of Washington, Seattle, WA.*

Human exposure to dithiocarbamates occurs from their many uses in agriculture, industry and medicine. Previous studies have demonstrated the ability of the dithiocarbamate disulfiram to produce a peripheral neuropathy in humans and experimental animals and have provided evidence that N, N-diethyldithiocarbamate (DEDC) is a proximate toxic species of disulfiram. The ability of DEDC to elevate copper levels in the brain suggests that it may also elevate levels of copper in peripheral nerve possibly leading to oxidative stress and lipid peroxidation from redox cycling of copper. The study presented here investigates the potential of DEDC to promote copper accumulation and lipid peroxidation in peripheral nerve. Rats were administered either DEDC or buffered saline by ip osmotic pumps and fed a normal diet or diet containing elevated copper and the levels of metals, F2 isoprostanes and severity of lesions in peripheral nerve and brain assessed by ICP/MS, GC/MS and light microscopy. Copper was the only metal that demonstrated significant elevations relative to controls; and elevations occurred in both brain and peripheral nerve in animals administered DEDC on both diets. In contrast, demyelination and elevated F2 isoprostanes were significantly increased only in the peripheral nerve samples of rats administered DEDC on both diets. Elevated levels of serum homocysteine were also observed in the DEDC animals on both diets. Autometallography staining of peripheral nerve was consistent with increased metal content along the myelin sheath but in brain, focal densities were observed. These data suggest that DEDC chelates copper to form a lipid soluble complex that then accumulates in peripheral myelin and undergoes redox cycling leading to lipid peroxidation and segmental demyelination. Supported by NIEHS grant ES06387.

432 ANATOMICAL AND PHYSIOLOGICAL ASPECTS AND ROUTINE AND SPECIFIC EXAMINATIONS OF MINIPIG EYE AS EXPERIMENTAL MODEL IN NON-CLINICAL SAFETY STUDIES. O. LOGET, F. HOFFMANN-LA ROCHE LTD, BASLE, SWITZERLAND.

O. M. Loget. *PRBN-S, F.Hoffmann-La Roche, Basel, Switzerland.* Sponsor: M. Stephan-Gueldner.

Non-clinical toxicology studies for a new drug are required before EIH (Entry Into Hunan). One of the most important points to consider is the choice of the non-rodent species which should have a metabolic assimilation of the drug under investigation as close as possible to that of Human. For several substances the minipig, which is omnivorous like man and is equipped with similar enzyme systems meets this need. Several minipig organs have also similarities with those of Human. This species is therefore being used more and more as experimental model in non-clinical safety studies in order to replace or to complement the dog or monkey. Notably aspects of the minipig eye, show a number of anatomical and physiological similarities with the human eye. The minipig is therefore considered to be a good experimental model for the assessment of adverse ocular effects. We describe the normal minipig ocular pattern, as well as some ocular findings, in order to define the main variants of the minipig eye. The results of some routine and specific ocular examinations performed in 372 minipigs are also presented. These data are helpful to differentiate between treatment-related and spontaneous findings which may be observed in non-clinical safety studies.

433 COMPARISON OF INTERACTIVE CYTOTOXIC EFFECTS OF SELECTED MYCOTOXINS ON RENAL CELLS.

A. H. Heussner, E. O'Brien, J. Haehnlein, M. A. Biester and D. R. Dietrich. *Environmental Toxicology, University of Konstanz, Konstanz, Germany.*

Of the about 300 mycotoxins identified to date, approximately 30 are found regularly in foodstuffs and animal feed, often in combination. Particularly ochratoxins and citrinin have already been shown to be responsible for porcine nephropathies and are suspected to play a role in the genesis of human urinary tract tumors and BEN (Balkan Endemic Nephropathy). Various animal studies have indicated interactive renal responses to certain mycotoxins. To date *in vitro* data is controversial. In the present study, the mycotoxins citrinin, patulin, ochratoxin A (OTA) and ochratoxin B (OTB) were investigated. Porcine (LLC-PK1) and human (IHKE) renal cell lines were used as models and the MTT reduction assay after 48 hours of exposure served as cytotoxicity endpoint. After various range-finding assays, concentration-response curves were generated for each individual toxin. Based on these data, binary mixtures were then tested. Comparison of predicted (additive) and tested mixture effects resulted in higher toxicity of citrinin and patulin in combination with OTA or OTB in LLC-PK1 cells, whereas IHKE cells showed higher than predicted effects only with combinations of citrinin with ochratoxins, predominantly with OTB. Hence, our results support the hypothesis of a superadditive or synergistic mode of action of these mycotoxins in renal cells.

434 DETECTION OF ORGANIC ANION TRANSPORTERS (OAT) IN HUMAN KIDNEY HOMOGENATE AND PRIMARY HUMAN KIDNEY CELLS (HKC).

R. Blum, E. O'Brien, A. H. Heussner and D. R. Dietrich. *Environmental Toxicology, University of Konstanz, Konstanz, Germany.*

Ochratoxin A (OTA), a secondary metabolite of several mould species, is associated with kidney carcinomas in male mice, in rats of both sexes and with nephropathies in pigs, as well as with the human disease, Balkan endemic nephropathy (BEN). The mechanism underlying OTA's toxicity is still unknown. One plausible explanation for the renal specificity of OTA could be accumulation in proximal tubular cells during the course of excretion (transport and/or reabsorption) thereby reaching toxic concentrations within the cells. This study aimed to detect organic anion transporter (OAT1, OAT2, OAT3) proteins in rat renal cortex homogenate, human renal biopsy samples, and primary human kidney cells (HKC). All three transporter isoforms could be detected in human renal cortical tissue *via* RT-PCR. No signal was detected in HKC (P1, P2). Anti-rat Oat1 antibodies detected both rat (Oat) and human (OAT) in Western blots of cortex homogenates. Anti-Oat2 showed no detection in either rat or human homogenates, while anti-Oat3 showed strong detection in human homogenate. A weaker signal was obtained using the specific anti-human antibody, anti-OAT3. The results show the presence of organic anion transporter proteins in human kidney. Expression of these transporters is, however, lost during passaging of HKC.

435 USE OF AN *IN VITRO* HUMAN RENAL CORTICAL CELL MODEL FOR TOXICITY AND TRANSPORT STUDIES.

N. S. Jensen, R. Challmes, L. A. Hyde and P. M. Silber. *In Vitro Technologies, Baltimore, MD.*

Different *in vitro* models for assessing renal toxicity have been developed and used with variable success. However, renal toxicity can be due to a variety of mechanisms including the ability of the cell to transport and concentrate xenobiotics. We describe the evaluation of cryopreserved human renal cortical cells (HRCC) as a model to determine both transport and cytotoxicity. For toxicity studies three lots of HRCC were thawed and plated on collagen coated 96-well plates and grown to ∽80% confluence. Cells were dosed for 48 hours with 0-50 µM cisplatin or transplatin in serum-free medium. MTT was used as an endpoint for toxicity. Organic anion and cation uptake assays were performed on HRCC monolayers that had been 100% confluent for 3 days. [^{3}H]p-aminohippuric acid (PAH, 5 µM) was the substrate for organic anion transporter (OAT), while [^{3}H]MPP$^+$ (0.1 µM) and [^{14}C]Tetraethylammonium bromide (TEA, 5 µM) were used for organic cation transporter (OCT) assays. Uptake assay incubations were performed by adding substrates to the HRCC monolayers and incubating for 15 minutes in a humidified 37°C, 5% CO_2 incubator. Following incubations, monolayers were washed with buffer, lysed, and radioactivity of samples was measured by liquid scintillation. HRCC toxicity results indicated that incubation with cisplatin, a compound known to have renal toxicity, resulted in a dose dependent decline in *via*bility of >40%, while transplatin showed no loss in *via*bility at up to 40 µM. However, at 50 µM cisplatin or transplatin, all cells demonstrated 40-60% loss in *via*bility. All substrates were successfully transported by HRCC. In addition, inhibitors were added to the different uptake assays to demonstrate that transport could be blocked. Bromosulfophthalein (1 mM) inhibited uptake of PAH by 63.4±21.1%, pseudocyanine iodide (0.1 mM) inhibited MPP$^+$ uptake by 77.9±3.86%, and procainamide (1 mM) inhibited TEA uptake by 72.5±6.10%. These results indicate that HRCC can serve as a useful model for evaluating various activities that can be associated with renal cytotoxicity.

436 TRANSPORT OF MERCURIC CONJUGATES OF HOMOCYSTEINE BY THE AMINO ACID TRANSPORTER, SYSTEM $B^{0,+}$.

C. C. Bridges and R. K. Zalups. *Basic Medical Sciences, Mercer University School of Medicine, Macon, GA.*

The kidneys, specifically the epithelial cells lining the proximal tubule, are the primary targets where mercuric ions accumulate and exert their toxic effects. Our laboratory has shown recently that mercuric-conjugates of cysteine (Cys), primarily 2-Amino-3-(2-amino-2-carboxy-ethylsulfanylmercuricsulfanyl)-propionic acid

(Cys-S-Hg-S-Cys), are substrates for the luminal, Na+-independent amino acid transporter, system $b^{0,+}$. Homocysteine (hCys), which binds readily to inorganic mercury, is a homolog of Cys that is also present in blood at low micromolar concentrations. Because of structural similarities between Cys and hCys, we hypothesized that mercuric conjugates of hCys, primarily as 2-Amino-4-(3-amino-3-carboxy-propylsulfanylmercuricsulfanyl)-butyric acid (hCys-S-Hg-S-hCys), are taken up by the same system that mediates the uptake of Cys-S-Hg-S-Cys, i.e. system $b^{0,+}$. To test this hypothesis, we analyzed the saturation kinetics, time-dependence, temperature-dependence, and substrate-specificity of the transport of hCys-S-Hg-S-hCys in Madin Darby Canine Kidney (MDCK) II cells stably transfected with system $b^{0,+}$. These data show that hCys-S-Hg-S-hCys is transported across the luminal plasma membrane of renal epithelial cells by the amino acid transporter, system $b^{0,+}$. Furthermore, our data indicate that this transporter likely plays a role in the nephropathy induced following exposure to inorganic forms of mercury. Our data are the first to implicate a specific membrane transporter (present in the luminal plasma membrane of proximal tubular cells) in the transport and toxicity of mercuric conjugates of homocysteine.

437 CADMIUM DECREASES MNRA OF MOUSE KIDNEY SODIUM-GLUCOSE COTRANSPORTER 1 (SGLT1) *IN VITRO*.

N. Tabatabai[1], S. S. Blumenthal[2], K. Dolan[2] and D. H. Petering[1]. *[1]Chemistry, University of Wisconsin-Milwuakee, Milwaukee, WI and [2]Nephrology, VA Medical Center, Milwaukee, WI.* Sponsor: J. Eells.

Chronic exposure to cadmium results in glucose malabsorption by kidney. We had shown that exposure of primary cultures of mouse kidney cortical cells to micromolar concentrations of cadmium results in a decrease in uptake of the glucose analogue, a-methylglucopyranoside (AMG). Semiquantitative analysis of sodium-glucose cotransporter 1(SGLT1) mRNA demonstrated that cadmium decreased levels of SGLT1, one of four sodium-glucose cotransporters in the mouse kidney. To investigate the mechanism of cadmium-induced repression of SGLT1, quantitative PCR was performed. Kidneys of mice were removed, cortical cells were cultured, and 5, 7.5, and 10 microM cadmium was added to nearly confluent cultures. Cells grown in media alone were used as controls. After 24 hours, uptake of AMG (0.1 mM) was measured and total RNA was prepared. For quantification of SGLT1 mRNA, RT-PCR was performed with cellular RNA and appropriate dilutions of SGLT1 mimic RNA. Intensities of PCR products were measured and number of SGLT1 mRNA molecules was calculated. Also, to determine the effect of cadmium on stability of SGLT1 mRNA, control and cells exposed to 7.5 microM cadmium were treated with transcription inhibitor Actinomycin D (10 microg/ml) and degradation of SGLT1 mRNA was monitored by RT-PCR. Results show that cells exposed to 5, 7.5, and 10 microM cadmium expressed 57%, 46%, and 28% of control cell SGLT1 mRNA molecules, respectively. We also found that cadmium had no effect on stability of SGLT1 mRNA. In addition, AMG uptake by cadmium treated cells were measured to be 57%, 36%, and 25% of untreated cells. AMG uptake was highly correlated with corresponding number of molecules of SGLT1 mRNA ($P< 0.001$, $R2 = 0.713$). These findings suggest that the observed decline in the uptake of AMG by cultured kidney cells in response to cadmium is due to a decrease in the mRNA molecules of SGLT1. Since cadmium did not affect the stability of SGLT1 mRNA, this metal can either directly or indirectly modulate the transcription of SGLT1.

438 CADMIUM DECREASES GLUCOSE UPTAKE IN PORCINE KIDNEY CELLS POSSIBLY BY ALTERING SODIUM-DEPENDENT GLUCOSE TRANSPORTER EXPRESSION.

J. R. Pennell and A. R. Villalobos. *University of Rochester, Rochester, NY.*

In humans, chronic exposure to cadmium (Cd) causes several renal abnormalities, such as proteinuria and glycosuria. Animal studies suggest that Cd-induced glycosuria involves a defect in glucose reabsorption by the renal proximal tubule (RPT) and moreover, that the attenuation of glucose reabsorption may be due to decreased levels of sodium-dependent glucose transporters (SGLTs). The goal of this study is to elucidate the mechanism by which Cd decreases SGLT levels, using LLC-PK1 cells, a model for porcine RPT. Cells were treated with 3.5 μM CdCl2 (no FBS) for 24 hrs and various endpoints were examined. Carrier-mediated uptake of α-methyl glucoside (AMG), a non-metabolizable analogue of glucose, was assayed by radiotracer technique. Total SGLT protein was measured by western blot analysis. mRNA levels of two SGLT isoforms, SGLT1 and pig SGLT3, were determined by RT-PCR. Cytotoxicity was evaluated by trypan blue exclusion and LDH release, and cell *via*bility was based on WST-1 conversion. Cd did not induce cytotoxicity or adversely affect cell *via*bility. Kinetics analysis of AMG uptake showed that Cd decreased V_{max} by 59% (4.2 vs. 1.7 nmoles/mg^{-1}min^{-1}; n=2); immunoblot analysis indicated a 50% decrease in total SGLT protein. These observations suggest a possible loss of active SGLTs in the apical membrane. Interestingly, K_m decreased by 51% (769 vs. 381 μM; n=2). Pig SGLT3 mRNA levels decreased by 50%, which is consistent with the observed decrease in V_{max} and SGLT protein (n=2). However, SGLT1 mRNA levels increased by 40% (n=2). Since SGLT1 has a relatively high affinity for AMG, the decrease in K_m may be a subsequent result of the observed increase in SGLT1 mRNA. Collectively, these data suggest that Cd may decrease glucose reabsorption by reducing SGLT expression. Still, the exact molecular mechanism(s) by which Cd modulates SGLT mRNA levels is uncertain. Further investigation will determine whether Cd alters message levels *via* dysregulation of PKA and PKC signaling. NIEHS-T32-ES07026; NIEHS-P30-ES01247; NIEHS-ES10439; NINDS-NS39452.

439 A HUMAN RENAL EPITHELIAL CULTURE MODEL FOR *IN VITRO* NEPHROTOXICITY STUDIES.

M. J. Powers and S. Damian. *Cambrex BioScience, Walkersville, MD.* Sponsor: S. Tannenbaum.

We have developed a cell culture model to re-create human renal tubular function in an *in vitro* environment. Primary epithelial cells were isolated from human renal cortices and seeded on extracellular-matrix-coated Transwell® membranes, which allow for bi-compartmental (apical and basal) exposure to culture medium. Isolation and culture parameters (*e.g.*, culture medium, matrix coating protocols, cell seeding density) have been optimized for the development of a functional renal tubular epithelial monolayer as assessed through tubule-specific markers. The apical brush border enzyme γ-glutamyl transpeptidase (γ-GTP), specific to proximal tubule epithelium, is consistently and stably expressed in these cultures through 14 days after seeding. Apical concentrations of γ-GTP are typically between 10 and 20 times higher than basal concentrations ($p<0.01$, Student's t-test), demonstrating the presence both of physiologically accurate polarity and of epithelial barrier properties in the culture model. Consistent ammoniagenesis, critical in renal acid-base homeostasis, is confirmed through at least 14 days in culture. Uptake of rhodamine-123 from the basal chamber is greater than twofold higher than uptake from the apical chamber ($p<0.05$, Student's t-test), illustrating that an active directional organic ion transport mechanism is present. This culture model therefore provides a polarized, functional renal tubular epithelium in an *in vitro* environment. In conjunction with specific functional assays such as those described here, the system provides a physiologically relevant platform technology through which the human kidney's response to nephrotoxins and other xenobiotics can be probed for all phases of renal ADME/Toxicology.

440 ESTROGEN THROUGH ESTROGEN-RECEPTOR INDEPENDENT PATHWAY INDUCES PROLIFERATION OF HUMAN KIDNEY EPITHELIAL CELLS.

K. P. Singh and D. Roy. *Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL.*

Kidney is a target organ of estrogen-induced cancer in animal model. However, the role of estrogen in human renal cell proliferation and carcinogenesis is not clear. In this study, we have examined the influence of 17-b estradiol and diethylstilbestrol (DES) on human embryonic kidney cells, HEK-293, and adult kidney cells, HK-2 of epithelial origin. Cells were treated with six different concentrations (1pg-100 ng/ml) of DES or 17 b-estradiol, and cell growth was measured at 24, 48, and 72 h periods. Both, E2 and DES treatment resulted in significant ($p<0.05$) stimulation of cell proliferation of embryonic kidney epithelial cells. Co-treatment of HEK-293 cells with E2 and Tamoxifen, an estrogen antagonist, did not inhibit the E2 induced cell growth. Analysis of expression of ERa and ERb by RT-PCR in untreated control as well as in E2-treated HEK-293 cells revealed that the transcripts of these two estrogen-receptors were not detectable. Transcripts of S phase- specific cell proliferation marker Ki-67 and G1-S phase -specific cyclinD1 were increased in E2 treated HEK-293 cells as compared to the untreated control. Based on these data, we conclude that (1) estrogens are mitogenic in human kidney epithelial cells, (2) E2 is more mitogenic in embryonic kidney cells than in adult kidney cells, and (3) estrogen-induced growth in human kidney epithelial cells are mediated by estrogen receptor- independent pathway.

441 MODULATION OF RENAL GLOMERULAR MESANGIAL AND PODOCYTE CELL NUMBERS CORRELATES WITH FIBRONECTIN ACCUMULATION IN BENZO(A)PYRENE-TREATED SPRAGUE-DAWLEY RATS.

A. Nanez[1, 2] and K. S. Ramos[1, 2]. *[1]Center for Genetics and Molecular Medicne, University of Louisville, Louisville, KY and [2]Biochemistry and Molecular Biology, University of Louisville, Louisville, KY.*

This laboratory has shown that repeated, weekly exposure of adult Sprague-Dawley rats to benzo(a)pyrene (BaP) (10 mg/kg i.p.) for up to 16 weeks results in loss of renal function, as evidenced by increases in total urinary protein, protein/creatinine

ratios, urinary albumin, structural deficits associated with activation of glomerular mesangial cells, disruption of podocyte foot processes and glomerular hypercellularity. At the cellular level, BaP selectively injures mesangial cells to allow expansion of the podocyte population. The present studies were conducted to determine if changes in glomerular cell numbers *in vivo* correlate with alterations in extracellular matrix fibronectin deposition. Immunohistochemical markers of cellular identity examined included Wilms' tumor suppressor protein (Wt1) for podocytes, Thy1 for mesangial cells, CD68 for macrophages, CD3 for T cells, and Factor VIII related antigen for endothelial cells. Thy1 expression and fibronectin accumulation were reduced by approximately 20% after 8 weeks BaP treatment. These reductions were transient, as significant increases in both Thy1 and fibronectin immunoreactivity were observed by 16 weeks. Changes in mesangial cell numbers correlated inversely with podocyte numbers at both 8 and 16 weeks, suggesting that BaP influences mechanisms involved in reciprocal control of glomerular cell densities. In contrast, CD68, CD3, and Factor VIII related antigen expression was not altered by BaP exposure. These data suggest that the glomerular hypercellularity induced by BaP involves non-inflammatory mechanisms that are regulated by cell:cell and cell:matrix interactions within the adult rat kidney. (Supported by NIH grants ES04917, ES09106, and CA90301).

442 PYRUVATE ATTENUATION OF P-AMINOPHENOL (PAP) TOXICITY IN RENAL SLICES FROM FISCHER 344 (F344) RATS.

M. Valentovic and R. Harmon. *Pharmacology, Marshall University School of Medicine, Huntington, WV.*

The acetaminophen metabolite, PAP is a known nephrotoxicant. The purpose of this study was to investigate the effect of pyruvate on PAP *in vitro* nephrotoxicity. Renal cortical slices were isolated from F344 rats and washed at 25 degrees Celsis in Krebs buffer. Slices were then incubated with 0 to 0.5 mM PAP for up to 120 min and constant oxygen. Renal slices exposed to 0.1 mM PAP showed toxicity as measured by increased lactate dehydrogenase (LDH) leakage and decreased pyruvate-stimulated gluconeogenesis. PAP decreased ($p<0.05$) both total glutathione (GSH) and glutathione disulfide (GSSG) within 30 min when compared to vehicle controls. Co-incubation with pyruvate (PYR) protected against PAP-induced time and concentration dependent effects on LDH leakage. PYR also reduced PAP effects on GSH and GSSG. Adenine nucleotides were reduced relative to control values within a 30 min exposure to 0.25 and 0.50 mM PAP. Co-incubation with PYR also completely protected renal slices from PAP induced changes in ATP and ADP. PYR also reduced the effects of PAP on 4-hydroxynoneal adduct formation. PYR pretreatment protected renal slices from PAP associated depletion of NADPH. Pretreatment with another energy substrate (5.55 mM glucose) did not provide protection similar to PYR. These data show that PYR protects against PAP *in vitro* toxicity in renal slices and that PYR protection is mediated by preventing PAP induced oxidative stress.

443 PENTAMIDINE-INDUCED INJURY IN LLC-PK1 CELLS IS NOT MEDIATED BY NMDA RECEPTOR ANTAGONISM.

A. L. Piskac and M. A. Smith. *Environmental Sciences / Toxicology, University of Texas School of Public Health, Houston, TX.*

The aromatic diamidine, pentamidine, is an antimicrobial drug used in the treatment of Pneumocystis carinii pneumonia (PCP) in HIV patients, leishmaniasis, and African trypanosomiasis. The dose-limiting nephrotoxicity restricts the clinical utility of pentamidine. Pentamidine-induced renal injury is targeted to the proximal tubule. Pentamidine is a known antagonist of the N-methyl-D-aspartate (NMDA) receptor, a subgroup of glutamate receptors. NMDA receptor subunits are predominantly expressed in the renal proximal tubule in comparison to the distal tubule. To determine whether the NMDA receptor is involved in pentamidine-induced *in vitro* cytotoxicity, LLC-PK1 cells (a proximal tubule cell line) were treated with pentamidine at concentrations of 1mM, .5 mM, and .1 mM, as well as 200 uM of D-AP-5, an NMDA receptor competitive antagonist, and 10 uM of MK-801 (dizocilpine), an NMDA channel blocker, or non-competitive antagonist, over a 24-hour period. Cytotoxicity was evaluated morphologically and by LDH leakage. For all time points, control, D-AP-5, and MK-801-treated cells maintained normal morphology, with no evidence of cellular damage or toxicity. Pentamidine induced time and dose-dependent morphological changes associated with apoptosis. The earliest apoptotic changes were observed at the highest concentration at 2 hours. Initial morphologic changes for cells treated with the intermediate concentration of pentamidine were seen at 6 hours. Early apoptotic changes for the lowest concentration of pentamidine were noted at 16 hours after treatment. LDH leakage following treatment with D-AP-5 and MK-801 were comparable to control for all time points. Pentamidine induced a time- and concentration-dependent increase in LDH leakage. The LDH leakage appears to be attributed to secondary necrotic changes following apoptosis. Pentamidine toxicity does not appear to be mediated through the NMDA receptor, as D-AP-5 and MK-801 did not induce morphological changes and toxicity in LLC-PK1 cells.

444 ASSESSMENT OF THE MODULATION OF RENAL ANTIOXIDANT DEFENSE MECHANISMS AND CYCLOOXYGENASES BY COMMON RODENT DIETS IN MALE SPRAGUE-DAWLEY RATS.

S. Cooper, B. Blaydes, F. Xin and B. Delclos. *NCTR, Jefferson, AR.*

Both specific components of the diet and total calorie intake are known to affect responses to toxins. In recent years, considerable attention has been directed to the possible effect of soy-containing diets with variable phytoestrogen levels on the response to estrogenic agents, but often the possible effect of the base diet used in toxicity studies on the measured response is given little consideration. We had previously found that the renal toxicity of nonylphenol appeared to be enhanced when fed to rats in the soy- and alfalfa-free 5K96 diet (Latendresse *et al.*, Toxicol. Sciences. 62: 140, 2001) and that the base diet affected the observed fluctuation of renal cyclooxygenase-2 during the estrous cycle of female rats (Blaydes *et al.*, Toxicologist 72: 348, 2003). As a prelude to our investigation of the modulation of renal toxicity by diet, we examined the effect of four common rodent diets that differ to varying degrees in several components, including soy content, protein composition, and fatty acid and cholesterol levels, on systems potentially involved in renal toxicity. Specifically antioxidant defenses and cyclooxygenases were examined. At weaning on PND21, male rats (five per group) were assigned to one of four diets: NIH-31, Purina 5K96, Purina 5001, or Purina 5002. Food consumption and body weight gain were monitored throughout the study and were found not to differ among the diet groups. On PND51, animals were sacrificed and kidneys removed. Expression of cyclooxygenases-1 and -2 and hemoxygenase-1 was evaluated in cortex and medulla by realtime PCR. Total and oxidized glutathione were determined by colorimetric assays and the activities of superoxide dismutase, catalase, and glutathione peroxidase were measured. Except for marginal differences in superoxide dismutase and oxidized glutathione, no diet-related differences were observed in the endpoints evaluated. While baseline levels of these enzymes did not differ among these diet groups, whether diet modulates the response to toxin exposure remains to be investigated.

445 DIFFERENTIAL EXPRESSION OF E-CADHERIN AND N-CADHERIN IN PROXIMAL AND DISTAL SEGMENTS OF THE RAT NEPHRON.

W. C. Prozialeck[1], P. C. Lamar[1] and D. M. Appelt[2]. *[1]Pharmacology, Midwestern University, Downers Grove, IL and [2]Biomedical Sciences, Philadelphia College of Osteopathic Medine, Philadelphia, PA.*

E- and N-cadherin are calcium-dependent cell adhesion molecules that play important roles in the development and maintenance of renal epithelial polarity. Recent studies have shown that alterations in cadherin function may be associated with various types of nephrotoxic injury. In the present study, we have employed dual immunofluorescent labeling procedures that utilized specific antibodies against either E- or N-cadherin, along with antibodies that target markers for specific nephron segments, to characterize the patterns of cadherin expression in frozen sections of adult rat kidney. The results showed that N-cadherin was the predominant cadherin in the proximal tubule, but was essentially absent in other nephron segments. By contrast, E-cadherin was abundant in the distal tubule, collecting duct and some medullary segments, but was present only at very low levels in the proximal tubule. Additional results revealed different patterns of N-cadherin labeling along various segments of the proximal tubule; the S1 and S2 segments exhibited a fine threadlike pattern of labeling at the apical cell surface, whereas the S3 segment showed intense labeling at the lateral cell-cell contacts. These findings raise the possibility that differences in cadherin expression may contribute to the differences in the sensitivity of various nephron segments to toxic injury. Supported by Grant #R01 ES006478 from the NIEHS.

446 FORMIC ACID EXCRETION IN RATS EXPOSED TO BROMODICHLORO-METHANE: POSSIBLE LINK TO RENAL TUBULE CELL PROLIFERATION IN LONG-TERM STUDIES.

E. A. Lock, L. Cottrell, M. Jacobsen, T. Soames and R. Williams. *Central Toxicology Laboratory, Syngenta, Macclesfield, United Kingdom.*

Bromodichloromethane (BDCM) is a renal carcinogen in male and female F344 rats. The basis for the renal toxicity and carcinogenicity of BCDM is unclear. Several mechanisms have been proposed, oxidation to form phosgene, reduction to

generate a dichloromethyl radical and metabolic activation *via* glutathione S-transferase theta to form a mutagenic intermediate. This study aimed to provide indirect evidence for a radical mechanism for BDCM-induced renal toxicity by looking for excessive formic acid excretion in urine. Male F344 rats were given BDCM po at 50 or 100mg/kg/day for 5 days/week for 28 days. Controls received corn oil with 5 rats/group. Urine was collected over 24h on days 4-5, 11-12 and 17-18 for 1H-NMR profiling and pH determination. Osmotic minipumps containing 3H-BrDU were implanted on day 24 and animals killed on day 28. Blood was taken for creatinine and urea and kidneys for histopathology and determination of S-phase. Rats exposed to BDCM excreted large amounts of urinary formic acid, accompanied by a change in urinary pH. Formate excretion was dose dependent being elevated >100-fold after 4 doses and remained elevated over the 3 weeks at 100mg/kg. At 50mg/kg formate excretion was elevated 40-fold after 4 doses and declined to 5-fold after 3 weeks. Histopathology revealed mild renal tubule injury in 2 rats at 100mg/kg BDCM. Blood urea and creatinine values were normal. Cell proliferation in the renal cortex was markedly elevated at 100 but not 50mg/kg BDCM. Long-term exposure to formic acid is known to cause renal tubule injury. Our study suggests, for the first time, that prolonged excretion of formic acid following BDCM may be a contributory factor to the increased cell proliferation and kidney injury seen in longer term studies. Studies with trichloroethylene, which also leads to excess formic acid in urine, suggest this may occur *via* a free radical mechanism inducing a B12 deficiency and hence a folate deficiency [Dow & Green (2000) Toxicology) 146, 123].

447 RENAL GLUTATHIONE PEROXIDASE AND GLUTATHIONE REDUCTASE ACTIVITY ARE ALTERED BY 3, 4-DICHLOROANILINE (3, 4-DCA) AND 2-AMINO-4, 5-DICHLOROPHENOL (2A45CP).

G. O. Rankin, M. A. Valentovic, N. Noureddine and B. Dunlap. *Pharmacology, Marshall University, Huntington, WV.*

Toxic insult to the kidney can result from the generation of free radicals that are deleterious to cell function. The two enzymes glutathione peroxidase and glutathione reductase are involved in cellular detoxification processes. The following studies examined the effect of 3, 4-DCA, an intermediate in the production of propanil (a pre-emergent herbicide extensively used in the United States), and its metabolite, 2A45CP on glutathione reductase and peroxidase activity in the kidney. Renal cortical slices prepared from male Fischer 344 rats were rinsed 3 times each for 3 min in oxygenated Krebs buffer. Renal slices (50-100 mg) were equilibrated in 3 ml oxygenated Krebs at 37oC. Tissues were next incubated for 30-60 min with vehicle (DMSO) or a final concentration of 2 mM 3, 4-DCA or 0.5 mM 2A45CP. DMSO, 3, 4-DCA and 2A45CP were added in a total volume of 30 µl. Upon completion of the incubation period, glutathione peroxidase or glutathione reductase activity was measured in renal cortical slices. A 60 min exposure to 2 mM 3, 4-DCA diminished glutathione reductase activity to 33% of control values (p=0.012) and diminished glutathione peroxidase activity to 50% of vehicle control (p=0.002). Glutathione reductase and glutathione peroxidase were decreased to 54% (p=0.022) and 56% (p=0.005), respectively, of vehicle control values following a 30 min exposure to 0.5 mM 2A45CP. These results indicate that based on concentration, 2A45CP is a more potent nephrotoxicant than 3, 4-DCA. Furthermore, 3, 4-DCA and its metabolite 2A45CP decrease glutathione reductase and glutathione peroxidase activity in renal cortical slices, which may contribute to the mechanism of their toxicity.

448 A NOVEL MITOCHONDRIAL MATRIX CALPAIN IN MITOCHONDRIAL INJURY.

D. D. Arrington, T. R. Van Vleet and R. G. Schnellmann. *Pharmaceutical Sciences, Medical University of South Carolina, Charleston, SC.*

Oncotic cell death is mediated by mitochondrial dysfunction and calpains, Ca^{2+}-activated cysteine proteases. Mitochondrial dysfunction is the end result of a variety of cellular insults, with ischemia/reperfusion and toxicant exposure being the most clinically relevant. Previously, we reported that calpain inhibitors protected renal proximal tubular mitochondrial function during hypoxia/reoxygenation and isolated renal cortical mitochondria (RCM) exposed to Ca^{2+}. A mitochondrial calpain-like activity was associated with Ca^{2+}-exposed RCM. We hypothesized that this calpain-like activity is due to a novel mitochondrial calpain. Rabbit RCM were isolated by differential centrifugation, further purified by sucrose/percoll gradient centrifugation, and sub-fractionated into matrix, inter-membrane space, and inner and outer membrane fractions. All fractions were analyzed for proteolytic activity by FITC-casein and SLLVY-AMC zymography. The addition of Ca^{2+} did not induce cleavage of the FITC-casein substrate in any of the RCM fractions. In contrast, Ca^{2+}-dependent SLLVY-AMC cleavage was observed in the matrix fraction, one prominent and two minor bands, but not in the other RCM fractions. Purified µ- and m-calpain were used as controls, migrated differentially during zymography, and cleaved both substrates. The bands of calpain activity in the matrix fraction did not co-migrate with µ- or m-calpain. In-gel cleavage of SLLVY-AMC by the matrix fraction calpain was inhibited by the calpain inhibitors E64 (100 µM) and calpeptin (100 µM) by 70 and 80%, respectively. Additionally, the calpain inhibitors E64 and calpeptin inhibited calpain activity using matrix suspensions with an IC_{50} of 26 and 2 nM, respectively. In summary, we identified a novel mitochondrial calpain activity in the matrix fraction of RCM that is different than cytosolic µ- and m-calpain, and is potently inhibited by calpain inhibitors. This mitochondrial matrix calpain appears to play an important role in mediating mitochondrial dysfunction following ischemia/reperfusion or toxicant injury.

449 EFFECT OF CALCIUM OXALATE ON KIDNEY MITOCHONDRIAL FUNCTION.

K. McMartin[1], C. Guo[1] and K. Wallace[2]. *[1]Department of Pharmacology, LSU Health Sciences Center-Shreveport, Shreveport, LA and [2]Biochemisty and Molecular Biology, University of Minnesota-Duluth, Duluth, MN.*

Ethylene glycol (EG) poisoning can produce acute renal failure, requiring long-term hemodialysis to restore function. The mechanism of renal failure is unknown, but associated with tubular cell necrosis and EG metabolism. The end metabolite of EG is oxalic acid, the precipitation of which as calcium oxalate monohydrate (COM) crystals in the tubular lumen has been linked with the renal toxicity. Our recent studies suggest that COM is an intracellular toxicant to normal human proximal tubule cells in culture. The present studies were designed to assess whether COM or ionic oxalate alters mitochondrial function so as to lead to renal cell death. Rat kidney mitochondria were prepared by homogenization of cortical tissue, then by differential centrifugation techniques. Substrate-induced respiration was measured in isolated mitochondria using a sealed reaction chamber fitted with Clark-type electrode. Mitochondrial swelling was measured by the decrease in absorbance at 540 nm. COM produced a dose-dependent decrease in State 3 respiration (40% decrease at 0.05 mM COM with either succinate or glutamate/malate as substrate), without affecting either State 4 respiration or the ADP/O ratio. The inhibition of State 3 respiration was not reversed by cyclosporin A (CsA) administration. COM, from 0.01-0.05 mM also dose-dependently increased mitochondrial swelling, which was completely blocked by CsA. Potassium oxalate, at concentrations up to 5 mM did not affect mitochondrial respiration. These results suggest that COM, and not the oxalate ion, damage rat kidney mitochondria and induce the mitochondrial permeability transition, which then leads to renal cell death. Since COM is transported intracellularly by kidney cells, the renal toxicity of EG may result from inhibition of mitochondrial respiratory function in proximal tubular cells by COM crystals. Supported by American Chemistry Council.

450 MODULATION OF MITOCHONDRIAL GLUTATHIONE (GSH) TRANSPORT IN NRK-52E CELLS ALTERS SUSCEPTIBILITY TO CHEMICALLY INDUCED APOPTOSIS.

L. H. Lash[1], D. A. Putt[1], F. Xu[1], J. Wang[1], C. S. Wood[1], J. Hartman[1] and L. H. Matherly[2, 1]. *[1]Pharmacology, Wayne State University, Detroit, MI and [2]Karmanos Cancer Institute, Wayne State University, Detroit, MI.*

Two anion carriers of the mitochondrial inner membrane, the dicarboxylate carrier (DCC) and oxoglutarate carrier (OGC), are the primary, if not sole, determinants of mitochondrial GSH status. To study the function of this critical GSH pool, NRK-52E cells (an immortalized cell line derived from normal rat kidney proximal tubules) were stably transfected with both wild-type and mutant cDNAs for the DCC and OGC, using total rat kidney RNA as template. Mutants were created by RT-PCR, with targeted residues being those that are conserved across species, are in predicted transmembrane domains, and are thought to be important in catalytic function and/or protein structure. Endogenous mitochondrial transport activity is relatively low, and these cells are highly sensitive to apoptosis induced by either tert-butyl hydroperoxide (tBH; 10 or 50 µM) or S-(1, 2-dichlorovinyl)-L-cysteine (DCVC; 50 or 200 µM). Overexpression of either of the wild-type carriers markedly increased the ability of NRK-52E mitochondria to accumulate GSH and markedly protected cells from apoptosis induced by either tBH or DCVC. By contrast, expression of one of three DCC mutants (DCC-H24Q, -P25A, -D120K) or a double-cysteine mutant of the OGC (OGC-C221, 224S) resulted in markedly lower transport of GSH and dicarboxylates, whereas two cysteine mutants of DCC (DCC-C21S, -C211S) exhibited only modestly lower GSH transport activity. NRK-52E cells stably overexpressing OGC-C221, 224S exhibited a similar sensitivity to tBH- or DCVC-induced apoptosis as wild-type cells, indicating absence of protection. These results support the hypothesis that susceptibility to cellular injury from either an oxidant or a mitochondrial toxicant can be directly modulated by mitochondrial GSH status. Moreover, these results show that genetic manipulation of mitochondrial GSH carriers is an effective means to alter cellular susceptibility to toxicants. (Supported by NIH Grant DK40725.)

451 CELLULAR TOXICITY OF CALCIUM OXALATE: IS IT RELATED TO DISRUPTION OF MEMBRANE INTEGRITY.

C. Guo, T. Dugas and K. McMartin. *Department of Pharmacology, LSU Health Sciences Center-Shreveport, Shreveport, LA.*

A key metabolite in ethylene glycol (EG) poisoning is calcium oxalate monohydrate [COM], which may be responsible for the EG-related acute renal failure. Previous studies have suggested that COM induces cytotoxicity by producing plasma membrane damage, leading to leakage of cell contents and cell death. For example, COM but not the oxalate ion, produces hemolysis in rat erythrocytes and increase LDH leakage by normal human proximal tubular (HPT) cells in culture. Pretreatment of COM with aluminum citrate, which appears to decrease aggregation of COM, prevents the cellular membrane damage . In the present studies, we have investigated the mechanism of the damage induced by COM. We have used liposomes composed of dipalmitoylphosphatidylcholine (DPPC) as model membranes and calorimetric techniques to explore the COM-lipid interactions. Unilamellar vesicles (ULVs) were prepared in PBS by extrusion through a 0.1 mm polycarbonate membrane. A differential scanning calorimeter (DSC) was used to collect the DSC thermograms by raising the temperature at a constant rate of 0.5oC/min in the range of 25-50oC. COM at 5 and 10 mM did not significantly change the main thermotropic phase transition of DPPC. The effect of COM on the thermotropic phase transition of the other kind of lipid membranes is therefore being explored. These studies suggest that the membrane damaging effect of COM may not result from direct lipid-COM interactions in PBS. Supported by American Chemistry Council.

452 EVIDENCE FOR THE DIRECT INACTIVATION OF ENDOPLASMIC RETICULUM BOUND CA^{2+}-INDEPENDENT PHOSPHOLIPASE A_2 IN RENAL CELLS DURING OXIDATIVE STRESS.

B. S. Cummings[1, 3], G. R. Kinsey[1], A. K. Gelasco[2], J. Mchowat[4] and R. G. Schnellmann[3]. [1]*Pharmacology and Biomed. Sciences., University of Georgia, Athens, GA,* [2]*Nephrology, Med. University of South Carolina, Charleston, SC,* [3]*Pharmacology Sciences., Med. University of South Carolina, Charleston, SC and* [4]*Pathology, St. Louis University, St. Louis, MO.*

While inhibition of endoplasmic reticulum bound Ca^{2+}-independent phospholipase A_2 (ER-iPLA$_2$) in primary cultures of rabbit renal proximal tubule cells (RPTC) potentiates oxidant-induced oncosis, the effect of oxidant exposure on ER-iPLA$_2$ activity has not been examined. Exposure of RPTC to the model oxidant tert-butylhydroperoxide (TBHP, 0-400 μM) resulted in time- and concentration-dependent decreases in ER-iPLA$_2$ activity. Exposure of RPTC to cumene hydroperoxide and cisplatin also decreased ER-iPLA$_2$ activity. TBHP-induced ER-iPLA$_2$ inactivation was reversed by the addition of dithiothreitol (DTT, 1 mM) to microsomes isolated from treated RPTC. TBHP also directly inactivated ER-iPLA$_2$ in microsomes isolated from untreated RPTC. TBHP-induced microsomal ER-iPLA$_2$ inactivation was time- and concentration-dependent with 200 μM TBHP inhibiting microsomal ER-iPLA$_2$ activity 50% after 15 min. Similar to RPTC, DTT (300 μM) prevented TBHP-induced ER-iPLA$_2$ inactivation as did the reactive oxygen scavengers butylated hydroxytolulene (10 μM) and N, N'-diphenyl-p-phenylenediamine (2 μM), and the iron chelator deferoxamine (500 μM). These data suggested that TBHP-induced ER-iPLA$_2$ inactivation was the result of reactive oxygen species. Electron paramagnetic resonance spin trapping demonstrated that TBHP initiated a carbon-centered radical after 5 min of exposure in microsomes, preceding ER-iPLA$_2$ inactivation by 10 min. Further studies suggested that the formation of the carbon-center radical occurred after, or in concert with, the formation of an oxygen-center radical. These data suggest TBHP-induced formation of radical species at the ER is responsible for ER-iPLA$_2$ inactivation. These data demonstrate the novel finding that ER-iPLA$_2$ is inactivated by oxidants, and that the mechanism of inactivation involves the oxidation of ER-iPLA$_2$ sulfhydryl groups.

453 DIABETES PROTECTS FROM LETHAL NEPHROTOXICITY OF S-1, 2-DICHLOROVINYL-$_L$-CYSTEINE (DCVC).

A. V. Dnyanmote[1], S. P. Sawant[1], E. A. Lock[2], J. R. Latendresse[3] and H. M. Mehendale[1]. [1]*Toxicology, ULM, Monroe, LA,* [2]*MUSC, Charleston, SC and* [3]*Pathology Assoc. Intl., NCTR, Jefferson, AR.*

Streptozotocin (STZ)-induced type I diabetic rats show attenuated renal injury from nephrotoxicants such as gentamycin, cisplatin and $HgCl_2$. We tested the hypothesis that STZ-induced diabetes protects Swiss Webster mice from a lethal dose (75 mg/kg ip) of DCVC. Diabetes was induced in mice with STZ (200 mg/kg ip) while the controls received only the vehicle. On day 10 the mice received either DCVC (75 mg/kg ip) or the vehicle alone. Ninety percent of the diabetic (DB) mice given DCVC survived in contrast to only 10% survival of non-diabetic (NDB) mice given DCVC. Time-course measurements of blood urea nitrogen (BUN), creatinine, and histopathology (H&E-stained kidney sections) were performed to assess renal injury. ^{3}H-thymidine (^{3}H-T) incorporation into renal DNA and PCNA immunohistochemistry were carried out to measure S-phase DNA synthesis and cell cycle progression, respectively. Apoptosis was estimated by TUNEL assay. In the NDB mice, DCVC produced a steep temporal increase in BUN and creatinine, which was associated with acute necrosis of proximal tubular cells (PTC) resulting in 90% mortality within 48 h due to acute renal failure (ARF). In contrast, in the DB mice, BUN and creatinine increased till 36 h (albeit less steeply than in the NDB mice) declining thereafter to completely resolve by 96 h. Activity of renal cysteine conjugate β-lyase, the enzyme that bioactivates DCVC, was unaltered in the DB mice, undermining the possibility that lower bioactivation of DCVC led to lower injury in the DB mice. ^{3}H-T incorporation and PCNA indicated an early and sustained tissue repair in the DB mice treated with DCVC. DB mice also exhibited higher apoptosis after receiving DCVC, further increasing the efficiency of this tissue repair by rapid removal of damaged PTC that might have otherwise died of necrosis extending the progression of injury. These findings suggest that prompt and efficient tissue repair rescues DB mice from DCVC-induced ARF and mortality. (Supported by DK 61650)

454 NF-κB MEDIATED TRANSACTIVATIONAL MECHANISMS OF G1-TO-S CELL CYCLE PROGRESSION IN AUTOPROTECTION AGAINST S-1, 2-DICHLOROVINYL-L-CYSTEINE INDUCED ACUTE RENAL FAILURE.

M. C. Korrapati[1], E. A. Lock[2] and H. M. Mehendale[1]. [1]*School of Pharmacy, ULM, Monroe, LA and* [2]*Medical University of South Carolina, Charleston, SC.*

Prompt stimulation of nephrogenic cell division and tissue repair were reported to be the mechanisms of protection against a normally lethal dose of S-1, 2-dichlorovinyl-L-cysteine (DCVC) in autoprotection. We tested our hypothesis that protection occurs because of up regulated G1 checkpoint genes like cyclin D1 (CD1) mediated by sustained NF-κB binding to nuclear DNA. Male swiss webster mice (n=4) received a priming dose (15 mg DCVC/kg, ip) in distilled water (10 ml DW/kg), 3 days prior to receiving a lethal dose (75 mg DCVC/kg, ip in DW). NF-κB binding to nuclear DNA was measured by electrophoretic mobility shift assay, total and phosphorylated IκBα, CD1 and cyclin dependent kinase (cdk4) protein levels were estimated by Western blotting at 3, 6, 12 and 24 h after the lethal dose with or without the priming dose. Autoprotected mice were compared with mice receiving the priming dose+DW, DW+lethal dose & DW+DW. Inhibited phosphorylation of IκBα restricted the nuclear translocation of NF-κB, and decreased levels of CD1 and cdk4 after lethal dose of DCVC alone. However, a prior priming dose led to sustained degradation of IκBα by its phosphorylation, increased translocation and NF-κB binding to nuclear DNA as early as 3 h after receiving the same lethal dose in the autoprotected group. CD1 and cdk4 expression stimulated by the priming dose remained at high levels in the autoprotected group. Therefore up regulated CD1 production facilitated by increased nuclear DNA-binding of NF-κB is the key signaling mechanism behind sustained stimulation of renal cell division in autoprotection. These findings raise the possibility that this signaling mechanism may be availed for potential pharmacotherapeutic modulation to restore lost proximal tubular structure and function. (Supported by DK 61650).

455 EFFECT OF MT-3 EXPRESSION ON APOPTOSIS IN THE HUMAN PROXIMAL TUBULE CELL LINE, HK-2.

S. H. Garrett[1], S. Somji[1], M. Sens[1] and D. A. Sens[2]. [1]*Pathology, University of North Dakota, Grand Forks, ND and* [2]*Surgery, University of North Dakota, Grand Forks, ND.*

Cadmium is a heavy metal known to induce nephrotoxicity in proximal tubule cells of the kidney. Most heavy metals can cause cell death by necrosis or apoptosis. Numerous studies have shown that metallothioneins (MT), a family of low molecular weight metal binding proteins can provide protection against heavy metal toxicity. The third isoform of metallothionein, MT-3, can also bind and sequester cadmium similar to that of the MT-1 and 2 isoforms, and could have the potential to play a role in mediating toxicity that cadmium elicits on the renal proximal tubule cell. The goal of this study was to determine if over-expression of MT-3 in the HK-2 kidney cell line would increase the resistance of these cells to the toxic effects of cadmium. The parental HK-2 cells and the MT-3 transfected cells were exposed to lethal and sub-lethal concentrations of cadmium chloride for various time periods. The effect of cadmium treatment on cell *via*bility was determined by the automated counting of the cell nuclei stained with the nuclear binding dye, 4', 6-diamidino-2phenylindole (DAPI). Continuous exposure of HK-2 cells at various concentrations of cadmium for 8 to 48 hr resulted in cell death with extensive chromatin condensation and DNA fragmentation, features typical for cells undergoing

apoptosis. This was further confirmed by agarose gel electrophoresis and caspase-3 assay. The MT-3 transfected cells did not show any increased resistance to cadmium compared to the HK-2 cells with very few cells showing morphological changes typical for apoptosis. These MT-3 transfected cells showed greater release of the enzyme lactate dehydrogenase compared to the parental cells indicating that the cells were undergoing necrosis. These results indicate that although the overexpression of MT-3 does not increase the resistance of the HK-2 cells to the toxic effects of cadmium but makes the cells more sensitive to necrosis.

456 STATINS INHIBIT ALBUMIN UPTAKE IN OPOSSUM KIDNEY PROXIMAL TUBULE CELLS VIA REDUCED PRENYLATION OF SIGNALLING PROTEINS.

J. Sidaway[1], R. G. Davidson[2], F. McTaggart[2], T. C. Orton[1], R. C. Scott[1], G. J. Smith[2] and N. J. Brunskill[3]. *[1]Safety Assessment, AstraZeneca, Macclefield, Cheshire, United Kingdom, [2]Cardiovascular and Gastrointestinal Discovery, AstraZeneca, Macclesfield, Cheshire, United Kingdom and [3]Department of Nephrology, Leicester General Hospital, Leicester, Leicestershire, United Kingdom.*

Statins are inhibitors of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA reductase) and are used for the reduction of cholesterol-containing atherogenic lipoproteins. They also affect cellular processes through depletion of non-sterol metabolites of mevalonate including geranylgeranyl pyrophosphate (GGPP), which is required for the prenylation of many proteins. During phase III studies of a new statin, rosuvastatin, transient proteinuria of tubular origin without impaired renal function was observed in some subjects taking the highest 80mg dose (above the recommended range). We hypothesised that this could be the result of reduced efficiency of protein re-absorption by renal proximal tubule (PT) cells through inhibition of HMG-CoA reductase. Using the PT-derived opossum kidney (OK) cell line, we measured protein uptake by incorporation of FITC-albumin and assessed HMG-CoA reductase activity by conversion of ^{14}C-acetate into cholesterol. Simvastatin, atorvastatin, rosuvastatin and pravastatin all inhibited albumin uptake into OK cells in a dose-dependent fashion (IC_{50} values at 24hr were 0.3, 1.0, 1.7 and 26.5 μM respectively) in the absence of cytotoxicity, and this was related to the degree of inhibition of HMG-CoA reductase. The inhibitory effect of simvastatin on albumin uptake was almost completely prevented by co-addition of mevalonate and GGPP, but not by cholesterol. Inhibition of geranylgeranyl transferase also reduced albumin uptake whilst simvastatin decreased prenylation of Rap1A, a protein associated with endocytosis. These data suggest that statins inhibit protein uptake in OK cells through reduced prenylation of critical signalling/transport proteins and establish the principle that inhibition of HMG-CoA reductase in PT cells may reduce tubular protein re-absorption *in vivo* providing a mechanistic explanation of statin-associated proteinuria.

457 ESTROGEN RECEPTOR α BUT NOT β IS A MAJOR MEDIATOR FOR ESTRADIOL INDUCED THYMIC ATROPHY.

Z. Lai[1], N. C. Fiore[1], S. C. Hewitt[2], K. S. Korach[2] and A. E. Silverstone[1]. *[1]Department of Microbiology and Immunology, SUNY Upstate Medical University, Syracuse, NY and [2]Lab. Reprod Develop Toxicology, NIEHS/NIH, Research Triangle Park, NC.*

It has been shown that estradiol (E2) induces thymic atrophy in all examined mammals. An estrogen receptor (ER) is required to mediate the effects of E2. Our previous study with ERα knockout (ERKO) mice demonstrated that loss of ERα could not completely protect from E2 induced thymic atrophy, suggesting that there may be other mediators for estrogen effects in the immune system. At least one other ER (β) has been identified. To investigate the role of ERβ in modulation of E2 induced thymic atrophy; we compared the effects of E2 on C57BL/6 (WT) and ERβKO mice in the present study. Our results show that E2 induced the same degree of thymic atrophy in either WT or ERβKO mice, whose thymii still contain ERα receptors. Analysis of thymocytes for expression of HSA, CD3, CD4, CD8, DP (CD4+8+), DN (CD4-8-) and CD44, CD25, also indicated that there was no difference for the effects of E2 between WT and ERβKO mice. Using radiation chimeras reconstituted from WT or ERβKO stem cells into WT mice, we found that there was no significant difference in reduction of the number of total thymocytes and the numbers of CD4, CD8, DP, and DN cells induced by E2 in both types of chimeras. As seen with WT donors, smaller thymii were seen in chimeras reconstituted by ERβKO stem cells into the ERαKO mice compared to those chimeras reconstituted into WT mice. We also found that the effects of E2 on thymocyte subset distribution, were partially inhibited in these ERαKO reconstituted with ERβKO BM, which lack thymic stroma ERα. We also did not see an increase of CD3 expression and a decrease of HSA expression in the ERαKO reconstituted with ERβKO BM chimeras; these effects are usually seen in the WT and ERβKO mice. Taken together, our results confirm that ERα but not ERβ is a major mediator for E2 induced thymic atrophy. (Supported by NIEHS ES 07216 and ES 04862, and Hendricks's fund)

458 EVALUATION OF THE POTENTIAL IMMUNOTOXICITY OF 3-MONOCHLORO-1, 2-PROPANEDIOL IN BALB/C MICE.

J. Lee[1], J. Byun[1], S. Park[1], H. Kim[1], J. Park[1], J. Eom[1], M. Ryu[1], Y. Heo[2] and H. Oh[1]. *[1]Division of Immunotoxicology, National Institute of Toxicological Research, KFDA, Seoul, South Korea and [2]Daegu Catholic University, Daegu, South Korea.*

3-monochloro-1, 2-propanediol (MCPD) is a well-known by-product of acid-hydrolyzed soy sauce during its manufacturing process. MCPD has been reported genotoxic *in vitro*, and reproductive toxicity and carcinogenicity in rats. However, no previous studies have investigated MCPD-induced alterations in the immune system. In the present study, MCPD was administered by gavage for 14 days at 0, 25, 50, 100, 120mg/kg to female Balb/c mice. The antibody-mediated immune response to sheep red blood cells (SRBC) was assessed using the plaque-forming cell (PFC) assay and splenic cell phenotypes were quantified by flowcytometry. Hematological and histopathological changes were assessed. The T lymphocyte blastogenesis by concanavalin A (Con A) and B-lymphocyte blastogenesis by lipopolyssacharide (LPS) were not significantly changed. The phenotypes of CD3+, CD45R+, CD4+, and CD8+ in spleen of MCPD-treated mice were not significantly changed. There were no significant changes in the hematological and histopathological changes of MCPD-treated mice. However, the significant decrease in thymus weight was observed in 100mg dose group, even though that did not change body weight gain. The cellularites of spleen and thymus were significantly reduced in 100mg dose group. Exposure to 100mg of MCPD decreased the PFC response to SRBC in mice. These results indicate that MCPD could reduce the immune system in Balb/c mice.

459 ACETONE: 4-WEEK DRINKING WATER IMMUNOTOXICITY STUDY IN CD-1 MICE.

P. K. Anderson, M. R. Woolhiser and J. M. Waechter. *Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI.*

Acetone exposure occurs from both natural (e.g., food and endogenous production) as well as through anthropogenic sources; previous studies have suggested acetone may modulate humoral immunity (Singh et al. Fund. Applied. Toxicol. 33: 129-139, 1996). This study investigated the immunotoxicity potential of acetone in accordance with the USEPA Immunotoxicology Test Guideline. Acetone was administered to mice at concentrations of 0, 600, 3000 or 6000 ppm (average daily doses of 121, 621, or 1144 mg/kg/day). The highest dose was about equivalent to the estimated dose dermally absorbed for Singh et al., and about 20-fold greater than normal, endogenous production by human adults. Mice were immunized with sheep red blood cells 4 days before the end of exposure to evaluate functional responsiveness of the immune system *via* the Antibody, Plaque Forming Cell (AFC) assay. Body weights, hematological parameters and thymus weights were also measured. Body weights and hematological parameters showed no treatment-related effects due to acetone consumption. Eosinophil percentages appeared slightly lower than controls in some groups of mice treated with acetone, but the results were neither dose related or statistically different from controls, and all values were within the range of historical controls. Spleen weights were unaltered by treatment while mean thymus weights of mice given acetone at 1144 mg/kg/day were about 25% lower, but not statistically different, than controls. No effects were noted for spleen cellularity as a result of acetone treatment. The AFC responses ranged from 1088-1401 AFC/10^6 splenocytes following acetone administration, and were not statistically different from control (1277 AFC/10^6 cells). The antibody response, WBC numbers, RBC counts, hemoglobin and hematocrit levels, and organ weights for positive controls (CYP) were all reduced. Acetone administration *via* drinking water for 28 days did not produce immunotoxicity in CD-1 mice at doses as high as 1144 mg/kg/day. This work was sponsored by the Acetone Panel of the American Chemistry Council.

460 EXPOSURE TO SODIUM DICHROMATE FROM DRINKING WATER DOES NOT ALTER IMMUNE FUNCTION IN B6C3F1 MICE OR SPRAGUE DAWLEY RATS.

R. D. Brown[1], K. L. White[1], D. R. Germolec[2], C. S. Smith[2], L. X. Zhang[1] and T. L. Guo[1]. *[1]Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA and [2]NIEHS, Research Triangle Park, NC.*

Hexavalent chromium [Cr(VI)] is a byproduct of chromium plating and as a result of significant use in the industry has resulted in environmental contamination of drinking water surrounding areas where it is produced. Cr(VI) in the drinking water has been associated with cancer clusters and increased incidences of various diseases. The USEPA drinking water standard is 100 mg/l (100 ppb). Studies were conducted by the National Toxicology Program to evaluate the effects of sodium

dichromate, Cr(VI), on the immune system in both rats and mice following exposure from drinking water. Female mice were exposed to Cr(VI) at concentrations of 15.6, 31.3, 62.5, 125, and 250 ppm while rats were exposed to 14.3, 57.3, 172, and 516 ppm in the drinking water for 28 days. Animals were evaluated for toxicological and immune function on day 29. In mice organ weights were not affected, while decreases in body weight and erythroid parameters were observed at the high dose level. Immune parameters such as the mixed leukocyte response, anti-CD3-mediated lymphocyte proliferation, splenic phenotypes, and natural killer cell activity were not effected. While a slight stimulation was observed in the plaque assay to sRBC in the middle dose levels, the effect was not dose-responsive in nature. No effect was observed on serum IgM ELISA to the T-dependent antigens, sRBC or KLH. In female rats, body weights, organ weights, and hematological parameters were not affected. Similarly, anti-CD3-mediated lymphocyte proliferation and natural killer cell activity were not affected. A slight increase was observed in the plaque assay at the lowest dose, similar to observations in the mouse. Overall, exposure from Cr(VI) in the drinking water did not produce marked effects on the immune responses of either rats or mice. Supported by NIEHS/NTP Contract ES-05454.

461 COMPARISON BETWEEN INTRAPERITONEAL AND ASPIRATION ROUTES OF EXPOSURE TO EVALUATE THE IMMUNOTOXIC EFFECTS OF A MIXTURE OF HERBICIDES.

K. Salazar[1], M. Chroussis[2], J. B. Barnett[1] and R. Schafer[1]. [1]*Microbiology, Immunology, & Cell Biology, West Virginia University, Morgantown, WV and* [2]*Alderson-Broaddus College, Phillipi, WV.*

The widespread use of herbicides in agriculture and the accumulation of herbicides in the environment create a need for understanding the mechanism of their toxic effects. Due to the ubiquitous nature of herbicides, exposure is frequently to a mixture. The herbicides 2, 4-D (2, 4-dichlorophenoxyacetic acid) and propanil (3, 4-dichloropropionanilide) represent two extensively used herbicides commonly sold as a mixture. Previous experiments in our lab have shown that intraperitoneal (i.p.) exposure to a mixture of these two herbicides produces synergistic immunotoxic effects on the thymus. This study utilized an aspiration model in C57Bl/6 mice to compare the immunotoxic effects on the thymus after i.p. or aspiration exposure to 2, 4-D and propanil. Mice were exposed to either a single 150 or 200 mg/kg dose of propanil or 2, 4-D, or a 150 or 200 mg/kg one to one mixture of the herbicides. Mice exposed *via* aspiration were lightly anesthetized and administered the herbicides in a DMSO and olive oil vehicle. Thymocyte populations were determined by flow cytometry 2 days post exposure. Mice exposed to a 150/150 mg/kg or 200/200 mg/kg mixture or to 200 mg/kg propanil alone had thymic atrophy following both routes of administration. CD4+CD8+ (DP) thymocytes were decreased approximately 50% compared to vehicle control. Intraperitoneal exposure to 150 mg/kg propanil resulted in thymic atrophy and a decrease in DP thymocytes. However, 150 mg/kg propanil administered *via* aspiration did not produce any significant thymic toxicity. This suggests that the mechanism inducing thymic toxicity following exposure to the mixture of herbicides is dependent on route of exposure. These data demonstrate that aspiration of the herbicides produce immunotoxic effects on the thymus. These experiments also suggest that the aspiration model is an effective method for evaluating immunotoxicity after herbicide exposure. Supported by the NIH Immunotoxicology Training Grant ES010953.

462 INDUCTION OF IFN-G CYTOKINE EXPRESSION BY CHLORPYRIFOS, CHLORPYRIFOS-OXON, AND ENDOTOXIN *IN VITRO*.

P. Duramad and N. Holland. *UC Berkeley, Berkeley, CA.*

Pesticides and their metabolites are ubiquitous in the environment as a result of widespread agricultural and domestic applications. In addition to pesticides, agricultural workers and their families are also exposed to elevated levels of allergens and endotoxin. Interactions among immunomodulatory agents have only recently become a focus of environmental health research. Few validated immunological markers are currently available to evaluate the immunotoxicity of pesticides. Cytokine profiling is a promising biomarker of the immune response since skewed Th1 and Th2 cytokine secretion is associated with autoimmune disorders, asthma, and cancer. Here, we used an *in vitro* based method to evaluate the effects of the organophosphate pesticide chlorpyrifos (CP) and its metabolite chlorpyrifos-oxon (CPO) on cytokine secretion by human lymphocytes. We also evaluated the effect of combined exposures to CP/CPO and endotoxin (LPS) in order to model the multiple exposures received by agricultural workers and their families. Human whole blood was treated with CP, CPO (both with doses of 1-10, 000mg/mL), and LPS (1.5-2.5mg/mL) either singly or in combination for 2-72 hours. At 2, 24, 48, and 72 hours supernatants were collected and assayed by ELISA for expression of interferon-g (IFN-g), the Th1 signature cytokine. Whereas no expression of IFN-g was detected in cultures treated with either CP or CPO alone, cultures treated with CP or CPO in combination with LPS expressed higher levels of IFN-g with some combinations inducing greater IFN-g expression than LPS alone ($p<0.01$). The levels of IFN-g secreted into the supernatants peaked at 48 hours and declined slightly at 72 hrs. In summary, CP and CPO alone do not induce cytokine expression, however the combinations of CP+LPS and CPO+LPS synergistically increased IFN-g expression. These results indicate a potential interaction between endotoxin and organophosphate pesticides. Finally, our results demonstrate that cytokine induction can be a sensitive marker of the immune response to combinations of environmental factors.

463 ROLE OF CYTOKINE NETWORKS IN DETERMINING SUSCEPTIBILITY TO DRUG-INDUCED LIVER DISEASE.

M. Bourdi[1], D. Eiras[1], M. Holt[1], T. Reilly[2], K. Welch[1], H. Amouzadeh[1] and L. Pohl[1]. [1]*Molecular and Cellular Toxicology Section/LMI/NHLBI, NIH/DHHS, Bethesda, MD and* [2]*Immunotoxicology, Drug Safety Evaluation, Bristol-Myers Squibb, Syracuse, NY.*

Using interleukin (IL)-10 deficient mice (IL-10-/-), we previously reported that IL-10 plays an important role in protecting the liver against acetaminophen (APAP)-induced liver injury. In the current work, we discovered a unique interaction that makes mice deficient in both IL-10 and IL-4 (IL-10/4-/-) highly sensitive to the hepatotoxic effects of APAP. C57BL/6 wild type (WT), IL-10-/-, IL-4-/-, or IL-10/4-/- mice were administered APAP at a dose of 120 mg/kg. Within 24 hours, 90% of the IL-10/4-/- mice died of massive hepatic damage. In contrast, all of the WT, IL-10-/-, and IL-4-/- mice survived and showed minor or no signs of liver injury. The high susceptibility of the IL-10/4-/- mice was associated with elevated levels of hepatic TNF-a mRNA, serum nitrite/nitrate, and hepatic APAP-protein adducts, which appeared to be due in part to depressed levels of hepatic glutathione but not elevated levels of CYP2E1. These findings suggest that hepatocyte homeostasis following drug-induced injury is controlled in part by the activities of both IL-4 and IL-10, which together help maintain adequate levels of glutathione and prevent overexpression of protoxicant factors including TNF-a and nitric oxide. Certain polymorphisms of IL-4 and IL-10 that lead to their under expression may have a role in determining individuals' susceptibility to drug-induced liver disease.

464 LIPOPOLYSACCHARIDE PRE-EXPOSURE SENSITIZES THE MOUSE TO DEOXYNIVALENOL -INDUCED PROINFLAMMATORY CYTOKINE EXPRESSION AND LYMPHOCYTE APOPTOSIS.

Z. Islam[1] and J. J. Pestka[1, 2]. [1]*Food Science and Human Nutrition, Michigan State University, East Lansing, MI and* [2]*Institute of Environmental Toxicology, Michigan State University, East Lansing, MI.*

Simultaneous exposure to lipopolysaccharide (LPS) and the trichothecene deoxynivalenol (DON, vomitoxin) synergistically induces proinflammatory cytokine gene expression *in vitro* and *in vivo*. The purpose of this study was to determine if LPS pre-exposure sensitizes the mouse to DON-induced TNF-alpha, IL-6, IL-1alpha and IL-1beta expression. To test this hypothesis, the effects LPS and/or DON treatment on production of these cytokines were assessed in male B6C3F1 mice using real time PCR and ELISA methods. Initially, mice were treated with LPS (1 mg/kg) for 8 h, orally gavaged with DON (12.5 mg/kg) and cytokine response measured beginning after 1 h. LPS-induced pro-inflammatory cytokines peaked at 2 h and were diminished within 4 to 8 h. However, subsequent DON exposure in LPS-exposed animals caused significant expression and release of proinflammatory cytokines as compared to DON alone. Expression of TNF-alpha mRNA peaked 5 folds at 1 h but the expression of IL-6, IL-1alpha and IL-1beta mRNAs was increased 5-, 4-, and 3-fold, respectively, 2 h after DON treatment compared to the mean additive responses of LPS alone and DON alone. While TNF-alpha and IL-6 were down regulated 8 h later, IL-1alpha and IL-1beta remained elevated until 24 h and was significantly higher compared to the mean additive responses of LPS alone and DON alone. Plasma levels of these cytokines were also elevated which was consistent with mRNA expression. Amplified cytokine expression was dependent on both LPS and DON dose. Mice pre-treated with LPS for 8 h and DON for 12 h exhibited a massive loss in thymocytes by apoptosis as confirmed by agarose gel electrophoresis and flow cytometry. Based on these and previous results, LPS pre-exposure appeared to sensitize mice to DON with resultant prolonged IL-1 up-regulation that might play a major role in subsequent stress-induced lymphocyte apoptosis.

465 INFLAMMATION AND TRAUMATIC SKELETAL MUSCLE INJURY.

M. Summan, T. Hulderman, J. M. Matheson and P. P. Simeonova. *Toxicology and Molecular Biology, DHHS/CDC/NIOSH, Morgantown, WV.*

Traumatic skeletal muscle injuries result in profound histopathological changes and loss of muscle function. These injuries result in local infiltration of large numbers of mononuclear cells, degeneration of injured myofibers and phagocytic removal of

cell debris. Macrophages are critical effector cells in host defense and release a diverse array of cellular mediators including cytotoxic and proinflammatory cytokines that contribute to tissue injury. The present study evaluated the role of systemic macrophages in the injury/repair mechanisms in a traumatic skeletal muscle injury model using liposome encapsulated clodronate, a drug with well characterized monocyte/macrophage depleting qualities. C57BL/6 mice (n = 4 per group) were injected with clodronate liposomes 48 and 2 hours prior to the freeze injury of the left tibialis anterior (TA) muscle and every third day during the post-injury period. Control mice received phosphate buffered saline (PBS) liposomes. At 1, 3, 9 or 14 days post-injury, TA muscles were harvested for histology, immunohistochemistry or gene expression evaluation by quantitative real time RT-PCR. Histopathology revealed less inflammatory cell infiltration in the injured muscle of clodronate treated mice at day 3 post-injury, delayed muscle tissue recovery and impaired clearance of necrotic myofibers at day 9 post-injury; and increased fat infiltration at day 14 post-injury. Immunohistochemical evaluation of injured muscle demonstrated that Mac-1 expression was dramatically reduced in clodronate treated mice at day 3 post-injury. Clodronate treatment also significantly attenuates inflammatory (TNF-alpha, Mac-1 and MCP-1), growth (IGF-BP) factor and antioxidant (thioredoxin) gene expression in injured TA muscle compared to injured non-treated TA muscle. In conclusion, the inhibition of the inflammatory response resulted in delayed phagocytosis of the necrotic myofibers and delayed the repair process of the injured muscle tissue. Selective and time dependent modulation of the innate immune system may provide optimal resolution of skeletal muscle injury.

466 IMMUNOGENICITY OF POLYETHYLENE GLYCOL (PEG)-BASED HYDROGEL SEALANT IN LAPAROSCOPIC PORCINE PARTIAL NEPHRECTOMY.

M. H. French, E. L. Park, W. Y. Ho, J. R. Talley, M. J. White, S. Ramakumar, N. F. Ullrich, J. A. Linehan and J. B. Ulreich. *Surgery, University of Arizona, Tucson, AZ.*

Our objective was to evaluate potential immunomodulatory effects of a PEG-based hydrogel sealant in a porcine model of laparoscopic partial nephrectomy. Female domestic swine underwent laparoscopic left lower pole guillotine nephrectomy. Hemostasis and tissue sealing were achieved solely by application of a biodegradable PEG-based hydrogel. Humoral and cell-mediated immunity (CMI) to the sealant were evaluated by enzyme linked immunosorbent assay (ELISA) and lymphocyte proliferation assay (LPA) at 0, 2, 6 and 12 weeks. No antibodies to the hydrogel were detected by ELISA in immunized rabbits. There were no significant differences between control and nephrectomized swine in the ELISA at 2, 6 or 12 weeks. Optical density values for all 4 groups of swine in the ELISA were near 0.2, essentially baseline. It is concluded that the presence of the hydrogel in the abdomen did not elicit IgG antibody formation. For the CMI response measured using the LPA, there were significant differences between the log of the stimulation indices for test and control swine for all three mitogens at 2 weeks post-surgery (ConA, $p \leq 0.01$; PHA, $p \leq 0.01$; PWM, $p \leq 0.05$). These differences disappeared at 6 and 12 weeks and may represent a temporal reactivity of the white cells to the surgery undergone by the test, but not the control, swine. For the cellular response to antigens in the LPA, the only significant difference between the test and control groups was a temporal response to Candida albicans at two weeks ($p \leq 0.03$). There was no significant difference between test and control pig reactivity to the hydrogel as antigen in the LPA at any time point. The PEG-based hydrogel effectively sealed the cut surface of kidneys following porcine partial nephrectomy. There were no immediate or long term (12 week) complications. There was no humoral or long term cell-mediated immune response to the hydrogel. We conclude that the hydrogel is safe and effective for abdominal use, a novel application. Supported by the UA UBRP.

467 IMMUNOTOXICITY OF A COPLANAR AND NONCOPLANAR POLYCHLORINATED BIPHENYL (PCB) CONGENER IN A FISH MODEL.

J. Duffy, Y. Li and J. Zelikoff. *Department of Environmental Medicine, New York University School of Medicine, Tuxedo, NY.*

Polychlorinated biphenyls (PCBs) are a well-studied class of environmental contaminants known to be immunotoxic for both mammals and fish. Depending upon the particular structure of a PCB congener (i.e., coplanar or noncoplanar), different degrees and/or types of immunotoxic effects have been observed in exposed species. For this study, the effects of a single intraperitoneal (i.p.) exposure of bluegill sunfish (*Lepomis macrochirus*) to either a coplanar (PCB 126) or noncoplanar (PCB 153) PCB congener on host immunocompetence was determined by examining non-specific and cell-mediated immune parameters. Specifically, phagocyte-mediated intracellular and extracellular superoxide ($O_2^{\cdot-}$) production as well as mitogen-stimulated T- and B-lymphocyte proliferation were examined using kidney or spleen cells recovered from fish on 7, 14 or 21 d post-exposure. Both PCB congeners acted to depress innate and cell-mediated immune functions at all post-exposure timepoints (compared to controls) with the exception of extracellular $O_2^{\cdot-}$ production which was unaltered following PCB exposure. This study demonstrates that structurally different PCB congeners, thought to act by different mechanisms to produce toxicological effects in exposed species, may actually impact the immune response in a similar manner. This work was supported by a Hudson River Foundation Graduate Fellowship and USACEHR contract no. DAMD 17-99-9011.

468 IMPACT OF MATERNAL TOXICITY ON POSTNATAL IMMUNE SYSTEM-RELATED PARAMETERS IN RATS.

A. H. Penninks, M. van Zijverden, F. Kuper, M. M. Tegelenbosch-Schouten, A. P. Wolterbeek and I. D. Waalkens-Berendsen. *Experimental Immunology, TNO Nutrition and Food Research, Zeist, Netherlands.*

Maternal toxicity during the entire gestation and lactation period might affect the pre-and postnatal development of the immune system of the offspring. As in the absence of a toxicological active substance maternal toxicity can also be mimicked by changes in body weight induced by food restriction, a postnatal developmental study was performed in which next to a control group two groups of pregnant rats were food restricted (approx. 85 and 70% of ad libitum) and two groups were treated with 20-or 100 μg dexamethasone/kg. In order to study the potential immunotoxic effects in these different groups enhanced immunopathology (paying attention to thymus, spleen, various lymph nodes and bone marrow), hematology and lymphocyte subset analysis in blood by flow cytometry was performed in the offspring on various post natal days (PND1, PND 21 and PND 70). Moreover, immune function was studied in offspring on PND 21 and PND 70 by means of a T cell-dependent antibody response to both SRBC (as measured by Plaque Forming Cell assay in spleen cells) and to KLH (as measured by ELISA). During gestation and lactation maternal body weight was affected on almost all days in the food restricted and dexamethasone-treated animals. Pup weights showed some non-significant differences at day 1-4 between the various groups, which became significant in all groups from day 7, except the low dexamethasone-treated group. Subset analysis of blood lymphocytes by flow cytometry revealed that at PND 4 lymphocyte subsets could hardly be determined, and at PND 21 they almost resembled those of adult rats. Mainly in the highest dexamethasone group lymphocyte subset numbers were affected. Immunopathology also revealed the immaturity of lymphoid organs at PND 4 which clearly was improved from PND 21. The further evaluation of the effects in the different treatment groups on immunopathology and the T cell-dependent antibody response on PND 21 and PND 70 (as measured by the SRBC-PFC assay and by KLH-ELISA) will be presented and discussed.

469 DEXAMETHASONE-INDUCED IMMUNOTOXICITY FOLLOWING FETAL VS. ADULT EXPOSURE.

R. R. Dietert, J. Lee, J. Olsen, K. Fitch and J. A. Marsh. *Microbiology and Immunology, Cornell University, Ithaca, NY.*

Immunotoxicity data using adult exposure to toxicants may not reflect perinatal vulnerability. As a result, direct life stage comparisons are needed to establish dose-response relationships, target organ sensitivities, and expected immunotoxic changes. This study compared gestational vs. adult life stages for suceptibility to dexamethasone-induced immunotoxicity including selected developmental-reproductive endpoints. Dexamethasone-21 phosphate was administered (s.c.) to pregnant CD rats at days 6-21 of gestation (0, 0.0625, 0.125, 0.25, 0.5 mg/kg/day) with identical exposure of non-pregnant adult females. Some reproductive (anogenital distance) and growth (body weight) measures of pups were altered. In the juvenile (5 wk.), the delayed type hypersensitivity response to KLH was significantly reduced at all doses examined and this pattern continued into adulthood (13 wk.). In contrast, the DTH response of adults exposed to DEX was unaltered even at the highest dose. Few DEX-induced changes were seen in offspring or adult blood parameters or in splenocytes analyzed for cell surface makers (by flow cytometry). The thymus of both exposed pups (both ages) and adults showed a marked reduction in the medulla/lobe area beginning with the 0.125 mg/kg/day DEX exposure level. Macrophage production of TNF and NO was only marginally affected as was splenocyte production of IL-4 and IFN gamma. In contrast, pups assessed as juveniles were significantly depressed in splenic IL-2 and IL-10 production. DEX exposure altered serum antibody levels across age groups with an increase of KLH-specific IgG (beginning with the 0.0125 mg/kg/day dose) while total IgE was reduced. These results suggest that while DEX exposure produces some common alterations following *in utero* vs. adult exposure, fetal exposure (even at the lowest doses tested) produces marked and persistent functional loss (DTH) not evident in exposed

adults. Furthermore, there was no apparent advantage in delaying immune assessment until the offspring reached adulthood. Supported by a grant from the American Chemistry Council.

470 DEVELOPMENTAL EXPOSURE TO A THYROID DISRUPTING CHEMICAL STIMULATES PHAGOCYTOSIS IN JUVENILE SPRAGUE-DAWLEY RATS.

A. A. Rooney[1], R. Matulka[2] and R. W. Luebke[3]. [1]*CVM Department of Anatomy, Physiological Sciences and Radiology, NCSU/USEPA, Raleigh, NC,* [2]*Department of Toxicology, UNC, Chapel Hill, NC and* [3]*USEPA/NHEERL, Research Triangle Park, NC.*

During development, exposure to xenobiotics that disrupt the thyroid-axis may adversely impact growth and reproduction through reduced thyroid hormone (TH) concentrations. The regulatory role of THs within the immune system suggests immune function may also be negatively effected. In a previous study we observed increased phagocytic activity of peritoneal macrophages from 7-week-old offspring of Sprague-Dawley (SD) dams exposed to the anti-thyroid drug propylthiouracil (PTU, 2.0 mg/kg/day). The current study tested phagocytic responses in offspring of dams exposed to 0.02, 0.2 or 2.0 mg PTU/kg/day from gestational day 10 through post-natal day (PND) 23. Serum triidothyronine (T_3) and thyroxine (T_4) were measured by RIA on PNDs 14, 21, 35, 49 and 101, while peritoneal macrophage phagocytosis was measured during the time frame that offspring recover from a hypothyroid to an euthyroid state (PNDs 21, 35 and 49) to examine the relationship between phagocytic responses and circulating THs. On PND 14, mid dose offspring had lower T_4 and high dose offspring had lower THs. Reduced T_4 persisted in high dose offspring through PND 21 (females) and PND 49 (males). Also in high dose offspring, an increased percentage of macrophages phagocytized latex beads on PNDs 35 and 49; however, phagocytic activity was not correlated to serum THs. These results demonstrate that only developmental exposure to high doses of PTU (sufficient to cause cretinism and severely retard growth) were associated with increased phagocytic response, and that increase was only observed in rats greater than 3 weeks of age. The high doses of PTU necessary to alter phagocytosis and the lack of a clear relationship between serum THs and phagocytosis indicate that phagocytic response of peritoneal macrophages is not a sensitive indicator of developmental hypothyroidism.(This abstract does not reflect EPA policy and was supported in part by the NCSU/EPA Cooperative Training Agreement CT826512010).

471 BOTH ADULT AND DEVELOPMENTAL EXPOSURES TO NEVIRAPINE INCREASED NK CELL ACTIVITY BUT DECREASED SPLEEN ANTIBODY-FORMING CELL (AFC) RESPONSE TO T-DEPENDENT ANTIGEN SHEEP ERYTHROCYTES (SRBC) IN FEMALE B6C3F1 MICE.

T. L. Guo[1], D. R. Germolec[2], D. L. Musgrove[1], R. P. Chi[1] and K. L. White[1]. [1]*Virginia Commonwealth University, Richmond, VA and* [2]*NIEHS, Research Triangle Park, NC.*

Nevirapine is a non-nucleotide reverse transcriptase inhibitor of HIV-1, and often used for combination therapy. Nevirapine binds directly to reverse transcriptase and blocks the RNA-dependent and DNA-dependent activities by causing a disruption of the enzyme function. In addition to being used for HIV adult patients, nevirapine has also been applied increasingly to prevent mother-to-child HIV-1 transmission. Thus, the immunotoxicity associated with both adult and developmental exposures to nevirapine is a great concern. We have evaluated the changes in immune responses in female B6C3F1 mice following exposure to nevirapine at dose levels of 0, 88, 176, and 266 mg/kg. In adult mice, exposure to nevirapine by gavage (twice/day) for 28 days had no effects on body weight, hematological parameters, mixed lymphocyte reaction and anti-CD3 mediated proliferation in spleen. However, a dose-related decrease in spleen AFC response to sRBC and an increase in NK cell activity were observed. In developmental exposure, pups were exposed gestationally and lactationally starting at gestation day 5 by dosing dams (gavage) twice a day and by gavage (once/day) starting at PND3. After weaning at PND21, pups continued to be dosed by gavage twice a day until PND45. There was no effect on hematological parameters. However, in agreement with the adult exposure, a dose-related decrease in spleen AFC response to sRBC and an increase in NK cell activity were observed. Furthermore, developmental exposure produced more decreases in spleen AFC response to sRBC than adult exposure. In conclusion, exposure to nevirapine decreases antibody-forming cell responses and increase NK cell activity in both adult and young mice (Supported by the NIEHS Contract ES 05454).

472 THE EFFECTS OF EARLY EXPOSURE OF ENDOSULFAN, PIPERONYL BUTOXIDE AND PERMETHRIN ON IMMUNE FUNCTION OF ADULT MICE.

G. E. Pimentel-Smith[1] and H. P. Misra[2]. [1] *Vet. Med., Virginia Tech, Blacksburg, VA and* [2]*Division of Biomedical Sciences, Edward Via College of Osteopathic Medicine, Blacksburg, VA.*

Newborns exposed to certain pesticides may not only have immediate effects on immune function but also may develop latent effects at adult life. We studied the effects of permethrin (PR) on adult (11 week-old) mice pre-exposed as juveniles (6-7 days old) with either the insecticide endosulfan (EN; a MFO inducer) or pesticide synergist piperonyl butoxide (PBO; a MFO inhibitor). Groups of 12 juvenile C57Bl/6 mice were injected i.p. with either EN or PBO on days 1 and 3, and allowed to grow up to 11 weeks. At 11 weeks, half of each group was given EN or PBO singly or in mixture with PR, i.p. Control groups received cyclophosphamide (positive) and PBS or corn oil (CO; negative). Doses were 1/8 LD50 (Merck Index) in 10-µl vehicle for juveniles and 1/4 LD50 in 80-µl vehicle for adults. One day after the last dose, all mice were injected with 1ml of 5% sheep red blood cell (SRBC) and sacrificed 4 days later. The immune parameters of splenocytes were analyzed. The results of flow cytometric analysis of cells, using PE- and FITC-labeled monoclonal antibodies, showed higher percentages of splenocytes in early apoptotic stage ($P<0.05$) in all chemical-exposed groups than the controls. All pesticide treated groups were found to have lower numbers of plaques ($P<0.05$) using the SRBC-PFC assay, except the EN+PR group. There were no differences observed in spleen weights. The PBO, PR and PBO+PR treatment groups exhibited higher number of total splenocytes ($P<0.05$). These results indicate that exposure of mice to these pesticides at early life may cause immune dysfunction later in life.

473 PRENATAL MERCURIC CHLORIDE [HGCL2] EXPOSURE IN BALB/C MICE: GENDER-SPECIFIC EFFECTS ON THE ONTOGENY OF THE IMMUNE SYSTEM.

I. A. Silva[1, 3], M. El Nabawi[2], D. Hoover[2] and E. Silbergeld[3]. [1]*Insttitute Molecular Cell Biology, Porto, Portugal,* [2]*University Maryland Medical School, Baltimore, MD and* [3]*Environmental Health Sciences, Johns Hopknis University Bloomberg School Public Health, Baltimore, MD.*

Mercury (Hg) is a well known developmental toxicant, with devastating effects associated with *in utero* exposure in humans and animal models. However, this characterization is based exclusively upon the neurodevelopmental effects of prenatal exposures. Because the immune system also undergoes prolonged development pre- and postnatally, and because Hg is known to be immunotoxic to adult animals, we tested the hypothesis that *in utero* exposure to HgCl2 would induce persistent immune dysfunction. Pregnant BALB/c females were treated every other day from GD5 for 11 days with s.c. injections of HgCl2 (200mcg/kg body weight); controls received equimolar injections of NaCl2. Offspring were sacrificed at PND10, 14, 21, and 60. Lymph nodes, (LN), spleens, and thymus were harvested for studies of proliferation and cytokine production *in vitro. In utero* HgCl2 exerted persistent organ specific effects on cell number, proliferation, and cytokine production in LN, spleen and thymus. The effects of prenatal HgCl2 exposure were expressed differently in adult females and males. In adult females, an inhibitory effect was observed on IL-4, IL-10 and IFN-gamma production in thymocytes, IL-2 in LN cells, and IL-10 in splenocytes. In males, a stimulatory effect was primarily observed. Overall, we conclude that *in utero* exposures to low doses of HgCl2 can induce persistent gender-specific immunotoxic effects, observable in adulthood. Research supported by Heinz Family Foundation and Cure Autism Now Foundation.

474 POSTNATAL EXPOSURE TO THIMEROSAL ALTERS IMMUNOLOGICAL FUNCTION IN ADULT MICE.

M. M. Peden-Adams[1], J. EuDaly[1], H. Lauren[1], J. Smythe[1] and D. E. Keil[2, 1]. [1]*Medical University of South Carolina, Charleston, SC and* [2]*NIOSH, Morgantown, WV.*

Infantile autism (IA) is a neurodevelopmental syndrome found in 1-5 of every 10, 000 children. The cause of IA is unknown; however, exposure to mercury is thought to contribute to this syndrome. More specifically, childrens vaccines containing mercuric compounds such as Thimerosal (TH) have been suggested to increase risk for acquiring neurodevelopmental problems such as autism. Pathophysiological links to autism include effects such as hyperserotoninemia, decreased T-cell proliferative function and activation, increased soluble IL-2 levels in serum, decreased CD8+ cells, decreased NK cells, development of anti-brain autoantibodies, decreased cerebellum volume and Purkinje cell number, and increased brain size. TH contains ethylmercury and the most commonly reported effects include contact hypersensitivity, allergic contact dermatitis, and altered T-cell responses. As several of the reported pathophysiologies are related to altered immunity we assessed the effects of TH on immune function. B6C3F1 mice were injected i.p. with TH (0, 20, 100, or 200 µg/kg) at 7, 10, and 12 days of age. A

suite of immune parameters including secondary immune organ weights and cellularity, T- and B-cell proliferation, NK cell function, and T-cell immunophenotyping were assessed along with developmental markers such as eye opening, ear unfolding, body weight and length, and brain weight at 8 weeks of age. Body weight was decreased while brain weight was increased following treatment with 20 μg/kg TH. NK cell function was increased after exposure to 200 μg/kg TH. Thymus weight and cellularity were not altered; however, splenic cellularity was increased by the 100 and 200 μg/kg treatments. Splenic B220 cells were increased by the 100 and 200 μg/kg treatments while CD4-/CD8+ and CD4+/CD8- cells were increased after the 200 μg/kg exposure. This study identifies that postnatal exposure to TH can modulate the developing murine immune system.

475 DERMAL EXPOSURE TO JP-8 JET FUEL DURING PREGNANCY ALTERS IMMUNOLOGICAL FUNCTION IN F1 MICE.

D. E. Keil[1, 2], L. Butterworth[1], S. Azadi[1] and M. Peden-Adams[2]. *[1]NIOSH, Morgantown, WV and [2]Medical University of South Carolina, Charleston, SC.*

Approximately 5 billion gallons of JP-8 jet fuel (JP-8) are used annually by the United States Department of Defense (DOD) making this the single largest chemical exposure to military personnel. In addition, the a*viat*ion industry extensively uses commercial jet fuel which is essentially JP-8 without certain additives for anticing, corrosion, and static dissipation. Civilian exposure can occur as well through environmentally contaminated sites where jet fuel has been reportedly spilled or leaked from supply lines or storage tanks. Many reports indicate that JP-8 alters immunological function in adult rodents. However, little is known regarding its impact on the developing immune system. Therefore, C57BL/6N pregnant dams (mated with C3H/HeJ males) were dermally exposed with 10, 25, 50, or 75 μL neat applications of JP-8 daily during gestation days 6-15. F1 offspring were evaluated for immunological alterations at 3 and 8 weeks of age. There were no treatment effects on body, liver, kidney, or thymus mass. Although slight, an increase in spleen mass and cellularity was observed at both 3 and 8 weeks. T-cell proliferation following anti-CD3 stimulation was not affected at either age. However, IgM plaque forming cell (PFC) responses were significantly suppressed in 3 week old pups exposed to 50 or 75 μL JP-8 and in all treatment groups at 8 weeks of age. Our data indicate that prenatal dermal exposure to JP-8 can affect the developing murine fetus resulting in impaired humoral immune responses detectable at adulthood.

476 PHENOTYPICALLY ALTERED MURINE BONE MARROW SUBSETS EXPRESS AND ACTIVATE THE AHR IN RESPONSE TO TCDD.

A. Wyman and T. A. Gasiewicz. *Environmental Medicine, University of Rochester, Rochester, NY.*

2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), acting through the aromatic hydrocarbon receptor (AhR), elicits numerous toxicological effects, including those to the bone marrow (BM). Phenotypic alterations to BM subsets have been demonstrated, but the mechanism for these alterations is unknown. Additionally, the presence and functionality of AhR has been shown in crude BM but not in phenotypically defined subsets. To address this, crude BM from male C57Bl6 mice was harvested, stained with either biotinylated anti-B220 or lineage specific antibodies, and enriched for B220+ cells or Lin- cells using magnetic separation. Real-time RT-PCR and Western blot indicated the presence of AhR in both subsets of BM. The presence of Arnt protein in Lin- subsets was also confirmed by Western blot. Activation of AhR was assessed using a transgenic mouse line expressing LacZ under the control of dioxin responsive elements (DREs) in the promoter region. After 10h treatment, LacZ mRNA was induced in mice treated with TCDD as compared to vehicle. Phenotypic changes in progenitor cells were assessed by dosing C57Bl6 male mice with 30 mg/kg BW TCDD in olive oil (or vehicle alone). BM was harvested at 12, 24, 48, 72 hours, and 5, 7, 9, 11, 13, and 15 days, enriched for Lin- cells by magnetic depletion and stained with a cocktail of antibodies: Sca-1 PE, cKit APC, and either CD34 FITC or TdT-FITC. When compared to vehicle, TCDD-treated cKit hi/Sca-1+ cell populations were significantly increased. The increase peaked at 250% of vehicle at 5 days, and decreased to near vehicle levels by day 15. cKit lo/Sca-1+ cell populations increased less, peaking at 150% of vehicle at 9 days. cKit/Sca-1/34+ cells showed even less of an increase, peaking at approximately 110% at 3 and 9 days post treatment. TdT+ cells decreased with TCDD treatment but recovered to vehicle levels by 13 days. The data suggests a more profound effect of TCDD treatment upon less committed cell populations, mediated by the presence of the AhR. (Funded by NIEHS Grant ES04862, Training Grant ES07026 and Center Grant ES01247)

477 REDUCTION IN THE NUMBER OF SUPERANTIGEN-SPECIFIC T CELL DIVISIONS INDUCED BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN RESULTS FROM INCREASED APOPTOSIS.

L. S. Faulconer[1], I. A. Camacho[1], P. S. Nagarkatti[1] and M. Nagarkatti[2]. *[1]Department Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA and [2]Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA.*

The precise mechanism by which 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) decreases antigen-specific T cell responsiveness is not clear. In the current study, we investigated the ability of T cells activated with staphylococcal enterotoxin A (SEA) to divide following exposure to TCDD by labeling the cells with 5, 6-carboxyfluorescein diacetate succinimidyl ester (CFSE) and analyzing them using a flow cytometer. To this end, female C57BL/6 mice were injected i.p. with 10 μg/kg bodyweight of TCDD followed by hind footpad injections of 10μg/footpad SEA. The popliteal lymph nodes (LN) were harvested on days one through four, labeled with CFSE and analyzed using a flow cytometer following 1-4 days of *in vitro* culture. In addition, the lymphocytes were analyzed for cells expressing Vβ3 or Vβ11 that become activated by SEA, as well as tested for apoptosis using TUNEL assay. Analysis using CFSE showed that TCDD-exposed lymphocytes exhibited a clear reduction in the number of cell divisions when compared to the vehicle controls. Even after reactivation *in vitro* with SEA, the lymphocytes from TCDD-treated mice demonstrated a decrease in the number of cell cycle progressions when compared to vehicle-treated mice. Also, exposure to TCDD caused a decrease in the percentage and total numbers of Vβ3+ and Vβ11+ cells. Moreover, increased levels of apoptosis were detected in the lymph nodes and in Vβ3/Vβ11 T cell subpopulations following TCDD treatment. Together, these data demonstrate that TCDD-induced apoptosis in SEA-activated T cells *in vivo* may account for decreased cell division and a consequent immunosuppression. (This work was supported in part by grants from National Institutes of Health R01ES09098, R01DA016545, R01AI053703, F31ES11562, R21DA014885, and R01HL058641)

478 EVIDENCE FOR INDUCTION OF APOPTOSIS IN T CELLS FROM MURINE FETAL THYMUS FOLLOWING PERINATAL EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).

P. S. Nagarkatti[1], I. A. Camacho[2] and M. Nagarkatti[2]. *[1]Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA and [2]Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA.*

Perinatal exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) causes thymic atrophy, but the precise mechanism of such toxicity remains unresolved. The current study investigated the role of apoptosis in TCDD-induced thymic involution following perinatal exposure to TCDD. To this end, C57BL/6 pregnant mice were injected intraperitoneally on gestational day (gd) 14 with a single dose of 10 μg/kg TCDD. Analysis of the thymus on gd-15, gd-16, gd-17, gd-18 and on postnatal day (PD) 1, showed a remarkable reduction in thymic cellularity 3-7 days after TCDD exposure. TCDD treatment also caused marked changes in the proportions of T-cell subsets, particularly on gd-17 and gd-18 thymocytes. *In vitro* culture of TCDD-exposed fetal or neonatal thymocytes showed increased apoptosis when compared to the controls, which peaked on gd-17. Triple-color staining involving CD4, CD8 and TUNEL showed that all four subpopulations of T cells underwent apoptosis following TCDD exposure, with the double-positive T cells undergoing the highest level. Moreover, increased cleavage of caspase-3 was seen when TCDD-exposed gd-17 thymocytes were directly tested. Furthermore, apoptosis-associated phenotypic changes were found in TCDD-treated neonatal thymocytes, which exhibited an increase in expression of CD3, αβTCR, IL-2R and CD44, but a decrease of CD4, CD8 and J11d markers. Finally, TCDD-exposed fetal and neonatal thymocytes had higher levels of Fas, TRAIL and DR5 mRNA, but the levels of Bcl-2, Bcl-xL and Bax were either unaltered or changed moderately. Taken together, these results suggest that TCDD-induced thymic atrophy following perinatal exposure may result, at least in part, from increased apoptosis mediated by death receptor pathway involving Fas, TRAIL and DR5. (This work was funded in part by grants from National Institutes of Health R01ES09098, R01 AI053703, R01 DA016545, F31ES11562, R21DA014885 and R01HL058641).

479 CONSEQUENCES OF TCDD EXPOSURE ON THE MIGRATION, PROLIFERATION, AND SURVIVAL OF ANTIGEN-SPECIFIC T CELLS.

C. Funatake[1], L. Steppan[1], E. Spanjaard[2], A. Marshak-Rothstein[2] and N. Kerkvliet[1]. *[1]Oregon State University, Corvallis, OR and [2]Boston University School of Medicine, Boston, MA.*

Previous studies have shown that TCDD causes a significant decline in the number of antigen-specific DO11.10 T cells in the spleens of mice following immunization with OVA. The loss of T cells follows what appears to be enhanced T cell activation

(primarily evident as decreased expression of CD62L and, to a lesser extent, enhanced cell cycling) in TCDD-treated mice on days 2 and 3 post-antigen. In the studies presented here, we examined three potential mechanisms for the loss of antigen-activated T cells in TCDD-treated mice: enhanced emigration out of the spleen, premature cessation of proliferation, and induction of cell death. TCDD significantly enhanced the number of antigen-specific T cells in the blood on day 3 post-antigen, which is consistent with TCDD promoting T cell activation. However the activated T cells in TCDD-treated mice failed to upregulate expression of CD11a, which is important for the extravasation of activated T cells. Thus, the T cells may have been trapped in the blood. CSFE staining showed that antigen-activated T cells continued to proliferate in vehicle-treated mice on day 4 after antigen exposure, whereas no additional cycling was observed in T cells from TCDD-treated mice after day 3. TCDD also induced an increase in apoptosis of activated T cells but not until days 5 and 6, arguing against enhanced cell death as the precipitating cause of the decline in T cells. Since T cells from D011.10$^{lpr/lpr}$ mice declined in a similar manner as normal T cells, the depletion of antigen-activated T cells by TCDD did not occur by a Fas-dependent process. Gene array analysis on day 3 indicated that TCDD increased the expression of several genes associated with cell survival/death. Taken together, these results suggest that TCDD induces an early but dysregulated activation of T cells, resulting in altered migration, truncated proliferation and reduced survival. Supported by NIH Grants P01ES00040, P30ES00210 and T32ES07060.

480 EXAMINING POSSIBLE MECHANISMS UNDERLYING PULMONARY NEUTROPHILIA IN VIRUS-INFECTED MICE TREATED WITH TCDD.

S. Teske[2, 1], L. Harrison[1], J. Neumiller[1] and B. Lawrence[1, 2]. [1]*Pharmaceutical Sciences, Washington State University, Pullman, WA and* [2]*Pharmacology/Toxicology Graduate Program, Washington State University, Pullman, WA.*

The immune system is one of the most sensitive targets of the pollutant 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). Our laboratory studies this immunosuppression using a murine-adapted model of human influenza virus infection. In addition to impairing the adaptive immune response to influenza virus, exposure to TCDD induces pulmonary neutrophilia. Neutrophils release cytotoxic meditors at the site of infection, and excess mediator release can damage healthy tissue. Neutrophil-mediated tissue insult may explain the enhanced mortality that we observe in virus-infected mice treated with TCDD. In fact, *in vivo* depletion of neutrophils improves host resistance in TCDD-treated mice. To determine the mechanism leading to pulmonary neutrophilia, we examined whether neutrophil chemoattractants are enhanced in the lung, adhesion molecules on neutrophils are up-regulated, or neutrophil apoptosis is delayed. Among neutrophil chemoattractants, we evaluated tumor necrosis factor (TNF)-α, interleukin (IL)-1, macrophage inflammatory protein (MIP)-1α, MIP-2, keratinocyte chemoattractant (KC), and lipopolysaccharide-induced CXC chemokine (LIX). Our results show similar levels of these mediators in both treatment groups. Using flow cytometry, we examined the following adhesion molecules on pulmonary neutrophils: CD11a, CD11b, CD11c, CD49d, CD31, CD38, and CD62L. Treatment with TCDD does not enhance the expression of any of these molecules. We studied neutrophil apoptosis using Annexin V and 7-AAD to distinguish *via*ble, apoptotic, and necrotic neutrophils. We also measured CD16 expression, since low levels of CD16 correlate with neutrophils undergoing apoptosis. Although we observed decreased CD16 expression on neutrophils from TCDD-exposed mice, exposure to TCDD did not alter the percent of apoptotic neutrophils. Collectively, our data indicate that TCDD likely promotes neutrophil recruitment *via* indirect mechanisms, such as changes in adhesion molecules on lung epithelium and endothelium.

481 EFFECTS OF SILICA ON *IN VITRO*-GENERATED MACROPHAGE SUBSETS.

C. T. Migliaccio and A. Holian. *Department of Biomedical & Pharmaceutical Sciences, Center for Environmental Health Sciences, University of Montana, Missoula, MT.*

Inhalation of silica can result in chronic inflammation and fibrosis with a decrease in lung function and an increase in susceptibility to autoimmune diseases. Under normal conditions the lung is considered an immune-suppressed organ; however, following exposure to silica the environment becomes chronically immune-activated. A major component of lung immunology is the pulmonary macrophage. Alveolar macrophages (AM) have been implicated as playing a pivotal role in silicosis, including phagocytic uptake of silica. In general, macrophages have the ability to regulate immune responses by performing the role of intermediary between the innate and adaptive immune systems. Recently macrophage subtypes have been described in the mouse with categories including alternatively activated, classically activated, and type-II activated. The subtypes are defined by surface protein expression (i.e. MHC class II, scavenger receptor) and cytokine profile and have potential counterparts in the human. Using bone marrow-derived macrophages we generated these three subtypes by culturing with interferon (IFN)-gamma, interleukin (IL)-13, or IL-4 with either soluble or complexed antigen. We then examined the effects of silica exposure on the subtypes for phenotypic changes as determined by flow cytometry and functional assays. Our functional assays include macrophage cytokine ELISA and antigen presenting cell (APC) cultures and cytokine ELISA. Results demonstrated differential surface marker expression among the subtypes, as well as APC capacity. The type II, and to a lesser extent the classic and alternative, subsets have presented with an increase in APC function when exposed to silica. Our current studies suggest that an increase in macrophage function as a result of silica exposure could be an explanation as to the chronic inflammatory environment presented in silicosis. This work is supported by NIH grants ES 04804 and RR-017670

482 SILICA STIMULATES PHOSPHORYLATION AND ACTIVATION OF AKT IN MURINE ALVEOLAR MACROPHAGES.

C. A. Wishcamper and A. Holian. *Pharmaceutical and Biomedical Sciences, University of Montana, Missoula, MT.*

Silicosis is an occupational lung disease caused by the inhalation of silica particles which results in chronic inflammation and progressive pulmonary fibrosis. Phagocytosis of silica by alveolar macrophages (AM) and subsequent activation is a crucial step in the pathogenesis of silicosis. AM release oxygen free radicals and lysosomal enzymes, as well as proinflammatory mediators that propagate inflammation, induce programmed cell death, and promote aberrant cell survival. However, the participation of specific intracellular signaling pathways in regulating the AM response to silica have not been fully elucidated. The serine/threonine protein kinase Akt is activated downstream of phosphatidylinositol 3-kinase and has been shown to regulate diverse cellular processes implicated in mediating a variety of biological responses that include inhibition of apoptosis, promotion of cellular growth, and control of metabolism. To elucidate the role of Akt in silica induced chronic inflammation, we examined lavage fluid and lung sections from Balb/c mice post-silica exposure. Mice received either no treatment or 500μg silica once a week for four weeks. Lavage fluid was collected using 1ml PBS, while whole lungs were fixed and processed for trichrome and immunostained for activated Akt (n=5). Following chronic silica exposure, significantly increased levels of IL-10 and TNFα ($p<0.05$, $p<0.10$, respectively) were observed in the lavage fluid of silica exposed mice compared to no treatment. Fluorescence immunostaining demonstrated visible increases in the presence of the activated form of Akt between 0 and 8 weeks, with decreased immunostaining for phospho-Akt observed by 16 weeks. These data emphasize the need to examine the Akt signaling cascade in the AM response to silica exposure. Furthermore, a better understanding of the molecular events involved in regulating the AM response to silica exposure may permit targeting of specific biochemical pathways for more effective diagnosis and treatment of occupational lung diseases.

483 REPEATED EXPOSURE TO DIESEL EXHAUST PARTICLES CAUSES SUPPRESSION OF CELL-MEDIATED IMMUNE RESPONSES TO *LISTERIA* INFECTION IN BROWN NORWAY RATS.

X. J. Yin[1], C. C. Dong[1], J. Y. Ma[2], J. M. Antonini[2], J. R. Roberts[2] and J. K. Ma[1]. [1]*School of Pharmacy, West Virginia University, Morgantown, WV and* [2]*HELD, NIOSH, Morgantown, WV.*

Diesel exhaust particles (DEP) have been shown to alter pulmonary immune responses to bacterial infection. However, the *in vivo* effect of DEP exposure on the T cell-mediated immune responses against bacterial infection has not been clearly demonstrated. Here, we examined the effects of repeated DEP exposure on pulmonary responses to bacterial infection. Brown Norway rats were exposed to DEP by inhalation at 20.62 ± 1.31 mg/m^3, 4 hr/day for 5 days, followed by intratracheal inoculation with 100, 000 *Listeria* at 2 hours after the last DEP exposure. DEP-exposed rats showed a significant increase in lung bacterial load at both 3 and 7 days post-infection. The repeated DEP exposure was shown to suppress both the innate, orchestrated by alveolar macrophages (AM), and T-cell mediated responses to *Listeria*. DEP inhibited production of interleukin-1β (IL-1β), tumor necrosis factor-α, and IL-12 by AM, but enhanced *Listeria*-induced production of IL-10, which has been shown to prolong the survival of intracellular pathogens such as *Listeria*. DEP exposure also suppressed the development of bacteria-specific lymphocytes from lung-draining lymph nodes, as indicated by the decreased numbers of T lymphocytes and their $CD4^+$ and $CD8^+$ subsets. In addition, the DEP exposure markedly inhibited the *Listeria*-induced lymphocyte secretion of IL-2 at day 7, IL-10 at days 3 and 7, and interferon-γ at days 3 to 10 post-infection when compared to air-exposed controls. These results show a sustained pattern of down-regulation of T-cell mediated immune responses by repeated low-dose DEP exposure,

which is different from the results of a single high dose exposure (100 mg/m^3, 4 hours), where the acute effect of DEP aggravated bacteria infection but triggered a strong T cell-mediated immunity.

484 PARTICULATE MATTER IMMUNOMODULATORY EFFECT ON THE PROGRESSION OF AUTOIMMUNE DISEASE IN NEW ZEALAND MIXED MICE.

M. Hassani, J. M. Brown and A. Holian. *Center for Environmental Health Sciences, The University of Montana, Missoula, MT.*

In this study we examined whether particulate matter exposures would impact the progression of autoimmune disease in a lupus-prone New Zealand mixed (NZM) model. NZM mice were intranasally instilled with either 30 µl saline or a 30 µl saline suspension of 500 µg acid washed PM1648, PM1648 or PM2.5 collected in Houston, TX, once a week for 4 weeks. Mortality and proteinuria levels did not significantly change among the groups. Levels of anti-nuclear antibodies (ANA) were evaluated 4 and 16 weeks following PM instillation by ELISA. All three particulates significantly suppressed the natural development of ANA in the NZM mice. There were no significant differences in the serum levels of IFN-γ, IL-12, and IL-4 among the groups 17 weeks post-PM instillation except for IL-10, which was significantly decreased in the particulate-instilled mice. IgG serum levels were significantly decreased in the PM1648 and PM2.5 instilled mice at 6 weeks following particulate instillation as compared to the saline and acid washed PM1648 instilled groups. Serum levels of IgG subclasses were also measured; IgG2b serum levels were significantly increased in the PM2.5 instilled mice as compared to the saline instilled group at 4 weeks following PM instillation, whereas the PM1648 and PM2.5 instilled mice showed significant decrease in IgG3 serum levels as compared to the control at the same time point. Significant reduction in the levels of serum IgM as compared to the saline group was observed in the PM1648 instilled mice during the first 6 weeks post-particulate-instillation period. There were also slight increases in the amount of immune cell infiltration and fibrosis within the PM 1648 and PM2.5 groups in comparison with the control group. Taken together, the results support the notion that PM modulates the immune system towards a Th2 response, thereby decreasing the effect of a Th1 mechanism of autoimmune disease in the NZM model. This work was supported by NIH grants ES 11120 and RR-017670.

485 TRICHLOROETHYLENE DOES NOT ACCELERATE AUTOIMMUNE DIABETES IN NOD MICE.

G. Ravel[1,2], M. Christ[1], F. Condevaux[1] and J. Descotes[2]. *[1]MDS Pharmacology Services, L'Arbresle, France and [2]Poison Center & INSERM University 503, Lyon, France.*

Trichloroethylene (TCE) is an environmental contaminant suspected of inducing immunological changes in human after exposure *via* the drinking water. Previous studies showed that TCE exposure could be linked to autoimmune diseases in susceptible human beings. Increases in antinuclear auto-antibodies and serum immunoglobulin levels, and activation of CD4+ T cells producing Th1-type cytokines have been observed in TCE-treated female MRL +/+ mice. In order to confirm these findings, the effects of TCE exposure were evaluated in another animal model of autoimmune diabetes, the female non obese diabetics (NOD) mouse. Sixty mice were given 0 or 5 mg/ml TCE *via* the drinking water for 12 weeks starting at 11 weeks of age. Blood glucose levels were measured every two weeks. Blood samples were analysed every 4 weeks for Th1/Th2 cytokine profile including IL-4, IL-5, IL-2, IL-12 and IFN-γ by flow cytometry. Cell surface expression of CD44, CD45RB and CD54 on peripheral and splenic CD4+ T lymphocytes was analyzed to evidence possible T cell activation in TCE-treated mice. The mice were killed after 4, 8 or 12 weeks of treatment and a fragment of the spleen was isolated for lymphocyte subset analysis. There were no treatment-related effects on blood glucose levels or on histopathology findings at any time points. TCE did not affect the Th1/Th2 cytokine balance and peripheral and splenic T lymphocyte analysis did not indicate T cell activation. Our results suggest that TCE exposure does not accelerate autoimmune diabetes in female NOD mice. This finding is in sharp contrast with effects previously reported in female MRL +/+ mice. It is also concluded that the selection of relevant animal species or strains for the prediction of autoimmunity in preclinical immunotoxicity evaluation is not straightforward.

486 ANALYSIS OF TARGET ANTIGENS FOR AUTOANTIBODIES ASSOCIATED WITH ASBESTOS EXPOSURE.

I. Leal, A. Holian and J. Pfau. *Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT.*

Environmental exposures to particles such as asbestos have been associated with systemic autoimmune disease. Previous studies on a population in Libby MT that have been significantly exposed to asbestos have found that these individuals appear to have an increased frequency of positive ANA (antinuclear antibodies) tests compared to a matched population in Missoula, MT. The goal of this study was to determine if previous positive ANA tests performed in this population were specific for chromatin and double stranded DNA (dsDNA) through the use of Enzyme Linked Immunosorbent assays (ELISAs) and Crithidia luciliae testing, respectively. Our study also aimed to determine if the anti-ENA (Extractable Nuclear Antigen) levels would also be higher in these individuals measured by ELISA, and addressable bead array (LUMINEX). Additionally, mean autoantibody titer for ANAs were performed to determine if there would be a significant change between exposed individuals and those in the control population. The results showed that the percent ANA positive and the mean autoantibody titer for ANAs were significantly higher in the Libby group than those of the Missoula group. The results of tests for antibodies to chromatin and dsDNA on 40 coded samples proved to be negative suggesting that the target for ANA antibodies in these individuals may be histone. The scl-70 ELISA and ENA addressable bead array indicated that those individuals exposed to asbestos had significantly higher ENA scores, particularly for Scl-70, SS-A and SS-B than those in the Missoula control population. These antibodies are commonly seen in autoimmune diseases such as scleroderma and Sjogrens syndrome. These results demonstrate the upregulation of specific autoantibodies in an asbestos-exposed population, and begin to identify some of the specific targets for these autoantibodies. This work was supported by NSF EPSCoR and CDC Grant CCR-822092.

487 DIFFERENCES IN HEPATOTOXICOLOGICAL PROFILE OF ET-743 BETWEEN SPRAGUE-DAWLEY RATS AND CYNOMOLGUS MONKEYS.

R. De Coster[1], J. Verbeeck[1], A. Vynckier[1], A. Looszova[1], K. Anciaux[1], L. Lammens[1], N. Bode[1], W. Coussement[1] and P. Aviles[2]. *[1]Global Preclinical Development, Johnson&Johnson Pharmaceutical Research&Development, Beerse, Belgium and [2]PharmaMar, Colmenar Viejo, Spain.*

Yondelis™ (trabectedin, ET-743) is a marine-derived compound under investigation in phase II trials and has shown activity in ovarian, breast and advanced pretreated soft tissue sarcoma. In patients, drug-induced toxicities are non-cumulative neutropenia, reversible increases in transaminases and fatigue. The hepatotoxic potential of Yondelis™ was evaluated in repeated dose studies in rodent (Sprague-Dawley rats) and non-rodent models (Cynomolgus monkeys). The latter were chosen as the preferred non-rodent species due to the similarities in metabolic profile to that of humans. In these studies, ET-743 was administered *via* a 3-hour intravenous infusion every 3 weeks for 3 (rat) or 4 (monkey) cycles, or weekly for 3 weeks followed by 1 week without treatment (3 cycles). After repeated dosing in rats, mortality was observed at 50 µg/kg in females and at 50-75 µg/kg in males. A pronounced dose-dependent and only partially reversible hepatotoxicity (increased transaminases, hepatocytic necrosis, bile duct inflammation and proliferation, cholangitis) was noted, and toxicity was cumulative and more pronounced in female rats. The difference in gender sensitivity in rats is likely to be linked to differences in the metabolic profile, biliary excretion and/or liver retention. Administration of a weekly 30 µg/kg-dose was better tolerated by Cynomolgus monkeys than dosing 70 µg/kg every 3 weeks. Hepatotoxicity was less pronounced than in rats and non-cumulative (increased transaminases, hepatocellular hypertrophy, hepatocytic degeneration/necrosis and inflammatory cells in the sinusoids and portal tracts). At 50 µg/kg, exposure to ET-743 was higher in monkeys (23-28 µg.h/L) than in rats (1.7-8.6 µg.h/L), without gender difference. The similar profile of hepatic changes induced by chronic ET-743 treatment in humans and monkeys suggest that the Cynomolgus monkey is a more relevant model for human Yondelis™ hepatotoxicity than the rat.

488 PROTECTION OF FUMONISIN B_1 hepatotoxicity by silymarin and myriocin in female balb/c mice.

Q. He, J. Kim and R. P. Sharma. *Department of Physiology & Pharmacology, The University of Georgia, Athens, GA.*

Fumonisin B_1 (FB_1) is a toxic and carcinogenic mycotoxin produced by *Fusarium Verticillioides* found on corn. The biological effects of FB_1 are mainly due to disruption of sphingolipid metabolism resulting from its inhibition of ceramide synthase. Silymarin has a strong protective effect against numerous hepatotoxicants. Myriocin is a selective inhibitor of serine palmitoyltransferase (SPT), the first key enzyme in *de novo* synthesis of sphingolipids. Both silymarin and myriocin protected porcine renal epithelial cells from FB_1 toxicity. In the present study we examined the protective effects of silymarin and myriocin on FB_1 hepatotoxicity in female Balb/c mice. Mice were treated daily with 750 mg/kg silymarin by gavage 16 hr prior or 1 mg/kg myriocin intraperitoneally 30 min before subcutaneous injections of either vehicle or FB_1 at 2.25 mg/kg.day for 3 consecutive days. Silymarin, to a greater extent than myriocin, attenuated FB_1-induced elevation of plasma alanine aminotransferase and aspartate aminotransferase, and hepatocellular apopto-

sis. Both agents reversed FB_1-induced expression of tumor necrosis factor (TNF) receptor 1, and selected cytokines, TNF α, TNF-related apoptosis-inducing ligand, lymphotoxin β, and interferon γ to the basal levels, except silymarin did not reduce FB_1 induced transforming growth factor β1. Silymarin augmented whereas myriocin attenuated FB_1-induced accumulation of free sphingoid bases. The increase of FB_1-caused free sphingoid bases by silymarin was not attributed to SPT activation. Both silymarin and myriocin blocked activation of hepatic SPT from FB_1 exposure. Results indicate that silymarin is more effective than myriocin in protecting FB_1 hepatotoxicity; the protective role of silymarin in FB_1 toxicity is likely in part through blocking the effects of free sphingoid bases and prevention of hepatic cytokine induction. Moreover, five-day treatment of mice with myriocin and FB_1 was lethal. (Supported by ES09403 from PHS).

489 COCAINE HEPATOTOXICITY AND ITS POTENTIATION BY LIPOPOLYSACCHARIDE: TREATMENT AND GENDER EFFECT.

T. Visalli[1], R. Turkall[2, 1] and M. S. Abdel-Rahman[1]. *[1]Pharmacology/Physiology, UMDNJ, Newark, NJ and [2]Clinical Laboratory Sciences, UMDNJ, Newark, NJ.*

The degree of hepatotoxicity after cocaine and lipopolysaccharide (LPS) doubled to tripled after seven-day administration as compared to 5-day administration in male mice. This study was conducted to investigate the effect of a seven-day treatment as well as the influence of gender on cocaine hepatotoxicity (CH) and upon LPS potentiation of CH. Male and female CF-1 mice were orally administered 20mg/kg body weight cocaine hydrochloride once daily for 7 days. Four hours after the last cocaine administration, the mice were administered 12 x 10^6 EU LPS/kg (or equal volume of sterile saline) intraperitoneally. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated as indices of liver injury. Blood and liver glutathione (GSH), glutathione reductase (GRx), and catalase (CAT) activities were also determined to investigate the oxidation stress of the treatment. The results demonstrate that gender has a profound impact on CH and LPS potentiation of CH. Serum ALT and AST concentrations were elevated in all males receiving cocaine alone or cocaine + LPS. Further, blood GSH and CAT were decreased and GRx activity was elevated in the same males. In addition, histological analysis revealed a high degree of focal necrosis in the male cocaine group, and severe sub-massive necrosis in the male cocaine + LPS group. Unlike males, females showed no effect of either cocaine alone or cocaine + LPS treatments. In conclusion, these results indicate that gender plays a significant role in CH and its potentiation by LPS. Also, lengthening the administration by two treatments increased the severity of cocaine + LPS hepatotoxicity dramatically. Investigation regarding the mechanism of action of the gender role is ongoing in our laboratory.

490 TNBS-INDUCED COLITIS IN THE RAT AS A MODEL FOR INFLAMMATORY BOWEL DISEASE.

S. Groom[1], K. Beard[1], E. Jacquinet[1] and N. Hamelin[1]. *[1]CTBR, Senneville, QC, Canada and [2]IPN, CTBR, Senneville, QC, Canada.* Sponsor: M. Vézina.

TNBS-induced colitis in the rat has been widely reported to be an appropriate model for inflammatory bowel disease, although the mechanism responsible for the colitis (i.e. immune-mediated or corrosivity) has remained the subject of discussion. This poster presents data generated from a series of 4-day studies that assessed the relative contribution of TNBS solution corrosivity to the induction of ulcerative colitis. In addition, dexamethasone was validated as a positive control such that this model can be used to screen compounds intended to treat the immune-mediated mechanisms associated with inflammatory bowel disease. Colonic instillation of TNBS doses of 4, 10 and 25 mg/rat each resulted in a severe ulcerative colitis. Colon weight, cytokine profiles and assessments of myeloperoxidase activity within colonic tissue provided data to support the efficacy of dexamethasone as a treatment for inflammatory bowel disease. However, efficacy was not evident within the colon macroscopic damage scores. These data suggest that the colitis induced by colonic instillation of TNBS is primarily one of corrosivity, however, reductions in the immune-mediated responses associated with inflammatory bowel disease were demonstrated by use of dexamethasone. Therefore, we conclude that TNBS-induced colitis in the rat can be used to provide data to support the proposed efficacy of compounds intended to treat inflammatory bowel disease.

491 HYPOXIA IN RAT LIVER AFTER MONOCROTALINE EXPOSURE.

B. L. Copple, P. E. Ganey and R. A. Roth. *Department of Pharmacology and Toxicology, Institute for Environmental Toxicology, and National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI.*

Monocrotaline (MCT) is a pyrrolizidine alkaloid plant toxin that produces hepatotoxicity in humans and animals. Administration of 300 mg MCT/kg to rats causes rapid sinusoidal endothelial cell (SEC) injury, hemorrhage, pooling of blood and fibrin deposition in centrilobular regions of liver. These events precede hepatic parenchymal cell (HPC) injury and produce marked changes to the structure of the microvasculature of the liver, which could interrupt blood flow and produce hypoxia in downstream regions. To test the hypothesis that hypoxia occurs in the liver after MCT exposure, rats were treated with 300 mg MCT/kg, and hypoxia was detected immunohistochemically. MCT produced significant hypoxia in centrilobular regions of livers by 8 hours after treatment. Inasmuch as fibrin deposition can impede blood flow, the effect of anticoagulant treatment on MCT-induced hypoxia and hepatocellular injury was determined. Administration of warfarin to MCT-treated rats reduced hypoxia in the liver by approximately 70% and decreased liver injury, suggesting that fibrin deposition plays a causal role in the development of hypoxia in the liver. Conversely, administration of L-NAME, a nonspecific inhibitor of nitric oxide synthases (NOSs), enhanced MCT-induced hypoxia and HPC injury. L-NAME did not, however, affect SEC injury or coagulation system activation. Results from these studies show that hypoxia occurs in the liver after MCT exposure. Furthermore, hypoxia precedes HPC injury, and manipulations that modify hypoxia also modulate HPC injury. (Supported by NIH Grants ES04139 and DK50728)

492 MODES OF CELL DEATH IN RAT LIVER AFTER MONOCROTALINE EXPOSURE.

R. A. Roth[1], B. L. Copple[1], C. M. Rondelli[1], J. F. Maddox[1], N. S. Hoglen[2] and P. E. Ganey[1]. *[1]Pharmacology and Toxicology, Michigan State University, East Lansing, MI and [2]IDUN Pharmaceuticals, San Diego, CA.*

Monocrotaline (MCT) is a pyrrolizidine alkaloid (PA) plant toxin that produces sinusoidal endothelial cell (SEC) injury, hemorrhage, fibrin deposition and coagulative hepatic parenchymal cell (HPC) oncosis in centrilobular regions of rat livers. Cells with apoptotic morphology have been observed in the livers of animals exposed to other PAs. Whether apoptosis occurs in the livers of MCT-treated animals and whether it is required for full manifestation of pathological changes is not known. To determine this, rats were treated with 300 mg MCT/kg, and apoptosis was detected by transmission electron microscopy and the TUNEL (TdT-mediated dUTP nick end labeling) assay. MCT produced significant apoptosis in the liver by 4 hours after treatment. To determine if MCT kills cultured HPCs by apoptosis, HPCs were isolated from the livers of rats and exposed to MCT. MCT caused a concentration-dependent release of alanine aminotransferase (ALT), a marker of HPC injury. Furthermore, caspase 3 was activated and TUNEL staining increased in MCT-treated HPCs. MCT-induced TUNEL staining and release of ALT into the medium were completely prevented by the pancaspase inhibitors z-VAD.fmk and IDN-7314, suggesting that MCT kills cultured HPCs by apoptosis. To determine if caspase inhibition prevents MCT-induced apoptosis in the liver, rats were cotreated with MCT and IDN-7314. IDN-7314 reduced MCT-induced TUNEL staining in the liver and release of ALT into the plasma. Morphometric analysis confirmed that IDN-7314 reduced HPC oncosis in the liver by approximately 50%. Results from these studies show that MCT kills cultured HPCs by apoptosis but causes both oncosis and apoptosis in the liver *in vivo.* Furthermore, caspase inhibition reduces both apoptosis and HPC oncosis in the liver after MCT exposure. (Supported by NIH ES04139)

493 ALTERED GENE EXPRESSION AS A CONTRIBUTING FACTOR TO LIVER INJURY IN RATS COTREATED WITH RANITIDINE AND LIPOPOLYSACCHARIDE.

J. P. Luyendyk[1], W. B. Mattes[2], J. F. Maddox[1], G. N. Cosma[2], P. E. Ganey[1] and R. A. Roth[1]. *[1]Pharmacology and Toxicology, Michigan State University, East Lansing, MI and [2]Investigative Toxicology, Pharmacia Corp., Kalamazoo, MI.*

Studies in rats have suggested a role for underlying inflammation in idiosyncratic liver injury from the histamine 2-receptor antagonist ranitidine (RAN). Coadministration of nonhepatotoxic doses of RAN and the inflammagen, bacterial lipopolysaccharide (LPS), to rats results in hepatocellular injury. We tested the hypothesis that hepatic gene expression changes could distinguish Vehicle-, LPS-, RAN-, and LPS/RAN-treated rats before the onset of significant liver injury in LPS/RAN-treated rats (i.e., 3 h post-treatment). Rats were treated with LPS (44 x 10^6 EU/kg, iv) or its vehicle, then two hours later with RAN (30 mg/kg, iv) or its vehicle. They were killed 3 h after RAN treatment, and liver samples were taken for evaluation of liver injury and RNA isolation. Hepatic parenchymal cell injury as estimated by increases in serum alanine aminotransferase activity was not significant by 3 h. Hierarchical clustering of gene expression data from Affymetrix U34A rat genome arrays grouped animals according to respective treatments. Relative to vehicle-treated controls, treatment with RAN and/or LPS altered hepatic expression of numerous genes, including ones encoding for products involved in inflammation, hypoxia, and cell death. Some of them were enhanced synergistically by LPS/RAN cotreatment. Within this latter group, real-time PCR confirmed robust changes in expression of B-cell translocation gene 2, early growth response-1, and

plasminogen activator inhibitor-1 (PAI-1) in cotreated rats. Consistent with the antifibrinolytic activity of PAI-1, significant fibrin deposition occurred only in livers of LPS/RAN-treated rats. This result suggests that expression of PAI-1 promotes fibrin deposition in liver sinusoids of LPS/RAN-treated rats and is consistent with the development of localized ischemia and consequent tissue hypoxia.

494 COMPARISON OF THE HEPATOCELLULAR TOXICITY OF THE ANTI-ANDROGEN FLUTAMIDE TO CYANO ANALOGS.

K. J. Coe[1], H. K. Ho[1], H. M. Holmes[1], Y. Jia[1], N. Fausto[2], S. A. Bruschi[1] and S. D. Nelson[1]. [1]*Medicinal Chemistry, University of Washington, Seattle, WA and* [2]*Pathology, University of Washington, Seattle, WA.*

Flutamide is an anti-androgen used in the treatment of metastatic prostate cancer. Its use has been curtailed due to its hepatotoxicity in a percentage of patients. Although it has been established that flutamide undergoes a high first-pass metabolism and can result in the formation of covalent adducts to microsomal proteins, the route of hepatocellular injury is not well understood. Flutamide possesses a nitroaromatic group, and it is postulated that this nitro group may mediate its route of toxicity. Two analogs of flutamide, where the nitro group is replaced with the cyano group, including the drug bicalutamide, were used to test this hypothesis in the established murine TAMH cell line. MTT cell *via*bility assays as well as confocal microscopy indicate that cells are more sensitive to flutamide than either of the cyano analogs. Western analysis shows that ATF3, a stress response transcription factor, is highly induced upon flutamide treatment, while HSP70i is not. Furthermore, drug-induced apoptosis does not appear to be a major route of toxicity of flutamide, as neither DNA laddering nor significant PARP cleavage were observed. (Supported by NIEHS Center Grant P30ES07033 and Pfizer Inc.)

495 ACETAMINOPHEN-INDUCED MITOCHONDRIAL PERMEABILITY TRANSITION IN ISOLATED MOUSE HEPATOCYTES.

A. B. Reid, R. C. Kurten, S. S. McCullough, R. W. Brock, G. Nowak and J. A. Hinson. *University of Arkansas for Medical Sciences, Little Rock, AR.*

Metabolism of acetaminophen (APAP) to N-acetyl-p-benzoquinoneimine, which depletes GSH and covalently binds to protein, is important in acetaminophen toxicity. However, cellular events leading to APAP-induced necrosis are poorly understood. We have utilized freshly isolated mouse hepatocytes to study mitochondrial permeability transition (MPT) in APAP toxicity. Incubation of APAP (1mM) with hepatocytes resulted in necrosis as indicated by increased permeability of the plasma membrane (measured using ALT release and propidium iodide-DNA binding). To separate metabolic events from later events in APAP toxicity, hepatocytes were preincubated with APAP (1mM) for 2 hr followed by centrifugation of the cells, washing the pelleted cells twice to remove the drug, and reincubation of cells in media. Control cells were treated identically except they were preincubated with media alone. At 2 hr, toxicity was not dramatically different between cells preincubated with APAP or media alone. Preincubation of hepatocytes with APAP followed by 5 hour incubation with media alone resulted in an 11-fold increase in ALT release and a 28-fold increase in propidium iodide fluorescence compared to control. Preincubation of hepatocytes with APAP followed by a 5 hr incubation with media containing cyclosporin A (10μM), a compound known to prevent MPT, resulted in a 79% decrease in ALT release and a 98% decrease in propidium iodide fluorescence. MPT was further analyzed using confocal scanning microscopy. The hepatocytes were preincubated with calcein AM followed by a wash to remove excess calcein AM. Subsequently, cells were incubated in media in the presence or absence of APAP (1mM) and analyzed by confocal scanning fluorescence microscopy. Cells incubated with media alone were shown to exclude calcein AM from the mitochondria. In cells incubated with APAP, the mitochondria did not exclude calcein AM suggesting MPT. These data indicate that MPT is an important event in APAP toxicity.

496 S-ADENOSYL-L-METHIONINE (SAME) REDUCES ACETAMINOPHEN HEPATOTOXICITY.

M. Terneus and M. Valentovic. *Pharmacology, Marshall University School of Medicine, Huntington, WV.*

The over-the-counter analgesic agent acetaminophen (APAP), when taken in excessive amounts induces hepatotoxicity in humans and animal models. The nutraceutical agent SAMe, has been reported to reduce the hepatic damage associated with repeated ethanol ingestion. Information is not currently available regarding the effect of SAMe on the hepatotoxicity of other xenobiotics. The following study examined whether SAMe could reduce APAP hepatic toxicity. Six to eight week old male C57BL/6 mice were treated with a single dose of 1 g/kg SAMe (intraperitoneal, ip) prior to administration of 500 mg/kg APAP (ip). Hepatic toxicity was evaluated 4 h after APAP administration. Liver weight (g/10g body wt) was increased from a vehicle (VEH) value of 0.48 ± 0.01 to 0.55± 0.01 ($p<0.05$) within 4 h in the APAP treated animals. Liver weight was comparable between the SAMe (0.45 ± 0.01) and the SAMe + APAP (0.46 ± 0.01) group. Alanine aminotransferase (ALT) activity was increased 70 fold by APAP relative to the VEH group. ALT levels were elevated less markedly in the SAMe + APAP group compared to the SAMe group. Hepatic lipid peroxidation was increased ($p<0.05$) 2 and 4 h after APAP treatment when compared to the VEH group. Administration of SAMe prevented the APAP induced rise in hepatic lipid peroxidation upon comparison of SAMe and SAMe + APAP groups. The increase in lipid peroxidation induced by APAP was associated with a marked depletion ($p<0.05$) of glutathione (GSH) at 2 and 4 h after APAP injection when compared to the VEH group. SAMe administration prevented GSH depletion associated with APAP treatment in the SAMe + APAP group at 2 and 4 h post APAP injection. These results indicate that SAMe reduces the hepatotoxicity of APAP. SAMe attenuation of APAP hepatotoxicity is mediated in part by maintaining GSH levels and reducing the extent of lipid peroxidation.

497 HEPATO-PROTECTIVE EFFECTS OF HC AGAINST ETHANOL-CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE IN RATS.

O. S. El-Tawil[1], A. M. Mohamadin[2] and A. B. Abdel-Naim[3]. [1]*Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt,* [2]*Tumor Marker Oncology Research Unit, Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt and* [3]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.*

Liver diseases represent a serious health problem in Egypt. Herbs are known to play a vital role in the management of various liver diseases. HC is a multi-herbal product containing clove, parsley, oak, ginger and cinnamon. The present work was designed to evaluate HC potential to protect against biochemical & histopathological alterations induced by alcohol-carbon tetrachloride (EOH + CCl4) in rats livers. An aqueous extract of HC was administered to rats with liver damage induced by EOH + CCl4. Biochemical parameters including serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phophatase (ALP) and bilirubin were estimated to assess the liver function. In addition, tissue total proteins, triglycerides, total cholesterol, reduced glutathione and thiobarbituric acid reactive substances (TBARS, as an index of lipid peroxidation) were determined. EOH + CCl4 caused profound liver damage, as evidenced by a rise in the levels of serum AST, ALT, GGT, ALP and bilirubin of hepatotoxic rats compared to the control group. Pre-treatment of rats with fresh aqueous extracts of HC reduced the EOH + CCl4- induced elevated levels of the above indices. Furthermore, HC significantly protected against EOH + CCl4-induced adverse effects on liver total proteins, triglycerides and cholesterol. EOH + CCl4 administration caused a significant decrease in liver GSH levels and a rise in TBARS concentration. HC pre-treatment restored liver GSH and prevented the elevation in liver TBARS levels. Histopathological examination clearly indicated that HC prevented centrilobular necrosis induced by EOH + CCl4 administration. In conclusion, HC exhibits significant hepatoprotective properties for treating EOH + CCl4-induced toxicity in rats.

498 ONSET OF 3-(3, 5-DICHLOROPHENYL)-2, 4-THIAZOLIDINEDIONE (DCPT)-INDUCED HEPATOTOXICITY IN RATS.

N. N. Patel, C. M. Crincoli and P. Harvison. *Department of Pharmaceutical Sciences, University of the Sciences in Philadelphia, Philadelphia, PA.*

Previously, we observed that DCPT caused progressive hepatotoxicity in Fischer 344 rats. Hepatic damage was evident within 24 h of administration of this compound. DCPT has a thiazolidinedione (TZD) ring, which is also present in insulin-sensitizing drugs, such as troglitazone, that are used for treatment of type II diabetes. Liver damage has been reported in some patients taking these drugs. Since DCPT may be useful for investigating the potential toxicity of TZD ring-containing compounds, it is important to fully characterize its effects in rats. The present study was therefore conducted to determine the onset of DCPT-induced hepatotoxicity. Male, Fischer 344 rats were administered DCPT (0.6 mmol/kg) i.p. in corn oil, and liver and kidney function were assessed at 3, 6, 12 and 24 h after dosing. Control animals received corn oil only. Compared to controls, rats that received DCPT exhibited mild to moderate ketonuria, starting at the 3 h time point. Diuresis was evident within 6 h post-dosing. Serum alanine aminotransferase (ALT) levels in corn oil-treated animals were 53.2±4.6, 51.6±0.8, 25.4±8.4 and 25.6±2.3 Sigma Frankel Unit (SFU) per ml at the 3, 6, 12 and 24 h time points, re-

spectively. In comparison, ALT levels were significantly elevated in DCPT-treated animals, with values of 107.0±12.0 (3 h), 148.7±27.6 (6 h), 407.3±77.9 (12 h) and 281.4±103.0 (24 h) SFU/ml. None of the animals exhibited any changes in liver or kidney weights, except for one DCPT-treated animal at the 12 h time point, which showed an elevated liver weight (8.96 g/100% body weight). Urine glucose and urine protein levels were not altered at any of the time points in either the corn oil or DCPT treatment groups. As serum ALT levels and some other parameters were altered within 3-6 h, absorption and distribution of DCPT is probably rapid. In conclusion, our results suggest that DCPT-induced hepatotoxicity occurs soon after administration of the compound. However, the mechanism behind this rapid onset of liver damage requires further investigation.

499 POTENTIAL ROLE OF CYTOCHROMES P450 IN 3-(3, 5-DICHLOROPHENYL)-2, 4-THIAZOLIDINEDIONE (DCPT)-INDUCED HEPATOTOXICITY IN RATS.

C. M. Crincoli, N. N. Patel and P. Harvison. *Department of Pharmaceutical Sciences, University of the Sciences in Philadelphia, Philadelphia, PA.*

3-(3, 5-Dichlorophenyl)-2, 4-thiazolidinedione (DCPT) is hepatotoxic in rats. This compound is structurally similar to insulin-sensitizing drugs, such as troglitazone, which have been shown to cause liver damage in some type II diabetic patients. Evidence in the literature suggests that cytochromes P450 may have a role in generating hepatotoxic metabolites from troglitazone. The purpose of this investigation was to determine whether biotransformation of DCPT is critical to induction of its hepatotoxicity. 1-Aminobenzotriazole (ABT) and troleandomycin (TAO) were chosen as non-selective and selective (CYP3A) cytochrome P450 inhibitors, respectively. Male, Fischer 344 rats were administered known hepatotoxic doses of DCPT (0.6 and 1.0 mmol/kg) i.p. in corn oil. Other rats were pretreated with either ABT (100mg/kg) in saline or TAO (500mg/kg) in acidified saline *via* i.p. injection prior to administration of DCPT. Control animals received equal doses of ABT or TAO, followed by corn oil only. Liver and kidney function were assessed at 24 hours post-dosing. Serum alanine aminotransferase (ALT) levels in rats treated with DCPT at 0.6mmol/kg and 1.0 mmol/kg were 140.0±35.1 Sigma Frankel Units/mL (SFU) and 506.9±99.4, respectively. ABT pretreatment markedly (85-95%) attenuated ALT values at both doses of DCPT; whereas TAO exerted a more modest effect on ALT levels (ca. 52% reduction). Control ALT values were 57.5±14.1 (ABT only) and 48.7±20.4 (TAO only). Liver and kidney weights, and urine protein concentrations were not significantly different among any of the treatment groups. In conclusion, these results suggest that cytochromes P450, including the CYP3A isozymes, are involved in DCPT-induced liver damage in rats. However, the nature of any hepatotoxic metabolites that are produced from DCPT requires further investigation.

500 UNDERLYING AGE-RELATED LIVER PATHOLOGY INCREASES AZOXYMETHANE TOXICITY IN FEMALE F344 RATS IN AN AGING STUDY ON COLON CANCER CHEMOPREVENTION.

S. Francke-Carroll[1], K. Daly[2], T. Wang[3] and B. Magnuson[2]. *[1]OSAS, USFDA, College Park, MD, [2]Department of Nutrition and Food Science, UMD, College Park, MD and [3]Agricultural Research Service, USDA, Beltsville, MD.*

The influence of aging and nutrition on the development cancer is of growing interest to the public health. Aging studies in rodents, however, hold many challenges; homogeneously aged laboratory animals are expensive, their availability is limited and they require careful maintenance since they are more stress sensitive than younger animals. Established cancer models, such as the Azoxymethane (AOM) colon cancer model in rats routinely require a standardized dose of AOM for all test groups. Applied to comparative aging studies, where test groups consist of animals that differ significantly in age this means that animals of all age groups receive the same standardized dose of AOM based on body weight, although older animals are potentially more sensitive. We report here inadvertent acute AOM-induced toxicity in female F344 rats due to underlying age related liver pathology. The rats were part of a study investigating the effect of dietary soy and aging on the development of AOM-induced aberrant crypt foci (ACF). Control and test animals of three age groups each (1, 11 and 21 month old), with seven animals per group, received one dose of 20 mg/kg AOM s.q., to induce the ACF. Three 21-month animals and one 11-month animal developed fatal toxic signs within 96 hrs post injection. Histopathological evaluation revealed, acute liver, adrenal gland and the gastro-intestinal toxicity. Preexisting age related changes, including hepatocellular adenomas, were also noted in the livers of all four animals. We conclude that age related liver pathology reduced the organ's ability to compensate, influenced drug metabolism and rendered these animals increasingly more sensitive to drug dosages well tolerated by younger animals in this established cancer research model. We further discuss unknown, underlying age related liver pathology as an additional factor influencing the outcome of aging research in laboratory rodents.

501 IMMUNOHISTOCHEMICAL ANALYSIS OF HEPATIC HEME OXYGENASE-1 EXPRESSION FOLLOWING ADMINISTRATION OF ETHINYL ESTRADIOL TO RATS.

L. A. Morio, A. Leone, J. B. Parker, P. Lord and M. K. McMillian. *Mechanistic Toxicology, Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Raritan, NJ.*

Heme oxygenase-1 (HO-1), also known as heat shock protein 32, is thought to have an important protective role against cellular stress during liver inflammation. Using PCR and microarray analysis we have previously demonstrated that HO-1 gene expression in whole liver tissue is elevated 1 day after administration of ethinyl estradiol to rats (manuscript submitted). In the present studies, we used immunohistochemical methods to analyze hepatic expression of this enzyme following administration of ethinyl estradiol, a steroid hormone which is an effective inducer of cholestasis. Treatment of female Sprague-Dawley rats with a single hepatotoxic dose of ethinyl estradiol (500 mg/kg, po) resulted in increased numbers of HO-1 positive hepatic macrophages at 1 day post-administration, while in parenchymal cells HO-1 expression remained undetectable. Interestingly, HO-1 immunoreactivity disappeared in hepatic macrophages after 4 days of repeated dosing with ethinyl estradiol while the cytoplasm of many hepatocytes now expressed the HO-1 protein. These data suggest a role for HO-1 in ethinyl estradiol-induced hepatotoxicity. The differential expression pattern of HO-1 protein in hepatic macrophages and hepatocytes suggests that HO-1 may play a role in ethinyl estradiol-induced liver injury.

502 INDUCTION OF PEROXISOME PROLIFERATION IN RAT HEPATOCYTES BY A SERIES OF HALOGENATED ACETATES.

J. McMillan, J. E. Walgren and D. J. Jollow. *Cell and Molecular Pharmacology, Medical University of South Carolina, Charleston, SC.*

Trichloroacetate (TCA) and dichloroacetate are hepatocarcinogenic metabolites of the environmental contaminant trichloroethylene. Induction of peroxisome proliferation *via* activation of the peroxisome proliferator-activated receptor α (PPARα) has been proposed as a mechanism for their hepatocarcinogenic action. However, it is unclear whether these compounds are direct ligands of PPARα or whether activation occurs by a ligand-independent process. Thus, the present studies were undertaken to determine whether a structure-related pattern of induction of palmitoyl-CoA oxidation could be observed in rat hepatocyte cultures exposed to a series of halogenated acetates. Significant differences were observed in both potency and efficacy of the halogenated acetates. The most potent inducers were the monohalo-substituted acetates, with the order of potency being iodo>bromo>chloro>>fluoro. Significant induction occurred at 50 and 100 µM, respectively for the monoiodo and monobromo derivatives. Cytotoxicity precluded obtaining full concentration-response curves for the monoiodo-, monobromo- and monochloroacetates. Trihaloacetates were modest inducers of palmitoyl-CoA oxidation, with no more than 2-fold induction at a concentration of 2 mM for TCA. The dihaloacetates were the most effective inducers of palmitoyl-CoA oxidation. Difluoroacetate (4mM) and dibromoacetate (3mM) induced palmitoyl-CoA oxidation by 10-fold and 7-fold, respectively, while treatment with the dichloro derivative (4mM) resulted in only a 3-fold increase. The slopes of the concentration dependence lines of the difluoro-/dibromoacetates vs. dichloroacetate were markedly dissimilar, suggesting a difference in PPARα activation or a substantial difference in metabolism or transport of these analogs. The disparity in efficacy for the dihalogenated acetates may also suggest a difference in how these peroxisome proliferators activate PPARα. Supported by DOE Cooperative Agreement DE-FC09-02CH11109.

503 ENDOTOXIN POTENTIATES AFLATOXIN B1-HEPATOCELLULAR INJURY BY A MECHANISM WHICH IS DEPENDENT UPON KUPFFER CELLS.

J. L. Wagoner[1, 4], E. X. Barton[1], D. F. Klein[2], T. T. Newton[2], R. A. Roth[3], L. H. Mortensen[4] and C. Barton[2, 4]. *[1]Iowa State University, Ames, IA, [2]Iowa Department of Public Health, Des Moines, IA, [3]Michigan State University, East Lansing, MI and [4]Des Moines University, Des Moines, IA.*

Exposure to a small, nontoxic dose of bacterial endotoxin potentiates the hepatotoxicity induced by aflatoxin B1 (AFB1), a mycotoxin commonly found in food products. The low level of expression of inflammatory mediators induced by endotoxin appears to be important for this increased susceptibility. In particular, tumor necrosis factor-alpha (TNF) is critical for the potentiation. Inasmuch as TNF is produced primarily by Kupffer cells, this study was conducted to explore the role of Kupffer cells in this pathogenic response. Male, Sprague-Dawley rats (300-400 g) were treated with gadolinium chloride (7.5 mg/kg, i.v.) or its saline vehicle, followed 20 hrs later with AFB1 at 1 mg /kg, i.p., or its vehicle (2% DMSO/water), and 4 hrs later with either endotoxin (E. coli LPS; 7.4 X 106 EU/kg, i.v.) or its

saline vehicle. Inhibition of Kupffer cell function by gadolinium chloride protected against hepatocellular injury as evidenced by alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increases in serum and histological examination of the liver. These results suggest that endotoxin potentiates AFB1 hepatocellular injury by a mechanism which is dependent on Kupffer cells. (Supported by a grant from the Iowa Osteopathic Education Foundation.)

503a TRANSCRIPTOMICS OF ACETAMINOPHEN IN RAT LIVER AND IN CULTURED DOG AND RAT HEPATOCYTES.

M. Jacquard[1], M. Renaud[1], J. Hoflack[1] and N. Claude[2]. *[1]Drug Safety, Biologie SERVIER, Gidy, France and [2]Drug Safety, IRIS, Courbevoie, France.*

Acetaminophen (APAP) induces liver centrilobular necrosis through conjugation routes overwhelming, P450-mediated toxic metabolization, followed by glutathione and macromolecular adduction. Whereas this mechanism is well described, little is known about early cell reactions prefiguring APAP-induced necrosis, and whether it is possible to reproduce the latter in cultured cells. Today, transcriptomics could allow a detection of cellular pre-necrotic status at the molecular level, before blood biochemical or histomorphological hallmarks. Therefore, APAP-induced transcriptomic profiles were monitored in Fischer rat liver and in beagle dog and Fischer rat hepatocyte cultures, under sub-necrotic treatment conditions evidenced by the relevant tissue and cellular parameters. Rats (3 males / group) were administered a single dose i.p. of 250 mg/kg APAP and liver collected at 6, 24, and 72 h time points. Freshly prepared rat and dog hepatocytes were treated for 4 h with 2 mM APAP. Transcriptomics profiles were established using 2-color fluorescence microarrays (Phase-1 Molecular Toxicology, USA; 270 genes for dog and 700 genes for rat microarrays) and analyzed using GenePix 5.0 and Acuity 3.1 softwares (Axon Instruments, USA). Comparison of rat *in vivo* and *in vitro* transcriptomic profiles showed a partial overlap regarding detoxication routes, but also a number of differences related to culture conditions. Higher APAP concentrations *in vitro* may have offered a better match between the two conditions. Similar responses were noted for dog and rat hepatocytes cultured *in vitro*, i.e. same metabolism, and detoxication processes indicative of an oxidative stress. These results show that identifying biomarker sets of genes within transcriptomic profiles may also improve prediction ability of *in vitro* transcriptomics.

504 BLESSINGS OF OLD AGE: PROTECTION FROM CHLORDECONE-POTENTIATED CCl_4 HEPATOTOXICITY.

B. Murali, M. C. Korrapati and H. M. Mehendale. *Toxicology, University of Louisiana at Monroe, Monroe, LA.*

Objective of the present work was to compare the toxic responses of chlordecone (CD) + CCl_4 between young adults (3 months), middle aged (14 months), and old aged (24 months) male Fischer 344 (F344) rats. After a non-toxic dose of CD (10 ppm in diet) or normal diet for 15 days, rats were challenged with a single non-toxic dose of CCl_4 (100 µl/kg, ip, 1:4 in corn oil) or corn oil (500 µl/kg, ip) alone on day 16. Liver injury was assessed by plasma ALT, AST, and histopathology during a time course of 0 to 96 h. Liver tissue repair was measured by [^{3}H]-thymidine incorporation into hepatic nuclear DNA, and PCNA immunohistochemistry. CYP2E1 protein, enzyme activity and covalent binding assay (2 & 6 h) were carried out in all the age groups with or without CD diet. Plasma glucose and hepatic glycogen were estimated. Exposure to CCl_4 alone did not cause any mortality. The combination led to 100% mortality of young adult rats by 72 h, whereas no mortality occurred in aged rats. Adult and middle aged rats exposed to CD + CCl_4 had identical liver injury up to 36 h. Thereafter, liver injury escalated only in young adults while it declined in middle aged rats. In the 24-month-old rats initial liver injury never developed to the level of the other two age groups further declining thereafter as in the 14-month-old rats. Neither hepatomicrosomal CYP2E1 protein nor the enzyme activity or covalent binding differed between the age groups with or without CD diet. Plasma glucose did not change in any age group. Significant depletion of hepatic glycogen was observed only in adult rats exposed to CD + CCl_4. Liver tissue repair ([^{3}H]-T, PCNA) was robust in aged rats allowing them to overcome liver injury by compensatory tissue repair. In young adults liver injury progressed due to failed compensatory tissue repair. These findings indicate that the aged rats are resilient to the lethal effect of CD + CCl_4 by virtue of early and robust compensatory liver tissue repair. (Supported by NIH/AG19058).

505 DEPLETION OF MITOCHONDRIAL COA BY PERFLUOROSULFONIC AND PERFLUOROCARBOXYLIC ACIDS *IN VITRO*.

T. M. O'Brien and K. B. Wallace. *Biochemistry and Molecular Biology, University of Minnesota, Duluth, MN.*

Perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA), and the substituted perfluorooctanesulfonamides perfluorooctanesulfonamidoacetate (FOSAA), and N-ethylperfluorooctanesulfonamidoacetate (N-Et-FOSAA) are widely used as surfactants on fabrics and papers, as anti-corrosion agents and fire retardants, as well as many other commercial applications. Their broad use, global distribution, and environmental persistence has generated considerable interest regarding the metabolic and potentially toxic effects of these compounds. We have previously shown that the perfluorooctanes disrupt mitochondrial bioenergetics and, more specifically, that perfluorinated carboxylic acids induce the mitochondrial permeability transition. The purpose of this study was to determine if, as structural fatty acid analogues, perfluorosulfonic acid and perfluorinated carboxylic acids deplete free coenzyme A. Freshly homogenized rat liver was incubated in the presence of the test compound at 37 C for 40 minutes. Caprylic acid was included as a positive control and the concentration of free coenzyme A determined by reversed-phase HPLC. The concentration of free CoA detected in the control incubations was 92.4 +/- 13.6 nmol/g liver. Incubation with caprylic acid caused a 5% decrease in free CoA, whereas there was a 15%-40% depletion of CoA when liver tissue was incubated with one or another of the perfluorinated acids. We conclude that PFOS and the perfluorinated carboxylic acids deplete free mitochondrial coenzyme A, and that this may contribute to the mechanism by which these compounds interfere with fatty acid metabolism *in vivo*. (Supported by a grant from the 3M Company).

506 EVALUATION OF THE SUBCHRONIC TOXIC POTENTIAL OF CHLOROFORM VIA AQUEOUS GAVAGE.

S. S. Anand[1], P. S. Palkar[1], M. M. Mumtaz[2] and H. M. Mehendale[1]. *[1]Department of Toxicology, University of Louisiana, Monroe, monroe, LA and [2]ATSDR, Atlanta, GA.*

The aim of the study was to develop a dose-response for hepatic, renal injury and tissue repair upon subchronic exposure to $CHCl_3$. Swiss-Webster mice were given 3 dose levels of $CHCl_3$ (150, 225, and 300 mg/kg/day) *via* aqueous gavage for 30 d. Liver and kidney injury were measured by ALT and BUN, respectively, and by histopathology. Tissue repair was assessed by (^{3}H-T) thymidine incorporation into hepato- and nephro-nuclear DNA and by PCNA. These were measured on 1, 7, 15, 21, and 30 d and from 24 to 96 h after the last dose (30 d). Blood, liver and kidney levels of $CHCl_3$ were quantified over 15 to 360 min. Only the high dose resulted in 20% mortality during the 30 d dosing regimen. After a single dose, $CHCl_3$ levels in blood, liver and kidney exhibited a dose response, peaking at 1 h and disappearing by 6 h. Upon repeated administration, on 7, 15 and 30 d, the initial levels were higher followed by steeper disappearance. Altered kinetics during repeated administration could be due to a combination of an adaptive response and altered CYP2E1. Moderate liver injury evident with all doses from day 1 to 15 regressed subsequently with the lower doses, whereas injury persisted with the high dose until 24 h after the last dose. Peak liver tissue repair was evident at 7 d with all doses. While the repair was persistent with the high dose, it declined to normal at lower doses. Significant elevation in BUN was evident on 1 d, persisted until 7 d, after which it declined to normal and remained so throughout the 30 d with all doses. Kidney repair peaked at 7 d and was robust with high dose. The biochemical observations were supported by histopathology and PCNA. Regression of injury after the initial rise was consistent with the higher elimination of $CHCl_3$. Absence of any significant elevation in liver tissue repair after 7 d excepting high dose, suggests that repeated exposure induces protection from injury at cellular level. The results indicate that subchronic exposure to $CHCl_3$ is unlikely to present a threat to humans (supported by ATSDR U61/ATD 681482).

507 PEROXISOME PROLIFERATOR CLOFIBRATE AFFECTS N-6/N-3 POLYUNSATURATED FATTY ACID (PUFA) COMPOSITION DIFFERENTIALLY IN RAT LIVER AND HEART.

J. T. Ahokas[1], Q. Tian[1], F. A. Grzemski[1] and S. Panagiotopoulos[2]. *[1]Key Centre for Applied and Nutritional Toxicology, RMIT University, Melbourne, VIC, Australia and [2]Austin & Repatriation Medical Centre, University of Melbourne, Melbourne, VIC, Australia.*

The peroxisome proliferator, clofibrate (CL) has been used to treat hyperlipidemia. Peroxisome proliferators are a structurally diverse group known to cause hepatocellular carcinoma in rodents with long-term exposure. Peroxisome proliferators act *via* activation of the nuclear receptor, peroxisome proliferator-activated receptor a (PPARα), which is known to play an important role in lipid metabolism The n-6 PUFAs have been shown to enhance tumorigenesis in lipid sensitive carcinogen-induced and tumor transplant tumor models, whereas n-3 PUFAs display anti-tumorigenic effects. This study investigated the effects of CL on PUFA composition in the liver and heart. Sprague-Dawley rats (n=6 / group) were treated with CL 300mg/kg per day or olive oil i.p. for up to 14 days. Fatty acid was then extracted and subjected to gas chromatography. CL pretreatment significantly decreased the plasma levels of all fatty acids except that of C20:3 n-6, which slightly increased after 14 day treatment ($P < 0.05$). In liver, CL administration reduced C18:2n-6,

but C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6 were significantly increased, . However, n-3 PUFAs including C18:3n-3, C20:5n-3, C22:5n-3 and C22:6n-3 were reduced by CL. These changes together caused a significant increase of hepatic n-6/n-3 ratio (P<0.0001). By contrast in the heart, C20:4 n-6 was reduced to about 50 percent of control level while C22:6n-3 was enhanced by 2-fold. Consequently, CL treatment led to a decrease of myocardial n-6/n-3 ratio (P<0.0001). The different effects of CL on n-6/n-3 ratio may contribute to its distinct actions in liver and cardiovascular system.

508 THE SIGNIFICANCE OF ELEVATED SERUM AMINOTRANSFERASE LEVELS IN THE ABSENCE OF HEPATIC NECROSIS: PRECLINICAL PREDICTIVE VALUE AND RELEVANCE TO HUMAN RISK.

D. D. Wiant[1, 2]. [1]*In Vivo Pharmacology, Adolor Corporation, Exton, PA and* [2]*Department of Health, West Chester University, West Chester, PA.*

Serum aminotransferase activities have been used broadly as surrogate markers for tissue injury and disease in human and veterinary settings and in safety assessment of chemicals and pharmaceuticals. This research study was conducted to demonstrate increased aspartate (AST) & alanine (ALT) aminotransferase activity in liver tissue without obvious hepatocyte death or damage and, in plasma as a sequel to hepatic necrosis. Rats were administered 4 or 10 oral doses of 12 mg/kg dexamethasone or 1000 mg/kg acetaminophen, respectively. Terminal plasma and liver samples were assayed by immunoblot and functional assay, while pre and post dose plasma was co-analyzed commercially and necrosis was histologically determined from processed liver slices. Dexamethasone (an aminotransferase inducer) increased liver and plasma AST & ALT levels. Terminal BWs decreased and absolute & relative (%BW) liver weights increased vs. vehicle controls. Dexamethasone group total liver protein decreased, but was similar in the acetaminophen vs vehicle groups. Acetaminophen (a hepatotoxicant) increased plasma ALT levels and slightly decreased liver ALT levels, while plasma AST levels stayed in reference range for age throughout dosing. Contrary to assumptions, acetaminophen liver injury was inconclusive. Dexamethasone induced rapid liver weight gain with necrosis, and increased liver tissue and plasma "liver" aminotransferases. The null hypothesis that there is no significant relationship between the presence of elevated plasma aminotransferase levels and the histologic finding of liver toxicity (necrosis or hepatocyte death) was rejected. Looking at ALT & AST induction in liver tissue by mRNA analysis is the next step in determining if observed liver reactions were toxic in nature, or adaptive. It is strongly suggested that preclinical liver toxicity determinations must include clinical chemistry (plasma ALT & AST), liver histopathology and corroborative liver tissue ALT & AST mRNA analyses.

509 IMPORTANCE OF MITOGEN-ACTIVATED PROTEIN KINASES (MAPK) IN ACETAMINOPHEN HEPATOTOXICITY IN MICE AND MURINE HEPATOCYTES.

M. Bajt and H. Jaeschke. *Liver Research Institute, University of Arizona, Tucson, AZ.*

MAPK (c-Jun kinase, JNK; p44/p42 MAPK, p38 MAPK) are involved in signal transduction and regulation of gene expression in a wide variety of pathophysiological responses of the cell. To evaluate which MAPK signaling pathway is important in acetaminophen (AAP) hepatotoxicity, phosphorylation of the individual MAPK or substrates was assessed after injection of 300 mg/kg AAP in C3Heb/FeJ mice with or without additional treatment of glutathione (200 mg GSH/kg; 1.5 h after AAP) as described (JPET 303: 468, 2002 and JPET 307:67, 2003). None of the MAPK were phosphorylated in untreated controls. However, after AAP alone, phosphorylation of p44 MAPK and p38 MAPK was observed at 6, 12 and 24 h without changes of the total amount of protein. Treatment with GSH, which attenuated liver injury, significantly reduced phosphorylation of p44 and p38 MAPK. In contrast, c-Jun protein levels, not detectable in controls, were moderately increased with AAP alone (6 to 24 h), reaching peak levels at 12 h. Treatment with GSH further increased c-Jun protein expression at all time points after AAP. There was only a minor increase in the phosphorylated form of c-Jun after AAP alone. However, treatment with GSH, substantially enhanced the amount of phosphorylated c-Jun with peak levels at 12 h after AAP. To assess the importance of MAPK activation, cultured murine hepatocytes were treated with 20-50 μM of PD98059 (p44/p42 inhibitor), U0126 (MEK1/2 inhibitor), SB203580 (p38 inhibitor) or SP600125 (JNK inhibitor) 1 h before adding 5 mM AAP. Cells treated with AAP showed a time-dependent loss of *via*bility (XTT assay: 24% of controls at 12 h; 80% of the cells were trypan blue-positive). Of the MAPK inhibitors used, only the p38 inhibitor SB203580 showed a significant inhibition of cell damage (XTT assay: 70% of controls and 20% trypan blue-positive cells at 12 h). Conclusion: Although AAP treatment differentially up-regulates several MAPK, only p38 MAPK may be involved in the intracellular signaling pathway resulting in necrotic cell death of liver parenchymal cells.

510 INTERLEUKIN-13 PROTECTS AGAINST ACETAMINOPHEN-INDUCED LIVER INJURY.

S. B. Yee, M. Bourdi, M. P. Holt and L. R. Pohl. *Molecular and Cellular Toxicology Section, Laboratory of Molecular Immunology, NHLBI, NIH, DHHS, Bethesda, MD.*

Recent studies have suggested that one predisposing aspect of idiosyncratic drug-induced liver disease may involve a deficiency of hepatoprotective factors, such as interleukin (IL)-6 and IL-10. In this regard, we tested the hypothesis that IL-13 is another hepatoprotective factor. Following treatment of male C57BL/6 wild type (WT) mice with acetaminophen (APAP; 200 mg/kg), hepatotoxicity, as determined by serum alanine aminotransferase activity, peaked by 24 hours with a concomitant increase in serum IL-13 concentration. Administration of an IL-13 antibody two hours before APAP treatment attenuated serum IL-13 concentration and significantly exacerbated liver injury up to 24 hours after APAP administration. While the qualitative nature of the resulting centrilobular lesions was similar, morphometric analysis revealed that IL-13 antibody pretreatment significantly increased lesion area. APAP administration to IL-13 knockout (KO) mice confirmed the protective role of IL-13 in APAP-induced liver injury. Serum tumor necrosis factor (TNF)-α concentration was significantly elevated in APAP-treated IL-13 KO mice and in WT mice co-administered with IL-13 antibody and then APAP. Protein adduct formation was also increased in IL-13 KO mice compared to WT. This increase, however, was not due to enhanced cytochrome P450 2E1 expression. Taken together, these results suggest that IL-13 is a critical hepatoprotective factor in APAP-induced liver injury and that increased TNF-α and protein adduct formation may be important in the mechanism of enhanced liver injury observed in APAP-treated mice with compromised IL-13 levels.

511 ROLE OF TOLL-LIKE RECEPTOR-4 (TLR-4) IN ACETAMINOPHEN (AA)-INDUCED HEPATOTOXICITY.

C. R. Gardner[1], L. Chen[1], J. D. Laskin[2] and D. L. Laskin[1]. [1]*Rutgers University, Piscataway, NJ and* [2]*UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

Acetaminophen (AA) is a mild analgesic and antipyretic agent known to cause hepatotoxicity at toxic doses. Increasing evidence suggests that that macrophages and inflammatory mediators contribute to acetaminophen-induced tissue injury. Toll-like receptor 4 (TLR-4) is a transmembrane protein expressed on macrophages. It has been shown to mediate endotoxin-induced inflammation and injury. In the present studies, we analyzed the role of TLR-4 in AA induced hepatotoxicity using control C3H/OuJ and TLR-4 mutant C3H/HeJ mice. Treatment of OuJ mice with AA (300 mg/kg) resulted in centrilobular hepatic necrosis and increased serum ALT levels within 3 h. This was correlated with a reduction in liver glutathione (GSH) levels. In HeJ mutant mice, tissue injury was significantly reduced. This was correlated with a delay in AA-induced GSH depletion. Immunohistochemistry showed that expression of proliferating cell nuclear antigen (PCNA), a marker of hepatocyte proliferation and tissue repair, was induced in hepatocyte nuclei 12 h after AA treatment in OuJ mice, but did not appear until 24 h in HeJ mice. Delayed induction of the hepatocyte mitogen IL-6, which is strongly induced at 3 h in OuJ mice, was also noted. TREM-1 is a macrophage receptor that has been implicated in the regulation of endotoxin-induced inflammatory responses. Evidence suggests that TLR-4 is required for TREM-1 expression early in the inflammatory process. We found that TREM-1 mRNA expression was induced in the liver of OuJ mice within 6 h of AA administration peaking at 12 h. In HeJ mice treated with AA, hepatic expression of TREM-1 mRNA was delayed until 12 h and maximal levels were not observed until 24 h. Taken together, these data demonstrate that TLR-4 plays a role in AA-induced hepatotoxicity. Reduced toxicity in TLR-4 mutant mice may be due to delayed generation of cytotoxic inflammatory mediators. Supported by NIH grants GM34310, ES04738 and ES05022.

512 IMPAIRED SIGNAL TRAFFICKING UNDERLIES FAILED ON-DEMAND LIVER TISSUE REPAIR UPON HEPATOTOXIC CHALLENGE IN TYPE 2 DIABETES.

S. P. Sawant[1], A. V. Dnyanmote[1], J. R. Latendresse[2] and H. M. Mehendale[1]. [1]*Department of Toxicology, School of Pharmacy, The University of Louisiana at Monroe, Monroe, LA and* [2]*NCTR, Jefferson, AR.*

The objectives of this study were to further characterize our type 2 diabetic (DB) model and to investigate mechanisms of increased CCl_4 hepatotoxicity in DB rats. Male SD rats were fed high fat and on day 14 injected streptozotocin (45 mg/kg). On day 24, diabetes was confirmed by hyperglycemia, normoinsulinemia, and oral glucose intolerance, all persisting beyond 6 months. Nonlethal doses of allyl alcohol (35 mg/kg), CCl_4 (2 ml/kg), and thioacetamide (300 mg/kg) yielded 80, 100, and 100% mortality, respectively, in DB rats within 48 h. A time course (0 to 96 h) study of CCl_4 (2 ml/kg)-induced liver damage revealed similar initial bioactivation-mediated injury in DB vs non-DB rats, markedly progressing with hyperammonemia and hyperbilirubinemia in the DB rats. Hepatomicrosomal CYP2E1 protein

and activity, $^{14}CCl_4$ covalent binding, and lipid peroxidation did not differ between DB and non-DB rats indicating that higher bioactivation is not the mechanism. Tissue repair was inhibited in DB rats leading to progression of injury, and liver failure. Prompt and robust tissue repair, regression of injury and survival were noted in non-DB rats. Epidermal growth factor receptor- mitogen activated protein kinases (EGFR-MAPK) signal trafficking were investigated. EGFR, MAPKs such as ERK1/2 and p38 expression and activation were downregulated as early as 3 h in the DB rats after CCl_4 adm. MAPKs facilitate the dissociation of NF-κB, which translocates in the nucleus, binds to DNA, and stimulates the transcription of cyclin D1, required for G1 to S phase checkpoint clearance. In the DB rats, NF-κB-DNA binding and cyclin D1 were down regulated compared to non-DB rats as early as 6 h after CCl_4 adm. These findings indicate high sensitivity of type 2 diabetes to diverse hepatotoxicants. The mechanism of high sensitivity to CCl_4 is impaired compensatory tissue repair owing to failed G1 to S checkpoint clearance, allowing unabated progression of liver injury. (Kitty DeGree Endowment)

513 PROTECTION AGAINST MECHANISTICALLY DISTINCT HEPATOTOXICANTS IS ASSOCIATED WITH ACUTE PHASE RESPONSE.

K. A. Ewald[1] and E. J. Calabrese[2]. *[1]KERA Environmental, LLC, Worthington, MA and [2]Department of Environmental Health Sciences, University of Massachusetts, Amherst, MA.*

The acute phase response (APR) is a mechanism of defense associated with a variety of stresses and injuries. Three experiments were conducted to evaluate the relationship between three temporal phases of APR-induction and chemically induced injury: 1) prior-, 2) simultaneous- and 3) post-APR-induction. APR-induction (subcutaneous (sc) injection of turpentine (T)) was tested with 6 toxicants reflecting a range of distinct toxic mechanisms: carbon tetrachloride (CCl_4), the chlordecone/carbon tetrachloride interaction (CD/CCl_4), galactosamine (GalN), allyl alcohol (AA), thioacetamide (TA) and mecuric chloride ($HgCl_2$). Each group of Sprague-Dawley rats was administered one of the 6 toxicants (5 hepatotoxicants and 1 nephrotoxicant; prior-induction) or one of 5 hepatotoxicants (simultaneous- and post-induction) by intraperitoneal (ip) injection. Serum alanine transaminase (ALT) or blood urea nitrogen (BUN; $HgCl_2$ only) and the acute phase protein, haptoglobin (Hp) were evaluated in all groups at 12, 24, 36, 48 and 72 hours after toxicant, along with histological evaluation at selected intervals (prior-induction only). While simultaneous and post APR-induction failed to reduce hepatotoxicity, results indicate that prior APR-induction causes a marked reduction (90 to 97%) in CCl_4, CD/CCl_4, GalN and TA induced liver toxicity. $HgCl_2$ and AA toxicity were not diminished by prior APR-induction, possibly due to chemical-specific mechanisms of toxicity. Findings suggest that the APR may be a significant adaptive response to a variety of mechanistically diverse hepatotoxicants.

514 MECHANISMS REGULATING TREM-1 EXPRESSION IN LIVER MACROPHAGES AND ENDOTHELIAL CELLS DURING ACUTE ENDOTOXEMIA.

L. C. Chen, M. A. Gordon, J. D. Laskin and D. L. Laskin. *Joint Graduate Program in Toxicology, Rutgers University and UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

Acute endotoxemia is associated with excessive release of proinflammatory and cytotoxic mediators in liver macrophages and endothelial cells. TREM-1 (triggering receptors expressed on myeloid cell-1) is a newly identified receptor on myeloid cells implicated in regulating host innate response. In the present studies, we analyzed the effects of acute endotoxemia on TREM-1 expression in liver macrophages and endothelial cells from endotoxin sensitive C3H/OuJ and endotoxin resistant C3H/HeJ mice, which have a mutant toll-like receptor 4 (TLR4). Cells were isolated from livers following perfusion with collagenase, centrifugal elutriation and density gradient centrifugation. Freshly isolated macrophages and endothelial cells from OuJ and HeJ mice constitutively expressed TREM-1 mRNA as determined by semi-quantitative RT-PCR. No major differences were noted between the mouse strains. In both cell types TREM-1 mRNA expression decreased with time in culture. This was overcome by the addition of IL-1 alpha (50 ng) or TNF-alpha (1 ng) to the cultures. Treatment of the mice with endotoxin (3 mg/kg, ip) caused significant induction of TREM-1 mRNA expression in macrophages from OuJ and HeJ mice, which was most prominent at 3 hr. Whereas in OuJ mice, TREM-1 expression remained elevated, in HeJ mice, expression decreased. Endothelial cells from both mouse strains also expressed TREM-1 which was most prominent 3 hr after endotoxin. Subsequently, TREM-1 decreased. To study mechanisms mediating induction of TREM-1, IL-1 beta and TNF-alpha mRNA expression was analyzed since these proinflammatory cytokines were found to induce TREM-1 in the cells. We found that IL-1 beta and TNF-alpha were highly induced 3 hr post endotoxin. These data implicate TREM-1 expression in acute endotoxemia. Moreover, this may be regulated *via* TLR4, IL-1 beta, and TNF-alpha. Endotoxin-resistance in C3H/HeJ mice may be due to limited expression of the TREM-1 protein. Support: NIH GM34310, ES04738 and ES05022.

515 EXPRESSION OF HEPATIC AND RENAL BILE ACID TRANSPORTERS DURING CHOLESTASIS.

K. Allen[1], A. L. Slitt[1], N. J. Cherrington[2], C. Chen[1], M. Dieter[1], J. Maher[1] and C. Klaassen[1]. *[1]University of Kansas Medical Center, Kansas City, KS and [2]University of Arizona, Tucson, AZ.*

The liver synthesizes and excretes bile acids (BA) to aid in intestinal emulsification and absorption of fats, and also extracts BA from portal blood to complete the enterohepatic cycle. Obstruction of the common bile duct can impede bile flow into the intestine, cause toxic BA to accumulate in liver, and increase urinary BA excretion. In general, the sodium cotransporting polypeptide (Ntcp) and the organic anion tranporting polypeptides (Oatps) are thought to transport bile acids into the liver and kidney, whereas the bile salt export pump (Bsep) and the multidrug resistance proteins (Mrps) are thought to export bile acids out of liver and kidney. Therefore, we hypothesized that during cholestasis the liver would alter gene expression to prevent BA accumulation, but the kidney would alter gene expression to enhance urinary excretion of BA. To test this hypothesis, C57BL/6J mice underwent either sham or bile-duct ligation (BDL) surgery. After BDL, liver, kidney, and serum were collected at day 1, 3, 7, and 14. Serum bile acid concentrations were 121μmol/L in sham-operated mice and were elevated to 450, 894, 1687, 1799μmol/L at 1, 3, 7, and 14 days in mice after BDL, respectively. In liver, Ntcp, Oatp1, Oatp3, and Oatp4 mRNA levels decreased, while Bsep, Oatp2, Oatp5, Oatp12, and Mrp1-5 mRNA levels increased. In kidney BDL decreased Oatp1 mRNA levels, and increased kidney Mrp1-5 mRNA levels. The elevated serum BA concentration after 14 days of BDL indicates that liver and kidney are limited in their ability to compensate for the loss of hepatic BA excretion. mRNA expression data indicates that hepatic and renal Oatp and Mrp genes are regulated similarly during cholestasis, favoring a gene expression pattern that lowers cellular BA concentrations. Based on the current knowledge of Oatp and Mrp function, these data suggests that liver and kidney do not work in tandem to protect the mouse during cholestasis. (Supported by grants ES-09716, ES-09649, ES-07079, ASPET SURF Program)

516 VOLATILE ORGANIC COMPOUND EXPOSURE ASSESSMENT FOR GOVERNMENT INSPECTORS AT GAS STATIONS.

M. J. Fedoruk[2, 3], R. Bronstein[3] and B. D. Kerger[1]. *[1]HSRI, Inc., Tallahassee, FL, [2]Center for Occupational and Environmental Health, University of California-Irvine, Irvine, CA and [3]Exponent, Inc., Irvine, CA.*

This study assessed occupational exposure to selected gasoline-derived volatile organic compounds (VOCs) among air quality inspectors at 23 gas stations selected to represent plausible high end exposures based on historical air quality violations. Five inspectors volunteered for the study and followed their normal routine inspection process including review of records, equipment inspection, and in one case calibration tests on selected gas pumps. Each inspector was fitted with three personal air monitoring devices: a full-shift badge monitor (approx. 8 hours) and two active sorbent tube monitors (battery pump, one for full shift and one for am/pm only, 2-3.5 hours each). Monitored analytes included TPH (total petroleum hydrocarbons, gasoline range), MTBE (methyl-tert-butyl ether), benzene, toluene, ethylbenzene and xylenes. TPH, MTBE and toluene were found in nearly all samples while benzene and xylenes were detected infrequently and at low levels, and ethylbenzene was never detected. There was generally good agreement between the badge and active monitoring results when normalized as time-weighted averages and after considering detection limit differences. Gas pump calibration tests were associated with the highest exposure to the VOCs tested, including at-facility-only maximum values of: TPH 29 ppm; MTBE 1.7 ppm; benzene 0.05 ppm; toluene 0.6 ppm; and xylenes 0.3 ppm. All measured VOCs were found at mean and maximum concentrations well below their respective permissible exposure limits set by the Occupational Safety and Health Administration. It is concluded that exposures of these government inspectors to the selected gasoline-derived VOCs at gas stations were relatively low compared to current workplace regulatory limits.

517 EXPOSURE ASSESSMENT OF VOLATILE ORGANIC COMPOUNDS AND METALS FOR GOVERNMENT INSPECTORS AT AUTO BODY REPAIR FACILITIES.

B. D. Kerger[1], R. Bronstein[3] and M. J. Fedoruk[2, 3]. *[1]HSRI, Inc., Tallahassee, FL, [2]Center for Occupational and Environmental Health, University of California-Irvine, Irvine, CA and [3]Exponent, Inc., Irvine, CA.*

This study assessed occupational exposure to selected volatile organic compounds (VOCs) and metals among air quality inspectors at auto body repair and painting facilities selected to represent plausible high-end exposures based on historical air

quality violations. Four inspectors volunteered for the study and followed their normal routine inspection process including review of records and equipment inspection. Each inspector was fitted with four personal air monitoring devices: a full-shift badge monitor for VOCs (approx. 8 hours), two active sorbent tube monitors for VOCs measured while at each facility (battery pump, one for full shift and one for am/pm only), and one active cassette monitor for metals measured while at each facility. Monitored analytes included a broad scan of metals (28 including all common heavy metals, total hydrocarbons in hexane equivalents, benzene, toluene, ethylbenzene, xylenes and styrene. None of the inspectors had detectable exposures to any of the metals assessed (e.g., Al, As, Be, Cd, Cr, Co., Cu, Pb, Mg, Mn, Se, Sr, Sb, Ti, Zn) at detection limits at or below 0.02 mg/cu.m. Benzene and styrene were not detected (detection limits generally below 0.05 and 0.2 ppm, respectively) in any air samples. Total hydrocarbons as hexane equivalents ranged from 0.4 to 2.9 ppm, toluene from 0.1 to 0.6 ppm, ethylbenzene from 0.006 to 0.03 ppm, and xylenes from 0.03 to 0.9 ppm during inspections only. There was good agreement between the badge and active monitoring results for VOCs when normalized as time-weighted averages and after considering detection limit differences. All measured VOCs were found at mean and maximum levels well below their respective permissible exposure limits set by the Occupational Safety and Health Administration. It is concluded that exposures of these government inspectors to the selected VOCs and metals at auto body repair facilities were relatively low compared to current workplace regulatory limits.

518 EXPOSURE CHARACTERIZATION FROM A SURROGATE FINE FRAGRANACE.

D. A. Isola[1], R. E. Rogers[2], R. Ansari[3] and L. W. Smith[1]. *[1]Reaearch Institute for Fragrance Materials, Inc. , Woodcliff Lake, NJ, [2]Toxcon Health Sciences Research Centre, Inc., Edmonton, AB, Canada and [3]Quest International Fragrances Company, Mount Olive, NJ.*

This study was designed to assess human respiratory exposure to a surrogate fine fragrance and address concerns about asthma, lung disease, and neurological effects. The test fragrance reflected a product with a low level of scent over time after topical application by an atomizer. The formulation contained 2.2% each of benzyl acetate (BA), eugenol (EU), α-hexylcinnamaldehyde (HCA), 1, 3, 4, 6, 7, 8-hexahydro-4, 6, 6, 7, 8, 8-hexamethylcyclopenta-γ-benzopyran (HHCB), hydroxycitronellal (HO-C), β-ionone (β-I), d-limonene (d-L), linalool (LL), and methyl dihydrojasmonate (MDJ) and 80% of a vehicle (80% EtOH and 20% HOH). The fragrance materials were chosen based on volatility, chemical structure, toxicity, and volume of use. The test product was sprayed toward a manikin at a distance of 3.5 inches. Three different anatomical areas were sprayed with 3 pump actuations each. The concentration of each fragrance material was measured at the adult-breathing zone (5 ft) and the child-breathing zone (1.5 ft) from the start of spray until 5 hrs post-spray. Particle size analysis was also conducted. The samples were collected on thermal desorption tubes and analyzed by GC/MS. The data showed that 0.89 g of test material was released after 9 actuations. Peak total fragrance air concentrations of 1256 mg/m^3 (adult zone) and 850 mg/m^3 (child zone) were seen at 8-18 min post spray. The more volatile fragrance materials (d-L, LL, BA) peaked at that time. The less volatile fragrance materials (β-I, MDJ, HCA, HHCB, EU, HO-C) peaked at later time periods, generally. Previous studies with air fresheners showed peak fragrance air concentrations of 2550 mg/m^3 (adult zone)/2070 mg/m^3 (child zone) at 1 min for aerosols and 2286 mg/m^3 at 1 hr for plug-ins. The product type and volatility of each fragrance material affect air concentration. The exposure data will be used for clinical study design.

519 EVALUATION OF EXPOSURE TO METALS ON REUSABLE SHOP TOWELS.

L. Beyer, M. R. Seeley and B. D. Beck. *Gradient Corp., Cambridge, MA.*

We evaluated health risks from use of reusable shop towels (RSTs) which may have metal and oil/grease contamination even after being laundered. We estimated oral intake of metals in laundered shop towels for two potential exposure pathways: a) hand contact with laundered shop towels and subsequent transfer to the mouth, and b) direct contact of RSTs with the lips. Because there are no standard methodologies for evaluating exposure to metals in towels, we developed an approach to estimate the amount of metal that could be transferred from the towels, and ultimately ingested. For exposure *via* hand contact with the laundered shop towels, we estimated transfer of metals from RSTs to hands based on empirical data regarding transfer of pesticide residues from surfaces to hands, using methodology developed by the US Consumer Products Safety Commission for evaluating exposure to dislodgeable residues on treated wood surfaces. We considered the number of RSTs used daily per person, the percentage of the towel surface area that would contact the hand, and the hand-to-mouth transfer efficiency. For exposure *via* direct contact with the lips, we estimated transfer of metals from laundered shop towels to the lips based on data regarding transfer of pesticide residues from surfaces to hands, the surface area of the lips, as well as estimates of the fraction of the lip surface area contacting the laundered shop towels, fraction of the metal on the lip that is ultimately ingested, and the number of times per day that lips are wiped with RSTs. We compared the results of this analysis to various allowable/recommended levels developed by USEPA, the Agency for Toxic Substances and Disease Registry, and California EPA (under Proposition 65), and concluded that: 1) laundered RSTs contain heavy metals, 2) metals on shop towels may get onto hands and then inadvertently be ingested, and 3) the amount of metals (especially lead) that someone might accidentally ingest from RSTs may potentially exceed regulatory and/or recommended limits. We performed a focused uncertainty evaluation to assess the impact of alternate assumptions on the conclusions.

520 POTENTIAL HEALTH EFFECTS OF EXPOSURE TO METHYLENEDIANILINE AND TOLUENEDIAMINE DURING POLYURETHANE FOAM MANUFACTURING.

T. A. Lewandowski[1], A. Hayes[3] and B. D. Beck[2]. *[1]Gradient Corporation, Seattle, WA, [2]Gradient Corporation, Cambridge, MA and [3]School of Public Health, Harvard University, Cambridge, MA.*

Methylene diphenylisocyanate (MDI) and toluenediisocyanate (TDI) are used by a wide number of industries to produce polyurethane foam products. Small amounts of methylenedianiline (MDA) and toluene diamine (TDA) are released during MDI and TDI polymerization and may be present in the newly finished polyurethane parts. MDA and TDA concentrations in foam decline exponentially within several hours of demolding. MDA and the 2, 5-isomer of TDA are known animal carcinogens and have also been reported to have significant non-carcinogenic health effects. Neither chemical is commonly found at sites of environmental pollution and thus fall outside the standard risk assessment process. Our goal was to determine whether worker exposures to MDA or TDA in freshly produced polyurethane foams were associated with unacceptable health risks. Unique aspects of this work were the lack of standard toxicity values (i.e., RfDs, CSFs) for these two chemicals and the unusual exposure pathway, which involved dermal transfer of a non-aqueous liquid from the foam surface to the hand; a process which varied across different worker tasks. Sampling and analysis of the fresh foam indicated that MDA and TDA concentrations at demolding varied considerably among production lines but MDA and TDA concentrations in all materials evaluated declined rapidly over time. For example, levels of TDA isomers declined about 4-fold within the first three hours. We found that under a worst-case exposure scenario, cancer risks from TDA exposure were approximately 5x10^{-6} whereas cancer risks from MDA exposure resulted in a tumorigenic MOE of 85, 000. Non-cancer hazard indices were also well below 1.0. Thus, we found that estimated cancer and non-cancer risks from MDA or TDA exposure from newly manufactured foam parts would fall well within normal occupational risk criteria. This analysis also suggested that risks for downstream workers and consumers would also likely be below levels of concern.

521 IRIDIUM TRACER USED TO MEASURE IN-VEHICLE DIESEL PARTICULATE MATTER CONCENTRATIONS.

M. D. Easter[1], M. L. Lakin[1], R. Ireson[2], C. Lapin[3], T. Hesterberg[4] and W. Bunn[4]. *[1]EnSIGHT, Walnut Creek, CA, [2]AQM Consulting, Greenbrae, CA, [3]Lapin and Associates, Glendale, CA and [4]International Truck and Engine Corporation, Chicago, IL.*

Previous studies reported diesel particulate matter (DPM) inside diesel-fueled school buses and potential exposure to occupants. These studies did not directly measure DPM, but assumed relationships between DPM and other pollutants present in the air, including black carbon, to estimate DPM. Numerous sources of black carbon other than diesel exhaust exist. This study used an analytical method capable of distinguishing particulate matter (PM) emitted by the study bus from other sources of PM that may enter it. Iridium (Ir) was used as a tracer added to the bus's fuel to identify the PM present in its exhaust and on-board air. Low ambient levels and the availability of sensitive analytical techniques made Ir an ideal tracer. Instrumental neutron activation analysis was used and is capable of measuring Ir to better than +/-1 picogram. Measurements of Ir inside the bus provide a basis for estimating exposure to PM from its own exhaust (DPM_{SB}) with high sensitivity. The average DPM_{SB} concentration inside the study bus was 0.22 μg/m3 - much less than other reported estimates of DPM. The California Air Resources Board reported that the average concentration of DPM in vehicles in a Los Angeles field study to be 33 μg/m3 - 150 times greater than the DPM_{SB} inside the bus in the current study. The concentrations of total fine particulate matter (PM2.5) and Ir were not correlated. Thus, the use of total PM2.5 as a surrogate for estimating the amount of DPM coming from the bus's own exhaust is inappropriate. The concentration of PM from the bus's own exhaust was a very small percentage of the total PM2.5 inside the bus, ranging from 0.09%-0.77%. Evidence suggests that the levels of PM2.5 and DPM found inside a school bus should be no greater than those found inside any other vehicle traveling on the same roads.

522 FREQUENCY OF EXPOSURE TO CONTAMINANT MIXTURES AT HAZARDOUS WASTE SITES.

M. Fay. *Toxicology, ATSDR, Atlanta, GA.* Sponsor: M. Mumtaz.

To determine the need for information on the joint effects of chemicals, the Chemical Mixtures Program of the Agency for Toxic Substances and Disease Registry (ATSDR) seeks to answer, "How often does exposure to chemical mixtures of environmental contaminants occur?" The frequency of exposure to mixtures was studied by analyzing contaminants found in completed exposure pathways (CEPs) in ATSDR's HazDat database (see www.atsdr.cdc.gov). CEPs are documented pathways that have led to exposure at hazardous wastes sites (HWSs). Evaluation of data from 1, 706 HWSs reveals that CEPs occurred at 743 (44%) of the sites. Of these, 588 sites had two or more substances in a CEP. Thus, exposure to mixtures occurred at 79% of the 743 sites with exposure. However, sites often have more than one pathway, and pathways with a single contaminant are common. The number of exposure pathways with two or more chemicals is estimated to be approximately half of all CEPs. This does not include exposure *via* multiple pathways/routes, so the extent of exposure to multiple chemicals at HWSs might be higher. Further analysis revealed that exposure to simple mixtures consisting of 3, 4, and 5 components occurred at 475, 390, and 321 sites, respectively. Although two chemicals constitute a mixture, 64% of the 743 sites had 3 in a CEP, 52% had 4 in a CEP, and 43% had 5 in a CEP. Exposure to mixtures by specific media was as follows: water, 413 sites; soil, 255 sites; air, 113 sites; and biota (mainly fish), 53 sites. Overall, water pathways had mixtures more often for lower numbers of chemicals (2 to 8 chemicals), but the number of sites with mixtures in soil pathways surpassed the counts for water at (and above) nine chemicals in the mixture. The maximum number of chemicals in a CEP was 62. In conclusion, this study shows that about four-fifths of HWSs with CEPs have chemical mixtures in a CEP, and, more importantly, that about half of all CEPs have a chemical mixture. Thus, exposure to mixtures of chemicals is quite common at HWSs.

523 FIBER GLASS AND ROCK/SLAG WOOL EXPOSURE OF PROFESSIONAL INSTALLERS.

L. D. Maxim[1], W. Eastes[5], J. G. Hadley[2], C. M. Carter[3], J. W. Reynolds[4] and R. Niebo[1]. *[1]Everest Consulting Associates, Cranbury, NJ, [2]Owens Corning Science and Technology Center, Granville, OH, [3]Johns Manville, Littleton, CO, [4]CertainTeed, Valley Forge, PA and [5]Consultant, Granville, OH.*

This study estimates the average cumulative lifetime exposure (fiber-months/cc) of professional installers of fiber glass (FG) and rock/slag wool (RSW) insulation and compares these estimates to the cumulative exposure of workers in plants that manufacture these materials. Key inputs to the exposure model include the frequency and duration of exposures, occupational tenure, mix of insulation types (e.g., FG, RSW) and forms (e.g., blown/loose fill, batts/blankets) installed, workplace concentrations, and extent of respirator usage. Based on best estimates/ranges for these inputs, the average cumulative exposures to FG (from 4 to 7 fiber-months/cc) and RSW (from 2 to 3.3 fiber-months/cc) are considerably less than the cumulative exposures experienced by manufacturing cohorts. Published epidemiological studies of FG and RSW manufacturing cohorts in the United States have shown that there is no significant increase in respiratory system cancer (RSC) compared to appropriate reference populations. The cumulative exposures of the manufacturing cohorts vary with insulation type and plant; maximum (benchmark) values are 23.5 fiber-months/cc for FG plants and 40 fiber-months/cc for RSW plants. Thus, based on fiber exposure, the professional installer cohort is unlikely to experience increased respiratory system cancer. Sensitivity analyses indicate that this conclusion is robust to estimation errors in key inputs.

524 AN OCCUPATIONAL EXPOSURE DATABASE FOR SYNTHETIC VITREOUS FIBERS (SVFS).

G. E. Marchant[1], D. Maxim[3], J. W. Reynolds[5], C. M. Carter[4], A. E. Crane[6] and J. G. Hadley[2]. *[1]College of Law, Arizona State University, Tempe, AZ, [2]Owens Corning Science and Technology Center, Granville, OH, [3]Everest Consulting Associates, Cranbury, NJ, [4]Johns Manville, Littleton, CO, [5]CertainTeed Corporation, Valley Forge, PA and [6]North American Insulation Manufacturers Association, Alexandria, VA.*

This poster presentation will describe an occupational exposure database for synthetic vitreous fibers that contains the largest number of exposure samples for fiber glass and mineral wool insulation products ever assembled. This exposure database has been created by the North American Insulation Manufacturers Association (NAIMA) and its members as part of the Health and Safety Partnership Program (HSPP), a comprehensive voluntary occupational safety and work practice partnership being implemented by the SVF industry under OSHA's oversight. The database now contains over 10, 000 validated exposure samples, which are individually classified by numerous parameters including fiber type, product type, industrial sector, job function, and respirator use. This capability to sort the exposure data by particular parameters can be used for several purposes, including (i) to provide a robust estimate of expected exposure levels for a particular occupational exposure scenario; (ii) to track changes in exposure levels over time; and (iii) to identify occupational exposure scenarios where additional protection (e.g., respirator use) may be needed or may no longer be needed. Cumulative exposure data are presented for the manufacturing, fabrication, installation, and demolition sectors for the major fiber types, along with data stratified for some specific product type/job function combinations that may be of particular interest (e.g., installation of blowing wool insulation). The overall and stratified data show that exposure levels in the industry are generally well below the 1 f/cc 8-hour TWA voluntary standard, with a relatively small number of specific exposure scenarios where exposures approach or exceed 1 f/cc.

525 OCCUPATIONAL EXPOSURE TO AIRBORNE CHRYSOTILE ASBESTOS DURING USE AND REMOVAL OF MASTICS, COATING, AND ADHESIVES (CIRCA 1940S-PRESENT DAY).

F. S. Mowat[1], M. Bono[2] and D. J. Paustenbach[3]. *[1]Exponent, Menlo Park, CA, [2]Exponent, Hudson, OH and [3]ChemRisk, San Francisco, CA.*

In recent years, questions have been raised about the health risks to persons who used asbestos-containing products from the 1940s to present day. Modern era sampling and analytical techniques are often needed to quantify exposure for many of these products, and exposure simulation studies have more frequently been used to estimate the historical airborne concentrations resulting from these products. This research measured the release of chrysotile asbestos during the physical manipulation of coatings, mastics, and an adhesive, which contained 1-8% asbestos within a tarry or a water-based medium. The purpose was to understand whether these products generated any measurable amount of airborne asbestos concentrations that workers or bystanders might be exposed during the use or manipulation of these products. These products were often used to adhere insulation to pipes and boilers or to cover the insulation so as to protect it from direct contact with water, as well as wear and tear. Tests were conducted to evaluate fiber release from such activities as application, spill cleanup, sanding, removal/cutting, and sweep cleaning. Each test activity was performed for 30 minutes, often in triplicate. Personal, area, and background/clearance air samples were collected during the tests, and analyzed for total fiber concentrations using phase contrast microscopy (PCM) and transmission electron microscopy (TEM). No asbestos fibers were identified in 446 of 452 samples collected during these activities. The calculated 8-hour time-weighted average (TWA) airborne concentration for the remaining six samples using hypothetical work scenarios ranged from 0.009 to 0.03 f/cc. Thus, actual and predicted airborne concentrations were well below the current and contemporaneous occupational exposure limits for chrysotile asbestos. Based on these results, these products did not pose an asbestos-related health risk to those who applied or interacted with them.

526 MERCURY BODY BURDEN AND INTAKE IN TWO CANADIAN COASTAL COMMUNITIES: GRAND MANAN AND ST ANDREWS/ST STEPHEN.

M. Legrand and H. Chan. *CINE - McGill University, Ste Anne de Bellevue, QC, Canada.*

Mercury exposure in two Canadian costal communities, Grand Manan and St Andrews/ St Stephen, New Brunswick, Canada was assessed with hair samples and sources of exposure through dietary methods identified. Mean arithmetic concentration of total mercury in hair was 0.7 +/- 0.55 mg/Kg at Grand Manan and 0.419 +/- 0.15 mg/Kg for St Andrews/St Stephen. All participants incurred daily total mercury ranging from near zero to 0.286 ug/kgbw/day which is below the 0.73 ug/kgbw/day providional tolerable daily intake of Health Canada. The low intakes and body burdens can be explained by the low level of mercury found in the species being consumed and their frequency of consumption. The top contributors to mercury intake included haddock, canned tuna, lobster, pollock and lake trout. The correlation between hair mercury and intake (defined as micrograms of mercury per spring season) yielded a 0.32 correlation coefficient. This supports the view that estimating mercury levels in hair using a focused food frequency questionnaire coupled with the 24 hour recall is a reliable technique. To our knowledge, this is the first reported study examining mercury body burden in human populations living along the Bay of Fundy and the first to report trends of fish and seafood consumption from that area. Mercury body burden and mercury intake show that, even though fish and seafood are consumed on a regular basis, particularly at Grand Manan, mercury intake is not a serious problem because the species being consumed contain low levels of mercury.

527 FETAL EXPOSURE TO PHYTOESTROGENS IN JAPAN: FOCUSING ON EQUOL.

E. Todaka[1, 2], H. Osada[3], M. Omori[1, 3], H. Miyakawa[4], M. Uzuki[4], Y. Ikezuki[5], O. Tsutsumi[5], K. Sakurai[6], H. Fukata[7], T. Iguchi[8, 9] and C. Mori[1, 9]. *[1]Bioenv Med., Chiba University, Chiba, Japan, [2]Center Env Health, Field Sciences, Chiba University, Chiba, Japan, [3]OB/GY, Chiba University Hospital, Chiba, Japan, [4]SRL Inc., Tokyo, Japan, [5]OB/GY, University of Tokyo, Tokyo, Japan, [6]Clin Cell Biol, Chiba University, Chiba, Japan, [7]Env Med. Science (SRL), Chiba University, Chiba, Japan, [8]Bioenv Research, Center for Integrated Bioscience, Okazaki National Research Institutes, Okazaki, Japan and [9]CREST, Kawaguchi, Japan.*

Phytoestrogens are estrogen-like chemicals derived from plants mainly soy. As there is a concern if it is safe when human fetus is exposed to excess amount of phytoestrogens, we investigated the feto-maternal relationship of serum concentration levels of phytoestrogens. We collected 51 maternal and newborn cord serum and measured the concentration level of genistein, daidzein, equol and coumestrol by liquid chromatography/tandem-mass spectrometry. Informed consent from each mother was obtained. This study was approved by the Congress on Medical Bioethics at Chiba University. Genistein was detected from 96% of maternal serum and 100% of cord serum. Daidzen was detected from 75% of maternal serum and 80% of cord serum. Equol was detected from 37% of maternal serum and 35% of cord serum. Coumestrol was detected from only one maternal serum. Equol is a metabolite of daidzein and is reported to be more estrogenic than daidzein or genistein. However, it is well known that there are equol producers and equol non-producers. In our study, the average concentration level of equol of maternal and cord serum were 0.89ng/ml and 2.04ng/ml each. Also, there was a tendency found that the endogenous estrogen level of newborns that were exposed to equol was lower than the newborns that were not exposed to equol. Furthermore, there was another tendency found that the concentration level of E2 was lower in female than male newborns when they were divided as equol exist group and non-exist group. The meaning of this finding should be cleared in the future. (This work was supported by the fund for endocrine disrupters from the Ministry of the Environment, Japan)

528 DIALKYL PHOSPHATES (DAP) IN PRODUCE CONFOUND BIOMONITORING IN ORGANOPHOSPHATE RISK ASSESSMENT.

X. Zhang[2, 3, 1] and R. I. Krieger[3, 1, 2]. *[1]Department of Entomology, University of California, Riverside, Riverside, CA, [2]Environmental Toxicology Graduate Program, University of California, Riverside, Riverside, CA and [3]Personal Chemical Exposure Program, University of California, Riverside, Riverside, CA.*

Organophosphate (OP) pesticides produce trace residues in fruits and vegetables protected from insect pests. DAPs are important as stable urine biomarkers of OP exposure. OP excretion may be estimated by the back calculation of absorbed daily dosage from urinary DAPs. DAPs may also be formed as a result of plant metabolism. In this study, DAPs were measured in produce for the first time as part of exposure assessment to evaluate whether preformed DAPs from plants could confound OP exposure assessment. Seventy-seven samples from the channels of trade in California were measured for DAPs and the parent OPs. Only produce known to contain an OP residue from routine monitoring by shippers and producers was selected for stduy. The OP pesticides were acephate, azinphosmethyl, chlorpyrifos, diazinon, dimethoate, dimethoate/omethoate, malathion, methamidophos, methidathion, oxydemeton-methyl and phosmet. All residues were below established residue tolerances. Fifty-one out of 77 samples (66%) contained more DAP residue than parent OPs. The mole ratios of DAPs to parent OP residues ranged from 0.1 to 73. The produce contained 4- to 5- times more oxidized APs (dimethylphosphate and diethylphospahte) than sulfurated products (dimethylthiophosphate and diethylthiophospahte). Urine of persons that consume OP treated produce will contain preformed DAPs as well as human metabolites resulting from biotransformation of the OP residue. All preformed DAPs represent false positives in any attempt to back-calculate OP exposure for children or adults. The practice of using urinary DAPs to estimate OP exposure may result in a substantial overestimate of the low dosages of the general population.

529 PROBABILISTIC METHODS TO ASSESS WORKER EXPOSURE TO AGRICULTURAL PESTICIDES.

E. Julien and . ILSI RSI Working Group. *Risk Science Institute, International Life Sciences Institute, Washington, DC.* Sponsor: P. Fenner-Crisp.

Assessing health risk posed by chemical contaminants in the environment requires the integration of data from two distinct disciplines: toxicology and exposure assessment. Methodology for exposure assessment has undergone rapid development over the last decade, particularly in the area of exposure modeling. Software tools now provide the computational power to conduct probabilistic exposure assessments, which explicitly incorporate variability in data for key determinants of exposure (e.g. variability in rate of contact with media containing a contaminant, variability in contaminant concentration). Thus, probabilistic analyses generate, for a specified population, distributions of exposure estimates (external dose estimates) that reflect the full range of potential exposure levels and their probabilities. Regulatory agencies have used probabilistic exposure modeling to assess risk from exposure to a variety of environmental toxicants. Such agencies are now examining probabilistic methods for occupational exposure assessment, specifically, worker exposure to agricultural pesticides. A number of scientific and statistical questions are raised, however, when probabilistic methods are applied in a novel way. To address these questions, ILSI RSI convened a Working Group with experts from diverse disciplines (occupational exposure assessment, dermal absorption assessment, probabilistic modeling, and statistics). This poster will present the Working Group's findings regarding: 1) developing model input distributions to characterize exposure to agricultural pesticides; 2) incorporating absorbed dose in a probabilistic assessment; 3) conducting a 2-dimensional assessment, which separates variability and uncertainty; and 4) interpreting and communicating results of a probabilistic worker exposure assessment. (Project sponsors include: USEPA, European Commission, Crop Life America, NIOSH, Health Canada, Crop Life International, European Crop Protection Association, California Department of Pesticide Regulation and ILSI RSI).

530 MEASURING CHILDREN'S HOME EXPOSURE TO TETRACHLORVINPHOS FROM FLEA CONTROL COLLARS APPLIED TO THEIR PET DOG.

J. S. Boone, J. W. Tyler and J. E. Chambers. *Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.*

Family units were chosen to determine the levels of the insecticide tetrachlorvinphos that could be transferred to children from a flea control collar from the family pet dog. One child (3-12 years) from each household wore t-shirts at home 5 different days before the application of the collar and for 5 consecutive days between days 6 and 12-post collar application (5 pre- and 5 post-). The dogs were petted to determine available transferable residues on days 6 and 12 using white cotton gloves. Commercially available flea control collars containing the organophosphate insecticide tetrachlorvinphos (14.55%, 33g collar) were applied to dogs as per package directions. Transferable residues were quantified by petting the dog for 5 minutes in 3 areas (neck with collar, neck, and back), and yielded residues ranging from 1300 to 63000 ug, 900 to 30000 ug, and 2 to 750 ug, respectively. A 100 square inch area was cut out of each t-shirt on the front chest area and weighed. The t-shirt samples were analyzed for tetrachlorvinphos; the pre-application t-shirts averaged <0.05 ng/g t-shirt and the post-application t-shirts averaged 2700 ng/g t-shirt. The t-shirt samples worn during the 5 days after placement of the collars ranged from <0.05 ng/g to 47000 ng/g. The variability between and among the families was high. The t-shirts from the child in family 119 averaged 40 ng/g (range 12 to 70 ng/g). The t-shirts from the child in family 120 averaged 13, 000 ng/g (range 290 to 47000 ng/g). The child from family 119 spent less time with the pet dog than the other children, but children from other families spent more time with their pet dog than the one from family120. The child from family 116 spent the most time with the pet dog and averaged 490 ng/g. The introduction of flea control collars containing tetrachlorvinphos into homes increases the amount of transferable residue of tetrachlorvinphos, to variable extents. (Supported by EPA R-828017).

531 THE ASSOCIATION BETWEEN MEDICATION USE AND TAMOXIFEN (TAM) AND TAM METABOLITE CONCENTRATIONS IN WOMEN WITH BREAST CANCER.

L. Gallicchio[1], K. Tkaczuk[2], L. Lewis[1] and J. A. Flaws[1, 2]. *[1]Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD and [2]The Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD.*

Tamoxifen (TAM) is a selective estrogen receptor modulator that is metabolized *via* cytochrome P450 enzyme pathways into several major metabolites, including N-desmethyltamoxifen (N-DMT) and 4-hydroxytamoxifen (4-OHT). Since the data regarding the interaction of TAM with other medications are limited, this study was carried out to determine whether other types of medications co-prescribed with TAM significantly influenced the plasma concentrations of TAM, N-DMT, and 4-OHT. Participants were 98 breast cancer patients who had been taking TAM for at least 30 days. Each participant completed a questionnaire and agreed to a medical chart review, which was used to obtain information on medication use at the time of enrollment. In addition, each woman provided a blood sample that was used to

measure plasma concentrations of TAM, N-DMT, and 4-OHT by high performance liquid chromatography. Results of the analysis showed that participants who were taking a diuretic had significantly higher mean plasma concentrations of TAM and N-DMT than participants not taking a diuretic, independent of age, race, body mass index, and months taking TAM. In contrast, arthritis/pain medication intake was negatively associated with plasma TAM concentrations. The use of other types of medications such as chemotherapeutic agents, allergy drugs, anti-depressants, and diabetes medications did not significantly alter plasma TAM, N-DMT, or 4-OHT concentrations. Our findings suggest that treatment with a diuretic or an arthritis/pain medication may significantly affect TAM metabolism. Future studies should investigate the clinical significance of the interaction of these drug types with TAM. This study was supported by DOD Grant DAMD17-00-1-0321 and the University of Maryland, Maryland Statewide Health Network through the Maryland Cigarette Restitution Funds.

532 BRAIN DERIVED NEUROTRPHIC FACTOR (BDNF) POLYMORPHISM ASSOCIATED WITH INCREASED SYMPTOM REPORTING AMONG DENTAL PERSONNEL.

N. J. Heyer[1], D. Echeverria[1, 2], J. S. Woods[1, 2], A. C. Bittner[1, 2] and F. M. Farin[2]. *[1]Battelle CPHRE, Seattle, WA and [2]Department of Environmental Health, University of washington, Seattle, WA.*

Associations were hypothesized between a BDNF polymorphism and increases in self-reported symptoms of poor concentration and memory among 191 male dentists and 231 female dental assistants exposed to mercury (Hg). DNA buccal cell samples were analyzed for the presence of a previously identified SNP at nucleotide 196 (G/A), which produces a valine to methionine substitution at codon 66 (V66M). Genotyping results identified 68% WT, 28% Het, 4% Mut within this population. Self-reported severity, frequency, and duration were collected for 45 symptoms. A priori groups resulted in 14 groups for current symptoms, 16 groups for recent symptoms lasting one month and chronic symptoms lasting more than one year. Alternatively, factor analyses resulted in 6 factors for current symptoms and 10 factors each for recent and chronic symptoms. Regression analyses were conducted for dentists and dental assistants separately controlling for Hg exposure. Among dentists, four factor scores and three a priori ratings were significantly ($p<.05$) associated with the BDNF polymorphism. Only one was in the unexpected direction. Among dental assistants, three factor scores and seven a priori ratings were significantly associated with the BDNF polymorphism. All associations were in the expected direction. Dentists reported increased symptoms for coordination, muscle weakness, and tremor. Dental Assistants reported increased symptoms for memory, anxiety and tremor. All but one association were within recent and chronic symptoms. Increased reporting for more diverse symptom groups, such as pain, cough and parasthesias were mixed among current, recent and chronic symptoms. These results confirm that the frequency and severity of self-reported symptoms are increased among people with BDNF variants. These symptoms cluster around memory, anxiety and peripheral muscle problems which are not inconsistent with mercury-related health effects. Supported by ES04696 and ES07033.

533 EFFECT OF TWO COMPLEX ENVIROMENTAL MIXTURES CONTAINING POLYCYCLIC AROMATIC HYDROCARBONS (PAHs), DIESEL EXHAUST AND URBAN DUST, ON THE METABOLIC ACTIVATION OF CYTOCHROME P450 1A1 IN MOUSE EPIDERMIS.

L. A. Courter, T. Musafia and W. M. Baird. *Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants, and are found in complex mixtures such as diesel exhaust and urban dust. We investigated the metabolic activation of cytochrome P450 1A1 (CYP1A1) through the Ethoxyresorufin O-Deethylase (EROD) and Western immunoblot assays. SENCAR mice were topically treated with two Standard Reference Materials (SRM) 1975 (diesel particulate organic extract) or SRM 1649 (urban dust) obtained from the National Institute of Standards and Technology (NIST), and either additional benzo[a]pyrene (B[a]P) or dibenzo[a, l]pyrene (DB[a, l]P for 24 hours. SRM 1975 treatment caused an increase in activity in the epidermis compared to the vehicle control, and a greater increase in activity with the addition of DB[a, l]P. However, with the addition of B[a]P to SRM 1975, there was no CYP1A1 activity. Similarly, SRM 1649 exhibited no activity. However, with the addition of B[a]P, SRM 1649 showed an increase and a greater increase in CYP1A1 activity with the addition of DB[a, l]P. These results indicate that components within PAH-containing environmental mixtures can either inhibit or promote the activation of known carcinogenic PAHs by CYP1A1. (This work was supported by NCI CA28825.)

534 FORMATION OF *CIS*- BPDE-ADDUCTS AND BASE-STACKED *trans*-BPDE-ADDUCTS IS INCREASED ON SUPERCOILED DNA.

G. Jiang[1], R. Jankowiak[2], N. Grubor[2], M. Banasiewicz[2], G. Small[2], M. Skorvaga[3], B. Van Houten[3] and J. States[1]. *[1]University of Louisville, Louisville, KY, [2]Iowa State University, Ames, IA and [3]NIEHS, Research Triangle Park, NC.*

Benzo[*a*]pyrene is metabolized to the highly reactive and mutagenic (+)-7*R*, 8*S*-dihydroxy-9*S*, 10*R*-epoxy-7, 8, 9, 10-tetrahydrobenzo[*a*]pyrene (BPDE). BPDE forms predominantly N^2-deoxyguanine DNA adducts in two stereoisomeric configurations (*cis* and *trans*). Both eukaryotic and prokaryotic nucleotide excision repair (NER) systems preferentially excise *cis*-BPDE-adducts. We examined the influence of DNA secondary structure on the stereospecificity of BPDE-adduct formation and on the incision of BPDE-adducts by the prokaryotic UvrABC NER endonuclease. Incision of BPDE-adducts formed at relatively low density (≤ 1 adduct/kb) on supercoiled plasmids by the thermoresistant *Bacillus caldotenax* UvrABC was 4 - 8-fold better than incision of BPDE-adducts formed on linear DNA. Incision efficiency did not decrease if supercoiled plasmid DNAs were linearized after BPDE adduct formation. These results suggested that adducts formed on linear and supercoiled DNAs differed in configuration and/or conformation. Low temperature fluorescence spectroscopy of adducted supercoiled and linear DNAs confirmed this hypothesis. The spectroscopy results indicated that *cis*-BPDE-adducts as well as base-stacked *trans*-BPDE-adducts formed more abundantly in supercoiled DNA than in linear DNA. That the *cis* to *trans*-adduct ratio was higher in supercoiled than linear DNA was confirmed by [^{32}P]-postlabeling analyses. The results of these studies indicate that DNA secondary structure influences both the configuration and conformation of BPDE adducts formed at low density, and suggest that the ratio of *cis* to *trans*-BPDE-adducts and the amount of base stacked *trans*-adducts formed under physiological exposure conditions may be higher than inferred from high dose experiments. (Supported in part by NIH Grant 1R01-ES06460 (JCS), the Commonwealth of Kentucky Research Challenge Trust Fund, USDOE contract no. W-7405-Eng-82.) Keyword: UvrABC ; benzo[*a*]pyrene ; stereochemistry

535 DNA ADDUCT FORMATION BY DIBENZO(C, P)CHRYSENE IN HUMAN CELL CULTURE.

J. Atkin[1], H. Garcia[1], B. Mahadevan[1], T. Musafia[1], A. Sharma[2], S. Amin[2] and W. Baird[1]. *[1]Oregon State University, Corvallis, OR and [2]Institute for Cancer Prevention, Valhalla, NY.*

Polyaromatic hydrocarbons (PAH) are ubiquitous environmental pollutanats that have been associated with cancer incidence. PAH are activated in human cells to active metabolites that adduct to DNA. Benzo(a)pyrene (BP) has a bay region that contributes to its carcinogenicity, while more potent dibenzo(a, l)pyrene (DBP) has a fjord region. Dibenzo(c, p)chrysene has both bay and fjord region and we wanted to compare its carcinogenic activation to BP and DBP. We studied the DNA adduct formation by dibenzo(c, p)chrysene and two of its metabolites, dibenzo(cp)C-1, 2-dihydrodiol and dibenzo(c, p) C-11, 12-dihydrodiol, by 33P postlabeling and HPLC analysis. We also assesed the toxicity of these compounds by MTT cytotoxicity assay. Our results show DNA adduct formation by the metabolites of DBC. The MTT assay showed no toxicity with DBC at concentrations up to 30ng/ul, while BP and DBP were toxic to MCF 7 cells at lower concentrations. H.G.P. was supported by ES 007316. The work was supported by grant CA 28825.

536 LUNG DNA ADDUCT FORMATION IN MICE EXPOSED TO DIBENZO[*A, L*]PYRENE: A DOSE-RESPONSE STUDY.

B. Mahadevan[1], J. Atkin[2], C. Bravo[3], A. Luch[4], L. Steppan[5], N. Kerkvliet[6] and W. M. Baird[7]. *[1]Environmental&Molecular Toxicology, Oregon State University, Corvallis, OR, [2]Environmental&Molecular Toxicology, Oregon State University, Corvallis, OR, [3]Environmental&Molecular Toxicology, Oregon State University, Corvallis, OR, [4]MIT Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, [5]Environmental&Molecular Toxicology, Oregon State University, Corvallis, OR, [6]Environmental&Molecular Toxicology, Oregon State University, Corvallis, OR and [7]Environmental&Molecular Toxicology, Oregon State University, Corvallis, OR.*

Polycyclic aromatic hydrocarbons (PAH) are environmental carcinogens present in the atmosphere from combustion sources such as cigarette smoke, diesel exhaust, urban dust, as well as industrial coal tar production. To date, dibenzo[*a, l*]pyrene (DBP) has been found to be the strongest tumor-initiating PAH ever tested in skin and rat mammary glands. In our study we show for the first time that systemic exposure to DBP causes DNA damage in mouse lung tissue. C57B1/6 mice were dosed by gavage with DBP at 1, 5 or 20 mg/kg body weight, daily for 10 days. Results indicated a decrease in body weight only at the highest dose. The lungs from treated mice were removed and DNA was isolated for DNA adduct analysis.

^{33}P postlabeling of DNA adducts analyzed by reverse phase high performance liquid chromatography (HPLC) revealed a dose-dependent DNA adduct formation in the mouse lung tissue. A major portion of the DNA adducts formed were contributed by (-)-*anti*-DB[*a, l*]PDE (DBP-DE), the ultimate carcinogenic metabolite of DBP. This data is consistent with and also support previous findings that the *anti*-isomer of DBP is more carcinogenic than the *syn* isomer.

537 ACCELERATED DNA ADDUCT FORMATION IN LUNG, NASAL MUCOSA, AND LIVER OF RATS EXPOSED TO URBAN AIR IN KAWASAKI, JAPAN.

H. Sato[1, 2], K. T. Suzuki[2], H. Sone[1], Y. Yamano[3], J. Kagawa[3] and Y. Aoki[1]. *[1]Research Center for Environmental Risk, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan, [2]Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan and [3]Department of Public Health, Tokyo Women's Medical University, Tokyo, Japan.*

Various polycyclic aromatic hydrocarbons (PAHs) generated by the combustion of fossil fuels, such as from diesel engines and industry, have been detected on suspended particulate matter in ambient air in urban areas. Some PAHs, e.g. phenanthrene, benzo(a)pyrene, and related compounds, are potent mutagens. The potency of ambient air for DNA adduct formation in rat organs was estimated by using a 32P-postlabeling method. Wistar rats were maintained in a small-animal facility located beside an intersection of main highways in the city of Kawasaki, Japan, for 4, 12, 24, 48, or 60 weeks, and exposed to roadside air (exposure group, EG) or clean air (control group, CG). The amount of carbon black deposited in EG lungs was 0.475 mg/g wet tissue after exposure for 60 weeks. Compared with CG, the relative adduct levels (RALs) were increased significantly in EG lungs (17.1-fold; $P < 0.05$), nasal mucosae (4.0-fold; $P < 0.01$), and livers (4.9-fold; $P < 0.01$) after exposure for 4 weeks. There were no significant differences in RALs between EG and CG after exposure for 12 weeks, but RALs were elevated again in EG after exposure for 48 or 60 weeks. The level of cytochrome P450 (CYP) 1A2 mRNA in EG rat liver was 2.3 times higher than in CG after 4 weeks of exposure, but gradually decreased with age in both CG and EG. No difference was observed between CG and EG in the levels of CYP 1A2 mRNA in liver after exposure for 60 weeks. These results suggest that roadside air in this region can cause the generation of DNA adducts. This influence of ambient roadside air can be estimated by using experimental animals, indicating that biological monitoring of DNA adduct formation may be a powerful tool for assessing the effect of ambient air on human health.

538 KINETICS OF REACTION OF EPOXIDE METABOLITES OF POLYCYCLIC AROMATIC HYDROCARBONS WITH HUMAN AND MOUSE HEMOGLOBIN.

S. R. Myers, C. Cunningham, T. Wright and H. E. Hurst. *Pharmacology and Toxicology, University of Louisville, Louisville, KY.*

Previous studies from this laboratory have focused on the detection of hemoglobin adducts to tobacco smoke carcinogens in maternal and fetal blood samples. The present study focuses on the kinetics of reaction of selected polycyclic aromatic hydrocarbon (PAH) epoxides with hemoglobin obtained from human and mouse. In this study, we hypothesize that the relative rate and extent of reaction with hemoglobin will be greatest with those PAH epoxides that are more carcinogenic than others. Specifically, we investigated the relative rates of reaction of benz(a)anthracene (BA), benzo(a)pyrene (BP), dibenz(a, h)anthracene (DBA), benzo(ghi)perylene (BghiP), fluoranthene (F), and benzo(b)fluoranthene (BbF) with hemoglobin. The overall carcinogenicity of these compounds is BP > BbF > DBA > F > BghiP > BA. The results obtained are in agreement with the overall carcinogenicity of the parent hydorcarbons with the PAHs having greater carcinogenicity having a greater extent of protein binding to hemoglobin. In addition, those compounds that were weakly carcinogenic diplayed slower kinetics of reaction with hemoglobin and less overall adduct formation. These results suggest that the formation of hemoglobin adducts of carcinogenic PAH serves as a potential biomarker of exposure and carcinogenicity of PAH. Future studies will determine the validity of these correlations *in vivo*.

539 B[A]P AND B[A]P-7, 8-DIOL INDUCED CELL CYCLE ARREST AND APOPTOSIS IN LNCAP CELLS.

O. F. Nwagbara[1], S. Reed[1] and R. Gragg[1]. *[1]Environmental Sciences Institute, Florida A&M University, Tallahassee, FL, [2]Environmental Sciences Institute, Florida A&M University, Tallahassee, FL and [3]Environmental Sciences Institute, Florida A&M University, Tallahassee, FL.* Sponsor: R. Thomas.

Carcinoma of the prostate, a steriod hormone dependent organ accounts for 25% of all cancer cases in the United States, is the second cause of death in males in the US it remains incurable once it has metastized. In 2002, there were about 189, 00 new cases of prostate cancer and estimated 30, 200 prostate cancer-related deaths in the US. Polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (B[a]P) and Benzo[a]pyrene-7, 8-dihydrodiol (B[a]P-7, 8-diol) are known to act through the aryl hydrocarbon receptor to elicit a variety of cancer and noncancer endpoints in exposed animal and cellular models, and humans. In addition, B[a]P modulates various endocrine functions by enhancing ligand metabolism, altering hormone synthesis, downregulating receptor levels and interfering with gene transcription. PURPOSE: This study investigates the effects of B[a]P and B[a]P-7, 8-diol on lymph node cancer of the prostate (LNCaP)carcinoma cell cycle progression in the presence and absence of synthetic androgen, R1881. METHODS:LNCaP cells were treated for 24 hour with B[a]P and B[a]-7, 8-diol (1.0uM, 5.0uM, 10.0uM, 20.0uM and 50.0uM) in the presence and absence of 0.1uM R1881 or DMSO vehicle. Cell *via*bility was measured by MTT assay. Cell cycle distribution was assessed using flow cytometry technique. RESULTS: Treatment with DMSO and B[a]P in the presence and absence of 0.1uM R1881 had no effect on cell *via*bility. Exposure to increasing concentrations of B[a]P-7, 8-diol alone does not affect cell cycle distribution while exposure to these same concentrations in the presence of R1881 induced apoptosis and S phase arrest at 50uM of B[a]P-7, 8-diol. CONCLUSIONS: These results show that the effects of B[a]P-7, 8-diol is concentration-dependent in the inhibition of G1 to S phase cell cycle progression in the presence of R1881.

540 THE PROLIFERATIVE EFFECT OF SELENIUM DIOXIDE AGANIST BENZO(A)PYRENE TOXICITY.

M. R. Smith[1], J. Ochieng[2] and A. M. Nyanda[1]. *[1]Pharmacology, Meharry Medical College, Nashville, TN and [2]Biochemistry, Meharry Medical College, Nashville, TN.*

The biotransformation of Benzo(a)pyrene [B(a)p], an ubiquitous environmental pollutant, is a two-step process. Initially B(a)p is hydrolyzed by cytochrome P_{450} and then reduced to form an ultimate metabolite, (±)-benzo[a]pyrene-7, 8-diol-9, 10-epoxide, which will bind to DNA and form an adduct at the N^7 guanine residue eventually leading to cellular transformation. Finally, B(a)p is then conjugated by multiple enzymes, particularly glutathione, for elimination. Used as a cofactor, glutathione is a vital component in the biotransformation of selenium compounds; however, selenium dioxide will react with the thiol group of glutathione to form superoxides and reactive oxygen species. We hypothesize that selenium dioxide (SeO_2) will enhance the formation of 10-selenoether-(±)-benzo[a]pyrene-7, 8, 9-triol, thus reducing DNA adduct formation in human mammary and mouse fibroblast cells . Therefore, the objective of this study is to analyze the proliferative effect of SeO_2 against B(a)p administration in MCF-10A, MDA-MB-435, MDA-MB-468 and 3T3. Preliminary data using $CellTiter_{96}$ (Promega) in actively growing cells indicate a dose-dependent decrease in cell proliferation by SeO_2 with maximum proliferation at $0.09 \times 10^{-2}\mu M$. Preliminary data, suggest that prior and subsequent treatment of SeO_2 has an inhibitory effect on B(a)p challenged cells. This study will contribute to the understanding of selenium dioxide in the reduction of xenobiotic associated cancer growth.

541 BENZO(A)PYRENE METABOLITES ACTIVATE EGFR PATHWAYS IN HUMAN MAMMARY EPITHELIAL CELLS: A POTENTIAL MECHANISM FOR TUMOR PROMOTION.

A. D. Burdick, K. Liu, L. G. Hudson, H. Shi and S. W. Burchiel. *College of Pharmacy, University of New Mexico, Albuquerque, NM.*

Polycyclic aromatic hydrocarbons, such as benzo[a]pyrene (BaP), are known mammary carcinogens in rodents and may be involved in human breast cancer. Classically, the carcinogenicity of BaP has been attributed to the formation of the bay-region epoxide, anti-BPDE, which has been shown to stably bind DNA. Our laboratory is interested in the mechanisms by which BaP metabolites may also act as tumor promoters. A hallmark of tumor promotion is the inhibition of cellular apoptosis and/or increased cell proliferation under conditions that would normally be apoptotic, such as growth factor withdrawal. We have reported previously that BaP mimicked growth factor signaling and increased cell proliferation in primary human mammary epithelial cells (HMECs) and the HMEC line, MCF-10A. Diones of BaP (i.e. BaP-quinones, BPQs) are important oxidative metabolites of BaP that have been associated with the production of reactive oxygen species (ROS). In these studies, we demonstrated by electron paramagnetic resonance spectroscopy (EPR) and flow cytometry that BPQs produced ROS in MCF-10A cells. Using a model of epidermal growth factor (EGF) withdrawal in MCF-10A cells, we showed that H2O2 production by BPQs lead to the activation of the EGF receptor (EGFR), resulting in increased cell number in the absence of EGF. Conversely, treatment of MCF-10A cells with anti-BPDE resulted in cell cycle arrest and apoptosis. Thus, the activation of the EGFR signaling pathways by BPQs may represent one pathway by which BaP contributes to tumor promotion, and ultimately the development of breast cancer. This work was supported by NIEHS RO1 ES-07259, P30 ES-012072 and ADB by the US Army CDMRP Grant DAMD17-02-1-0512.

542 MALIGNANT TRANSFORMATION INDUCED BY BENZO(A)PYRENE AND DISTILLATE MARINE DIESEL FUEL IN HUMAN KERATINOCYTES.

O. Lohitnavy, J. Campain and R. Yang. *Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO.*

Some polycyclic aromatic hydrocarbons (PAHs) are known to have carcinogenic properties. To explore the interactions among these PAHs, cytotoxicity and the ability to induce neoplastic transformation by distillate marine diesel fuel (DMA type) and benzo(a)pyrene (BaP) were investigated in an immortalized human keratinocyte cell line, RHEK-1. Cytotoxicity analysis for both DMA and BaP were carried out for this cell line using the clonogenic assay. In transformation studies, RHEK-1 cells were treated repetitively with LC_5 BaP, LC_{50} BaP, LC_{10} DMA, the mixture of LC_5 BaP and LC_{10} DMA, the mixture of LC_{50} BaP and LC_{10} DMA, or acetone as a solvent control. As RHEK-1 lacks metabolic capabilities, these experiments were carried out in the presence of rat S9 microsomal fraction. Anchorage-independent growth (AIG), an indicator of malignant transformation, was measured in cultures as a function of time. Our results demonstrated that the cells exposed to LC_{50} BaP and the mixture of LC_{50} BaP and DMA acquired the ability of AIG in a time-dependent manner. In contrast to AIG-negative control cells, the cloning efficiency in methylcellulose of transformed populations significantly increased from 1-2% at passage 26 to 22-25% by passage 42. Other transformation-associated characteristics being analyzed in AIG+ cultures and negative controls include morphological alterations, saturation density, and serum independent growth. Our findings thus far, based on the endpoints studied, do not demonstrate obvious interactions between DMA and BaP. However, gene expression alterations in these cultures will be characterized to increase our understanding of the process of malignant conversion in keratinocytes through identification of key molecular players. This study was funded by NIEHS Grant #RO1 ES09655.

543 TRANSFORMATION-ASSOCIATED CHARACTERISTICS IN CELL GROWTH AND DNA CONTENT IN HUMAN KERATINOCYTES, RHEK-1.

J. Campain, O. Lohitnavy and R. Yang. *Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO.*

The ability of benzo(a)pyrene (BaP) alone and a mixture of BaP and five non-carcinogenic polycyclic aromatic hydrocarbons (5PAHs) (anthracene, chrysene, fluoranthene, pyrene and phenanthrene) (Warshawsky et al, *Fundam. Appl. Pharmacology* 20:376-382, 1993) to induce malignant transformation of an immortalized human keratinocyte cell line (RHEK-1) has been previously demonstrated. To investigate properties of the transformed cells, saturation density, serum independence, cellular DNA content, and cell cycle distribution were analyzed at passages 17, 25, and 33. For comparison, a non-transformed control treated with DMSO was also characterized. Our results showed that the BaP- and BaP+5PAHs - transformed cells had significantly lower saturation density than the DMSO-control cells. This is likely a function of cell size. All transformed cells were still dependent upon fetal bovine serum for maximal growth in cultures. Changes in DNA content of the transformed cells have been demonstrated by staining with bromodeoxyuridine and propidium iodide followed by flow cytometric analysis. Our results demonstrated that the malignant cultures were highly aneuploid, with evidence for at least two different populations present. The DNA content of these populations rapidly increased with time in culture, strongly suggesting a high degree of genetic instability. In order to facilitate characterization of cell cycle parameters in these complex populations, we are currently isolating clones. These clones will be analyzed individually for transformation-associated characteristics, DNA content, and specific gene expression alterations in order to more accurately define correlative (or causal) relationships. This study was funded in part by NIEHS Grant RO1 ES09655 and ATSDR Cooperative Agreement U61/ATU 881475).

544 TOXICITY AND METABOLISM OF P-CHLOROANILINE AND P-CHLOROPHENYL UREA: CARCINOGENIC IMPLICATIONS.

R. A. Cardona[1], G. J. Putterman[1], D. L. Story[1], M. M. Gay[1], V. DeMatteo[1] and E. I. Goldenthal[2]. *[1]Crop Protection R&D, Crompton Corporation, Bethany, CT and [2]Department of Toxicology, MPI Research, Mattawan, MI.*

In a 1989 National Toxicology Program (NTP) bioassay, p-chloroaniline (PCA) was reported to be carcinogenic in male Fischer 344 rats based on an increased incidence of splenic sarcomas. In the NTP report, it is proposed that PCA undergoes N-hydroxylation to form the corresponding N-hydroxylamine metabolite. N-hydroxylation of aromatic amines is a well-known mechanism of aromatic amine carcinogenicity. The resulting hydroxylamine derivative of PCA, or the proximate carcinogen, can then be conjugated by sulfation, acetylation or glucuronidation to form the ultimate carcinogen capable of ionizing to produce a nitrenium ion that reacts with macromolecules (DNA) forming adducts, which result in splenic tumors. N-hydroxy PCA also causes methemoglobinemia in mammals. Consequently, methemoglobin formation can be used as an indicator for the presence of PCA and its N-hydroxy metabolite, or proximate carcinogen. Seven-day dietary and single-dose gavage studies in the Fischer 344 rat with PCA and its urea analog, p-chlorophenylurea (CPU), and a CPU rat metabolism study, were conducted to determine if CPU is metabolized to PCA or, as demonstrated by methemoglobin formation, a N-hydroxylamine derivative. Results demonstrated that CPU is neither metabolized to PCA nor N-hydroxylated, the required first step in the carcinogenic mode of action of PCA and other aromatic amines. Therefore, it can be concluded that CPU does not possess the same toxicity or carcinogenic mechanism of action as PCA. Further, monuron, the 1, 1 dimethyl analog of CPU, is carcinogenic. Its mode of action involves either formation of a methyl carbonium ion or N-hydroxylation and subsequent formation of a nitrenium ion, as proposed for PCA. Either mechanism is plausible given the presence of a dimethylamine biophore in the monuron molecule and the ability of monuron, like PCA, to produce methemoglobin formation in mammals. In light of the above it can be implied that, unlike PCA and monuron, CPU is not carcinogenic.

545 TOXICOLOGY AND CARCINOGENESIS STUDIES OF TRIETHANOLAMINE IN F344/N RATS AND B6C3F1 MICE.

F. A. Suarez[1], G. Pearse[1], M. R. Hejtmancik[2] and J. R. Bucher[1]. *[1]NIEHS, Research Triangle Park, NC and [2]Batelle, Columbus, OH.*

Triethanolamine is an ingredient in detergents and polishes, textiles, herbicides, pharmaceutical ointments, and in the manufacture of cosmetic products. The carcinogenicity of triethanolamine was evaluated in dermal studies in F344/N rats and B6C3F1 mice. In pre-chronic studies, male and female rats were topically administered 0, 25, 250, 500, or 1000 mg/kg in acetone or 2000 mg/kg neat triethanolamine for 13 weeks. Mice received 0, 25, 250, 500, 1, 000, or 2, 000 mg/kg in acetone or 4, 000 mg/kg triethanolamine (neat). Clinical findings in rats included irritation, scaliness and crustiness of the skin at the site of application. Similar changes were observed in mice but only in the 4, 000-mg/kg dose group. Microscopic examination indicated acanthosis and inflammation at the site of application. Based on the severity of the acanthosis and inflammation at the site of application, doses selected for the 2-year dermal studies in rats were 32, 63, and 125 mg/kg (males), and 63, 125 and 250 mg/kg (females). Mice received 200, 630, and 2, 000 mg/kg (males), and 100, 300, and 1, 000 mg/kg (females). In the 2-year study, the incidences of acanthosis, inflammation and ulceration were increased in female rats. Male rats in the 63-mg/kg dose group had an increased incidence of adenoma of the renal tubule epithelium. This finding was considered an equivocal response. In mice, hepatocellular adenomas occurred with positive trends in females, providing some evidence of carcinogenicity. Males had increased incidences of hemangioma (2, 000 mg/kg), and hemangiosarcomas (630 mg/kg). This finding was considered an equivocal response. Treatment-related lesions observed at the site of application occurred in males from the 300 and 1, 000 mg/kg groups. Microscopically observed changes were associated with the focal crusts observed macroscopically, and included epidermal hyperplasia, suppurative inflammation, and minimal focal ulceration and dermal chronic inflammation.

546 AN ORAL CARCINOGENICITY STUDY OF SENNA-MIS IN THE ALBINO RAT.

J. M. Mitchell[1], S. McPherson[2], University. Mengs[3], J. Tigner[1], J. Zijlstra[4], P. Dettmar[5], R. Gregson[2] and I. Lebish[1]. *[1]Purdue Pharmacology, Ardsley, NY, [2]CTBR Bio-Research Inc., Senneville, QC, Canada, [3]Madaus AG, Koln, Germany, [4]Novartis Consumer Health SA, Nyon, Switzerland and [5]Reckitt Benckiser Healthcare, Hull, United Kingdom.*

Senna-MIS (Madaus Indian Senna), a known laxative derived from plants, was administered by gavage to Sprague-Dawley (Crl:CD(SD)BR) rats once daily at dose levels of 0, 0, 25, 100 and 300 mg/kg/day for up to 104 consecutive weeks. Sixty animals per sex were assigned to the control and dose groups. The primary dose and treatment-related clinical observation was mucoid feces (seen at 300 mg/kg/day). Compared to controls, animals administered 300 mg/kg/day had reduced body weights, increased water consumption and notable changes in electrolytes in serum (increases in potassium and chloride) and urine (decreases in sodium, potassium and chloride) and darker urine. The changes in electrolytes are believed to be physiologic adaptations to the laxative effect of Senna. Dark discoloration of the kidneys was observed in animals in all treated groups. Histological changes were seen in the kidneys of animals from all treated groups and included slight to moderate, tubular basophillia and tubular pigment deposits. In addition, for all treated groups, mini-

mal to slight hyperplasia was evident in the colon and cecum. These histological changes together with the changes seen in the hematology, chemistry and urine parameters evaluated have been shown in previous studies of Senna to be reversible. No treatment-related neoplastic changes were observed. It is concluded that lifetime daily administration of Senna-MIS at dosage levels of up to 300 mg/kg/day did not reveal any evidence of carcinogenicity in Sprague-Dawley rats.

547 STABILITY OF Tg.AC PHENOTYPIC RESPONSE ACROSS MULTIPLE BREEDINGS.

G. Moser[1], J. W. Spalding[2], R. E. Cannon[2], R. W. Tennant[2], S. Stasiewicz[2], M. A. Streicker[1] and T. L. Goldsworthy[1]. *[1]Integrated Laboratory Systems, Research Triangle Park, NC and [2]NIEHS, Research Triangle Park, NC.*

Two-stage carcinogenesis in mouse skin has helped define the multistage nature of tumorigenesis. A consistent alteration in mouse skin tumors is activation of the Ha-*ras* oncogene. The Tg.AC mouse model has an inducible activated v-Ha-*ras* gene (ζ-globin promoted v-Ha-*ras* on a FVB background) and has been proposed as an alternative to the conventional two-year bioassay. Historically dermal exposure to 12-O-tetradecanoyl-phorbol-13-acetate (TPA) produces skin papillomas as a predictable reporter phenotype. However, in three studies conducted in 1997 and 1998, the incidence of papilloma-bearing Tg.AC animals was greatly decreased as compared to previous studies. Genomic DNA blots linked an altered genotype to the non-responder phenotype. By selective breeding of mice with the responder genotype, the TPA-induced papilloma response was shown to be consistent and robust in late 1998-1999. To expand upon these findings, we evaluated 19 breedings of hemizygous male and female Tg.AC mice from 2000-2003. After dermal administration of 2.5 μg TPA thrice weekly for at least 12 weeks, the incidence of papilloma bearing mice in male and female Tg.AC mice was greater than 85%. With one exception mean group tumor latency period was <10 weeks, peak number of papillomas was over 20, and tumor multiplicity was greater than 12 papillomas per tumor bearing mouse. The mean multiplicity in male Tg.AC mice was greater than that of female Tg.AC mice. With regard to tumors of odontogenic origin, over 50% of the Tg.AC studies produced animals with odontomas. The incidence of mice with odomotomas within these studies was 5-25%. The success of this selective Tg.AC breeding strategy is evidenced by the recovery of a reproducible and robust phenotypic response in TPA-exposed hemizygous Tg.AC mice over multiple breedings. These studies also validate the importance of positive controls in Tg.AC studies. This work was supported by NIEHS (N01-ES-95442).

548 TRANSPLACENTAL CARCINOGENICITY OF AZIDOTHYMIDINE IN B6C3F1 MICE AND F344 RATS.

D. M. Walker[1], J. F. Hardisty[2], F. A. Ruecker[3], K. A. Funk[2], M. J. Wolfe[2] and V. E. Walker[1]. *[1]Lovelace Respiratory Research Institute, Albuquerque, NM, [2]Experimental Pathology Laboratories, Inc., Research Triangle ParK, NC and [3]FAR Consulting, L.L.C., Manchester, MO.*

AZT has been demonstrated to be incorporated into host cell DNA and to cause DNA chain termination, and thus may act as a mutagen and carcinogen in transplacentally exposed animals and humans. Olivero et al. (JNCI 89:1602, 1997) previously demonstrated that prenatal exposure to clincally relevant cumulative doses of AZT resulted in treatment-related increases in lung, liver, and reproductive organ tumors of CD-1 mice. To evaluate the carcinogenic potential of transplacental AZT exposure in a second mouse strain and a second rodent species, pregnant B6C3F1 mice and F344 rats were exposed by gavage to 0, 80, 240, or 480 mg/kg bw/day AZT for the last 7 days of gestation. Complete necropsies were performed on 680 female and male B6C3F1 mice and 680 female and male F344 rats at 1 or 2 years after birth (40-45 mice and rats/gender/dose group/age group). There was a significant treatment-related increase in the incidence of lung neoplasms (adenoma and carcinomas combined) and a significant dose-related shift from benign to malignant hepatocellular neoplasms (with multiplicity and metastases to lung) in AZT-exposed male mice at 2 years-of-age. These effects in lung and liver are consistent with those reported earlier in CD-1 mice. The major histopathological finding in rats was an increase in the incidence of gliomas in AZT-exposed female rats. The average historical incidence of gliomas in control F344 rats is ~0.5% in females from 2-year bioassays (up to 26 months) versus ~1.5% in females allowed to live out their lifespan (up to 34 months), suggesting that the increased occurrence of gliomas in 2.2 to 4.4% of AZT-exposed female rats may be related to transplacental administration of the drug. Histopatholocial studies of B6C3F1 mice and F344 rats also revealed a treatment related complex of non-cancerous and inflammatory lesions, especially in mitochondrial rich tissues, including the heart.

549 PHENOTYPIC BASELINE DATA ON TRANSGENIC MOUSE MODELS FOR TOXICOLOGY.

E. Arlund and S. Swing. *Taconic Farms Inc., Germantown, NY.*

TG.AC, rasH2, TSG-p53, and mdr1a are genetically engineered (transgenic and knockout) mouse models used in carcinogenicity testing and drug transport study. To better define these models and provide baseline data, these animals in their naïve state were evaluated in a phenotypic screen. Phenotyping consisted of Urinalysis (a 5 element dipstick), Clinical Chemistry (a 9 component serum analysis), Hematology (a 9 component whole blood analysis), Differential count, organ weights and histology (9 tissues). Genotypes of interest (homozygous TG.AC, heterozygous and wildtype p53 and rasH2) were evaluated and compared to non-transgenic equivalents (FVB, B6). In all cases both males and females, 7 weeks of age, are compared. All of the urinalysis, clinical chemistry and hematology results are within the normal limits for mouse species, though variability is evident between specific models and genotypes. All histologic lesions diagnosed in various tissues during the evaluation were considered to be incidental findings, which are normally described as background pathology. These changes or lesions can usually be attributed to species characteristics, age, dietary influence, or are gender related. This data defines the general physiology of these animals as within normal species range, an important aspect for any genetically altered animal.

550 COMPARISON OF THE K6/ODC AND SKH-1 HAIRLESS MICE IN RESPONSE TO PHOTOCARCINOGENICITY OF LOMEFLOXACIN.

J. Bastien, T. O'Brien and Y. Chen. *ODC Mouse Group, Inc., Drexel Hill, PA.*

K6/ODC mice have been engineered to overexpress the enzyme ornithine decarboxylase (ODC) in skin and some internal organs and are extremely sensitive to tumor induction with both genotoxic chemicals and UV radiation. The Skh-1 hairless mouse strain is the standard mouse model for photocarcinogenesis studies. The purpose of this study was to compare the response of K6/ODC and SKh-1 mice to irradiated lomefloxacin in order to determine the more effective and sensitive model for use in testing photochemical carcinogens. Lomefloxacin and vehicle were administered by gavage to K6/ODC and Skh-1 mice five times every two weeks for a period of 12 weeks. Approximately 1.5 h after oral administration, mice were exposed to UV radiation. Unirradiated vehicle and chemical treated controls were also used. Tumor incidence was recorded weekly for 22 weeks. No signs of phototoxicity were seen in any group. With lomefloxacin plus UV irradiation, the tumor incidence was100% vs. 37% and multiplicity was 11.4 vs. 1.66 for K6/ODC and Skh-1 mice, respectively. The latency of tumor formation was greatly reduced in K6/ODC mice compared to Skh-1 mice. In animals exposed to UV alone, Skh-1 mice developed no tumors while K6/ODC mice had 4 tumors. There were no tumors in unirradiated control groups of either strain. The majority of the tumors were squamous papillomas in both strains, but some squamous cell carcinomas were also present. These results demonstrate the utility of the K6/ODC model for detecting photochemical carcinogens, especially at low doses and relatively short exposure periods.

551 A SUMMARY OF TOXICOLOGICAL AND CHEMICAL DATA RELEVANT TO THE EVALUATION OF CAST SHEET TOBACCO.

R. J. Potts, P. H. Ayres, M. A. Higuchi, K. Shreve, D. R. Meckley, E. R. Bombick, J. E. Swauger, A. T. Mosberg and D. H. Pence. *R J Reynolds Tobacco Company, Winston-Salem, NC.*

A tiered testing strategy based on a comparative chemical and biological testing program has been developed to evaluate the potential of tobacco processes, ingredients, or other technological developments to change the biological activity that results from burning tobacco. Cast Sheet tobacco is a specific type of reconstituted tobacco sheet currently used in the manufacture of cigarettes. As part of the toxicological evaluation of Cast Sheet tobacco, test cigarettes containing Cast Sheet at a blend level of either 10 or 15% were compared to a reference cigarette that did not contain Cast Sheet. Testing included analysis of mainstream smoke chemistry, *in vitro* studies using cigarette smoke condensate (CSC) and/or whole smoke (Ames assay, sister chromatid exchange assay, and neutral red cytotoxicity assay), and *in vivo* studies (13-week inhalation study of mainstream smoke in Sprague-Dawley rats and a 30-week dermal tumor promotion study of CSC in SENCAR mice). Compared to the reference cigarette, inclusion of Cast Sheet resulted in significant increases in mainstream smoke levels of "tar" and nicotine, as well as increases in certain target analytes. All analyte values were within the ranges for currently marketed products of similar "tar" category. CSC derived from the cigarettes containing 15% Cast Sheet demonstrated significantly higher Ames activity in strain TA100 on a revertants/cigarette basis but not on a revertants/mg "tar" basis when compared to the reference cigarette. Whole smoke generated from cigarettes containing 10% Cast Sheet was less cytotoxic compared to the reference cigarette. No other statistical differences were observed between the reference and test cigarettes in the remaining *in vitro* studies. *In vivo* studies demonstrated that the biological activity of the test cigarettes was comparable to that of the reference cigarette. Collectively, the studies demonstrated that inclusion of Cast Sheet up to 15% in the final blend did not increase the inherent biological activity of cigarette smoke/CSC.

552 EVALUATION OF THE DBA/2 MOUSE FOR ASSESSING THE DERMAL TUMOR-PROMOTING POTENTIALS OF CIGARETTE SMOKE CONDENSATES.

M. S. Stavanja, D. R. Meckely, P. R. Nelson, G. M. Curtin, P. H. Ayres, A. T. Mosberg and J. E. Swauger. *Regulatory Toxicology, R. J. Reynolds Tobacco Company, Winston-Salem, NC.*

The DBA/2 mouse has been shown to be as sensitive as the SENCAR mouse to two-stage tumorigenesis when initiated with N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) or high doses of 7, 12-dimethylbenz[*a*]anthracene (DMBA) followed by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) promotion. A 30-week dermal tumor promotion study was conducted to evaluate the overall utility of the DBA/2 mouse for assessing the relative tumor-promoting potentials of cigarette smoke condensates (CSC). CSC from the Kentucky 1R4F cigarette was the test article (promoter). Fifteen groups of 40 male DBA/2 mice were used. Seven treatment groups were initiated with single 75 or 150µg dermal applications of DMBA and promoted with 9, 18, 27, 36, or 45 mg "tar"/application. Four treatment groups were initiated with single 300µg dermal applications of MNNG followed by promotion with 9, 18, 27, or 36 mg "tar"/application. CSC was administered three times per week for 29 weeks. Control groups included initiated, non-promoted animals and sentinels. End-points included clinical signs, body weights, and mass tracking data. Neither control groups nor DMBA-initiated/CSC-promoted groups produced masses. The MNNG/1R4F (9mg) CSC group developed one mass-bearing mouse (MBM). The MNNG/1R4F (18mg) CSC group developed 2 MBM and the MNNG/1R4F (36mg) CSC group developed one MBM. These MBM developed only one mass each. This pattern of response indicates no treatment-related effect. The DBA/2 mouse was found to be unresponsive to dermal tumor promotion with CSC and would not be considered a useful substitute for the SENCAR mouse in the standardized dermal tumor-promotion assay used to evaluate CSCs. However, this model could potentially be used for understanding the mechanism by which the DBA/2 mouse is resistant to the tumor-promotion activity of CSC.

553 MICROSCOPIC EXAMINATION OF LUNG TUMORS ENHANCES EVALUATION OF TOBACCO SMOKE-INDUCED TUMORIGENICITY IN A/J MICE.

G. M. Curtin, M. A. Higuchi, P. H. Ayres, J. E. Swauger and A. T. Mosberg. *Regulatory Toxicology, R.J Reynolds Tobacco Company, Winston-Salem, NC.*

A/J mice were exposed whole-body or nose-only to mainstream tobacco smoke (MTS) from Kentucky 1R4F cigarettes. Whole-body exposed mice received filtered air or 0.20 mg wet total particulate matter (WTPM)/L for 20 weeks (6 hr/d, 5 d/wk), followed by 16 weeks recovery. MTS-exposed mice exhibited statistically significant reductions in body weight relative to shams, with a 2-fold higher weight gain evident during recovery. Gross evaluation of lung tumors post-exposure revealed >50% reductions for multiplicity and incidence compared to shams, with both indices exhibiting non-statistical increases following recovery. Microscopic examination of lungs confirmed tumor reductions during exposure, but revealed statistically significant increases for both multiplicity and incidence post-recovery. Nose-only exposed mice received 0.04, 0.125 or 0.40 mg WTPM/L for 28 weeks (3 hr/d, 5 d/wk), followed by 16 weeks recovery. MTS-exposed mice exhibited dose-related reductions in body weight compared to shams, with statistically significant changes noted for 0.125 and 0.40 mg WTPM/L exposures; body weight gains during recovery were likewise proportional to exposure concentration. Gross determination of tumors post-exposure yielded non-statistical reductions for multiplicity and incidence compared to shams; non-statistical increases for both indices were observed following recovery, but were limited to 0.125 and 0.40 mg WTPM/L exposures. Microscopic examination revealed exposure-related reductions for multiplicity and incidence that were less dramatic than observed grossly, as well as dose-related (non-statistical) increases for both indices post-recovery. These data suggest that microscopic examination of lungs is essential for adequately evaluating MTS-induced tumor formation. Moreover, development of an appropriate lung tumorigenicity model will likely require that the toxicological impact associated with tobacco smoke exposure (i.e., reductions in body weight and tumor formation) be examined more thoroughly.

554 NONYLPHENOL INDUCES MAMMARY CANCER IN MMTVNEU MICE.

H. Villanueva[1], R. Acevedo[1], P. Parnell[2], S. L. Gray[3], T. Gimenez[3] and W. Baldwin[1]. *[1]Biological Sciences, University of Texas at El Paso, El Paso, TX, [2]Clemson Veterinary Diagnostics Center, Clemson University, Columbia, SC and [3]Animal and Veterinary Sciences, Clemson University, Clemson, SC.*

4-Nonylphenol (4-NP) is a toxic degradation product of alkylphenol ethoxylates, which are nonionic surafactants used in institutional cleaning agents, textiles, agricultural chemicals, plastics, paper products, household cleaning agents and personal care products. 4-NP has been found in food and drinking water, and is an environmental estrogen that has been shown to increase proliferation of breast cancer cell lines. The threshold dose for increased development and proliferation of mammary tissue in ovariectomized rats is approximately 40mg/kg/day. To determine if 4-NP may increase mammary cancer incidence in mice, FVB/N-TgN(MMTVneu)202Mul mice susceptible to mammary cancer, were fed 4-NP in honey at 0, 30 or 45mg/kg/day, or provided 10µg/kg/day estradiol in honey for 32 weeks. One out of fourteen control mice developed a tumor, while 0/14 and 5/13 mice developed tumors in the 30 and 45mg/kg/day groups respectively. Overall, 4-NP increased both tumor incidence and latency at 45mg/kg/day. This data further supports a threshold dose of 4-NP of no greater than 45mg/kg/day. None of the mice receiving estradiol formed tumors. In addition, metastasis occurred in two of thirteen mice in the 45mg/kg/day group, but metastasis did not occur in other treatment groups. A 7-day pilot study indicated estriol levels were increased by 4-NP, but not estradiol or progesterone levels. Therefore, each month mice were tail bled and steroid hormone levels were analyzed. However, steroid hormone levels were not altered significantly by 4-NP. In conclusion, 4-NP induces mammary cancer formation in MMTVneu mice and has a tumor threshold between 30mg/kg/day and 45mg/kg/day in MMTVneu mice.

555 TOXIC AND CARCINOGENIC EFFECTS OF INHALED PROPYLENE GLYCOL MONO-*T*-BUTYL ETHER IN F344/N RATS AND B6C3F$_1$ MICE.

A. M. Doi[1], J. H. Roycroft[1], R. A. Herbert[1], J. A. Dill[2], S. L. Grumbein[2], R. A. Renne[2] and J. R. Bucher[1]. *[1]NTP, NIEHS, Research Triangle Park, NC and [2]Battelle Toxicology Northwest, Richland, WA.*

Propylene glycol mono-*t*-butyl ether (PGMBE) is a solvent used commercially in a wide variety of applications. Because of its high production volume and high potential for human exposure, studies were conducted to evaluate the toxicology and carcinogenicity of PGMBE in rats and mice. Male and female F344/N rats and B6C3F$_1$ mice were exposed to PGMBE by whole-body inhalation for 2 weeks, 14 weeks, or 2 years. The exposure concentrations of PGMBE in rats and mice were 0, 75, 150, 300, 600, or 1, 200 ppm in the 2-week and 14-week studies, and 0, 75, 300, or 1, 200 ppm in the 2-year studies. The kidney and the liver were sites of PGMBE toxicity in rats. In the 2-week and 14-week studies, renal lesions suggestive of α_{2u}-globulin nephropathy occurred in male rats. In the 2-year rat studies, exposure-related nonneoplastic renal lesions were accompanied by marginal increases in renal tubular neoplasms in males. Although not significant, the incidences of hepatocellular adenoma were increased in the liver of male rats of the high exposure group, and followed an overall increasing trend with exposure. In mice, the liver was the main target of PGMBE toxicity. In the 2-week and 14-week mice studies, liver weights and/or the incidences of centrilobular hypertrophy were increased in both sexes. In the 2-year studies, the incidences of liver neoplasms in male and female mice were significantly increased in the high exposure group, and followed an overall positive trend with exposure. In summary, PGMBE exposure for 2 years induced kidney lesions characteristic of α_{2u}-globulin accumulation in male F344/N rats, while the evidence of carcinogenic activity of PGMBE in F344/N male rats was equivocal. There was no evidence of carcinogenic activity of PGMBE in female F344/N rats. In B6C3F$_1$ mice, PGMBE induced liver neoplasms in males and females.

556 STRAIN-DEPENDENT SUSCEPTIBILITY TO TRANSPLACENTALLY-INDUCED MURINE LUNG TUMORS.

M. S. Miller[1], J. E. Moore[1], M. Xu[1], G. B. Nelson[2], S. T. Dance[1], N. D. Kock[1] and J. A. Ross[2]. *[1]Wake Forest University, Winston-Salem, NC and [2]USEPA, Research Triangle Park, NC.*

Previously, our laboratory demonstrated that fetal mice are more sensitive to *in utero* exposure to the polycyclic hydrocarbon, 3-methylcholanthrene (MC), than are adults, and that resistant strains had a high incidence of lung tumors 12 months after transplacental exposure to MC. We compared the effects of *in utero* treatment with MC on lung tumor induction in the offspring of intermediately susceptible Balb/c (Bc), resistant C57BL/6 (B6), and reciprocal crosses between the two strains. Pregnant mice were treated with 45 mg/kg of MC on day 17 of gestation and tumor incidence, multiplicity, and tumor size determined in the offspring 14-18 months after birth. Bc, B6Bc, and BcB6 mice exhibited a 100% tumor incidence whereas the resistant B6 mice had an incidence of 5.3%. B6 mice exhibited 1 small nodule after 18 months whereas Bc mice rarely survived beyond 14 months and BcB6 and B6Bc mice survived to approximately 16 months. Bc, B6Bc, and BcB6 mice exhibited significant tumor involvement in the lungs; in many cases multiple tumors coalesced into single large masses with the majority of lesions classified as adenocarcinomas. Counting only lesions that were discrete, individual nodules, tumor multiplicities in Balb, B6Bc, BcB6, and B6 mice were 7.7±2.9, 5.5±3.3,

6.0±3.0, and <0.1, respectively. Similarly, tumor size in Balb, B6Bc, BcB6, and B6 mice was 1.5±0.7, 1.6±0.9, 1.4±0.7, and <1mm, respectively. These results suggest that, similar to adults, crosses between susceptible and resistant strains results in a susceptible phenotype for lung cancer. We are currently examining differences in MC metabolism, DNA adduct levels and repair, and mutations in Ki-*ras* as possible mechanistic factors mediating strain susceptibility to lung carcinogens. These studies highlight the important interactions between genetic and environmental factors in determining individual susceptibility to environmental toxicants during development. (Supported by EPA STAR grant R829428-01-0. This abstract does not reflect EPA policy.)

557 INITIATION-PROMOTION STUDIES OF 1, 3-DICHLOROPROPENE (1, 3-D) IN MALE F344 RAT LIVER AND STRAIN A MOUSE LUNG.

S. Reel, L. M. Kamendulis, P. J. Klein and J. E. Klaunig. *Division of Toxicology, Indiana University School of Medicine, Indianapolis, IN.*

Chronic treatment with the nematocide 1, 3-dichloropropene (1, 3-D) resulted in the induction of neoplasia in the rat liver and mouse lung. While 1, 3-D does not appear to be mutagenic, the mode of action for the induction of these neoplasms has not been established. 1, 3-D may function to promote the growth of preneoplastic lesions. The present study examined the effect of 1, 3-D on hepatic focal lesion growth in the F344 male rat and lung adenoma growth in the male Strain A mouse. In the liver, 1, 3-D (25 mg/kg) had no effect on the number or volume of GSTp positive staining hepatic foci after 30 or 60 days of treatment whereas 1, 3-D increased the number and volume of GSTp negative staining hepatic focal lesions after both 30 and 60 days. In contrast, phenobarbital (PB; 80 mg/kg) significantly increased the number and volume of GSTp positive staining focal lesions at both 30 and 60 days of treatment and increased the number and volume of GSTp negative staining hepatic lesions after 60 days of treatment. Following a 30 day recovery period (treatment for 30 days, followed by no treatment for 30 days), the increases in number and volume of hepatic lesions induced by either 1, 3-D or PB returned to control levels. DNA synthesis in GSTp negative staining hepatic lesions was increased by 1, 3-D after both 30 and 60 days of treatment, and by PB after 60 days of treatment. In the mouse lung, 1, 3-D produced a slight increase in the incidence of grossly visible lung adenomas (26% compared to 10% in control). Treatment with vinyl carbamate (VC) ± 1, 3-D resulted in a 100% gross adenoma incidence. The total number of adenomas was similar between the VC group and those receiving VC and 1, 3-D (976 vs. 980 adenomas per 20 mice). Analysis of adenoma size distribution showed that 1, 3-D produced a slight increase in the number and size of adenomas compared to control. These results support the premise that 1, 3-D may function at the promotion stage of carcinogenesis.

558 CHROMIUM(VI) IN DRINKING WATER INCREASES THE INCIDENCE OF UV-INDUCED SKIN TUMORS IN HAIRLESS MICE.

M. Costa, T. Kluz, T. L. Davidson, T. Rossman, A. Uddin, A. Nadas, Q. Zhang and F. Burns. *Department of Env. Med., New York University, Tuxedo, NY.*

Hexavalent chromium (Cr(VI)) is a well known human carcinogen with exposures occurring in both occupational and environmental settings. Although lung carcinogenicity has been well documented for exposure *via* inhalation, the cancer-causing potential of Cr(VI) in drinking water has been much more controversial. Human exposure to chromium compounds alone is not relevant in the scenario of our daily lives, since we are exposed to many different toxicologically active environmental insults. We used a hairless mouse model to study the effects of Cr(VI) exposure *via* drinking water on ultraviolet radiation(UVR)-induced skin tumors. Hairless mice were divided into seven groups: control, UVR alone, 2.5ppm K_2CrO_4, 5.0ppm K_2CrO_4, UVR(1.2 KJ/m2) + 0.5ppm K_2CrO_4, UVR + 2.5 K_2CrO_4, and UVR + 5.0ppm K_2CrO_4. Mice were inspected weekly for the appearance of skin tumors larger than 2mm. Mice were sacrificed on day 182 and skin tumors were removed for histopathological analysis. No skin tumors were observed in the control or chromium only exposure groups. There was a dose dependent increase in the number of skin tumors greater than 2mm for mice exposed to Cr(VI) and UV when compared to mice exposed to UV alone. The increase in tumors larger than 2mm was significant for UV + the two higher doses of K_2CrO_4. Malignant tumors were observed in all UV treated mice and there was a statistically significant increase in the numbers of malignant tumors for the UVR plus K2CrO4(5ppm) group. Exposure to Cr(VI) *via* drinking water exposure has been a significant public health concern and drinking water standards have been set based on exposure to chromium alone. The data presented here indicate that Cr(VI) can increase the number of UV induced tumors in a dose dependent manner. This suggests that human exposure to Cr(VI) may increase the risk of carcinogenesis for other agents as well. Our results have important implications for risk assessment and suggest that the current drinking water standards for Cr(VI) exposure should be reassessed and adjusted downward.

559 RELIABILITY AND PRACTICALITY OF MEDIUM-TERM LIVER CARCINOGENESIS BIOASSAYS.

S. Tamano[1], A. Hagiwara[1], M. Kawabe[1], H. Yoshino[1], T. Ichihara[1], K. Imaida[2], T. Shirai[3] and N. Ito[4]. *[1]Daiyu-kai Institute of Medical Science, Ichinomiya, Japan, [2]Department Onco-Pathology, Kagawa Medical University, Kagawa, Japan, [3]Department Exp. Pathol. And Tumor Biol., Nagoya City University, Nagoya, Japan and [4]Nagoya City University, Nagoya, Japan.*

The promoting activities of carcinogens can rise to preneoplastic and neoplastic lesions by stimulating clonal proliferation. We have focused on developing medium-term liver carcinogenesis bioassays for the detection of carcinogens based on this property. Male F344/duCrj rats, six weeks of age, are initially given a single ip injection of DEN(200mg/kg b.w.) dissolved in saline to initiate hepatocarcinogenesis. After 2 weeks, they received test compounds and are then subjected to two-thirds partial hepatectomy (PH) at week 3. The animals are killed for quantitative analysis of glutathione S-transferase placental form (GST-P) positive liver foci at week 8. A positive response is defined as a significant increase in the quantitative values of GST-P-positive foci. Of a total of 314 chemicals examined, 61 out of 66 known hepatocarcinogens (92%) gave positive results. The five hepatocarcinogens which proved negative all belonged to the peroxizome proliferator group that depresses GST-P expression. Therefore, the positive rate for hepatocarcinogens excluding these 5 peroxisome proliferators was 100%. One of the 48 chemicals reported as non-carcinogenic was found to be positive in this assay, but the available information might suggest that it is liver tumor promoter. The results indicated that medium-term liver bioassay system is very useful tools for detection of not only genotoxic but also non-genotoxic carcinogens. Carcinogenicity of carcinogenic agents can be detected primarily by their promoting activity, because the results of the medium-term bioassay clearly mirrored those for long-term carcinogenicity.

560 THE MOST APPROPRIATE STRAIN FOR RAT CARCINOGENICITY BIOASSAYS.

L. D. Britton, N. Downes, P. Mullins and D. Mitchell. *Toxicology, Sequani Limited, Ledbury, United Kingdom.*

A lifespan study was initiated to evaluate the Hsd:Sprague-Dawley SD and Hsd:BrlHan:WIST (Han Wistar) rat strains. Data generated was compared with contemporaneous Crl:CD(SD) IGS BR rat data. The objective was to make an informed choice of the most suitable strain to recommend for future carcinogenicity studies. Males (100) and Females (100) of each strain were obtained from Harlan UK and allocated to the study at 6 weeks of age. All strains were housed under the same standard conditions and subject to the same routine handling/observations. The Harlan strains were consistently lighter in weight (mean weight 10%-30% lighter) and routinely consumed less food (up to about 25% less) than Crl:CD rats of the same age. In the second year, the Han Wistar ate around 5%-10% less food than the Hsd:SD but, despite this, ultimately became around 25% heavier (mean weight). Only 33% of Hsd:SD males and 35% of females survived to 104 weeks, which was comparable with the Crl:CD (males 39-40%; females 33-39%) but lower than that for Han Wistar rats (60% / 59% survival for males / females). About half of the Hsd:SD males that died early were found dead without previous indication of ill-health; this contrasts with around 15% of male Han Wistar and Crl:CD decedents. Only one Han Wistar female was found dead in 104 weeks (2%), compared with about 5% of Hsd:SD females and 10% of Crl:CD females. As survival of the Han Wistar rats was good (50% survival at 104 weeks is considered optimal), half the survivors were terminated at 104 weeks and the remainder continued on study for a further 3 months. For the Sprague-Dawley strains, it is clear that standard group sizes of 50 would not have provided optimal numbers for terminal assessment at 104 weeks. It can be concluded that the poor survival and associated pathology of the two Sprague-Dawley derived strains were linked to their common derivation and unaffected by differences in bodyweight and food consumption. Of the three strains assessed, the Harlan Hsd:BrlHan:Wist is considered the most suitable for carcinogenicity bioassays.

561 CHARACTERIZATION OF SUBCUTANEOUS TUMOR GROWTH FOR A549, PC3 AND CACO2 HUMAN CANCER XENOGRAFTS IN THE NUDE MOUSE.

A. Adamou, S. Groom and M. Vezina. *CTBR, Senneville, QC, Canada.*

The human tumor/mouse xenograft system is a recognized model for the in-vivo pre-clinical screening of potential anti-cancer drugs. This poster presents data for the subcutaneous tumor development and growth of 3 tumor cell lines with very different growth characteristics. PC3 prostate cancer is a relatively rapidly growing cell line; CACO2 colon cancer is a slow growing tumor cell line and A549 non-small cell lung cancer was selected as an intermediate. Six week old male and female mice (Crl:Nu/Nu-nuBR) were inoculated with 0.1 mL of the appropriate tumor cell suspension (containing approximately 106 to 107 tumor cells). At a mean

tumor volume of between 100 to 200 mm3, equal numbers of animals were randomly selected to receive paclitaxel treatment or remain untreated. The time taken to reach a mean tumor volume of 100 to 200 mm3 was 4 days for the A549 tumor cell line (117 ± 35 mm3) and 3 days for the PC3 tumor cell line (125 ± 35 mm3). The presence of a tumor was detected for most animals using these cell lines. In contrast, the weakly tumorigenic and slow growing CACO2 tumor cells failed to reach 100 mm3 within 45 days of inoculation despite detection of a tumor in approximately 80% of the animals within that interval. The time to 50% tumor growth inhibition (paclitaxel-treated mice vs untreated mice) was 31 to 32 days for A549 and 23 to 24 days for PC3. Animals inoculated with the CACO2 cell line were not treated with paclitaxel. These data validate A549 and PC3 for use in the nude mouse as effective *in vivo* models for the screening of potential anti-cancer drugs as well as paclitaxel as an effective reference compound. The suitability of slow growing tumor cell lines for this type of evaluation remains to be evaluated.

562 TIME TO FATAL TUMORS IN P53-/- RAD50S/S MICE.

D. Singh[1], A. O. Chiu[1], J. H. Petrini[2], N. H. Chiu[3] and J. Beaubier[4]. [1]*NCEA ORD, USEPA, Washington DC, DC,* [2]*Mol Biol Prog, MSKCC, New York, NY,* [3]*OST OW, USEPA, Washington, DC and* [4]*OPPTS, USEPA, Washington, DC.*

Mathematical modeling of individual animal data from established colonies of p53 knockout and rad50 hypomorphic mice were carried out in this analysis. Mre11, Rad50, and Nbs1 are critical for repairing DNA-double-strand-breaks. Rad50s/s mice die mostly with marrow or lymph node depletion, with attrition in spermatogonia. Rad50s/s mice had apoptotic cells in 50% of their seminiferous tubule. Cellular attrition was mitigated by p53 deficiency to 3% in p53+/- Rad50s/s or 6%, p53-/- Rad50s/s mice. Rad50s/s MEFs exhibited a 1.4 - 3.4 fold increases in subG1 DNA content. r-H2AX marker (% in 150 cells, >=3 nuclear foci per cell)increased from approximately 0.45 - 1.1 in Rad50+/+ cells to 0.67- 1.9 for rad50s/s cells. Upon exposure to 12 Gy radiation, r-H2AX increased 1.5- 4.3 fold in Rad50s/s as compared to Rad50+/+ cells. V(D)J recombination was normal in Rad50s/s mice. P53+/- mice developed osteosarcoma after 12 months, whereas p53+/-Rad50s/s died of thymic lymphoma (N=17, p=0.002) with an average of 5 months. The p53-/- strain died within 4.5 months while those with p53-/- Rad50s/s died within 3 months, with the same tumor spectrum. While most Rad50s/s mice died by 4 months with anemia, 20% survived 4-7 months and 20% of this latter group developed thymic lymphoma. In these lymphoma, changes in chromosomal number and structure were signific- antly increased. Our modeling suggests a decrease in critical steps from 6 to either 5 or 4 in p53-/- mouse tumor tissue and from 4 to 3 in p53-/-Rad50s/s carcinogenesis. Anemia development appears to involve 1-2 steps in the cell cycle, whereas the development of lymphoma requires 4 or more steps.

563 TIME TO FATAL TUMORS IN P53 +/-, -/- H2AX +/-, -/- KNOCKOUT MICE.

A. Chiu[2], M. Difilippantonio[1], A. Nussenzweig[1], N. H. Chiu[3], D. Singh[2] and J. Beaubier[4]. [1]*NCI, NIH, Bethesda, MD,* [2]*NCEA ORD, USEPA, Washington, DC,* [3]*OSTOW, USEPA, Washington, DC and* [4]*OPPTS, USEPA, Washington, DC.*

Carcinogenesis is a complex multistage, multipath process. In this analysis intermediate stages in cancer development are identified not only by pathologic changes but also at the cellular-molecular and genetic levels using mice with knockout or targeted-hypomorphic-antioncogenes. Cellular processes affected by antioncogenes include progression of cell-cycle, apoptosis and senescence, which in turn are controlled by gatekeepers such as p53 and Ku70/80. Applying mathematical modeling and computer simulation to individual animal data of established colonies, we found a limited number of critical cell-cycle events (N < 6) associated with p53 +/- , -/- mouse malignant lymphoma, soft- tissue sarcoma and various carcinoma development. This study used a time-to-death analysis of tumor-bearing animals employing parametric and nonparametric statistics. The quadruple knockout p53 -/- H2AX -/- mouse has a shorter latency and requires one less stage for carcinogenesis than the study strain double knockout p53 -/- mouse. We have also begun an analogous study of genes including H2AX focused on genomic stability and homologous and nonhomologous recombination. The number of stages in lymphoma development in p53 -/- H2AX +/- mice is also reduced compared to the p53 -/- strain. The genomic stability-caretaker "H2AX-Rad50-MRE11- BASC" complex plays probably just as an important role in cancer development as the gatekeepers do for poptosis- senescence. Disclaimer: Opinions and conclusions expressed in this abstract are those of the authors and do not necessarily reflect those of the NCEA, OST, OW, ORD, OPPTS, USEPA and NCI, NIH.

564 CARCINOGENIC RESPONSE OF K6/ODC MICE TO MELPHALAN.

Y. Chen, J. Bastien and T. O'Brien. *ODC Mouse Group, Inc., Drexel Hill, PA.*

K6/ODC mice have been engineered to overexpress the enzyme ornithine decarboxylase (ODC) in skin and some internal organs and are exquisitely sensitive to skin tumor induction by very low doses of genotoxic carcinogens. This model has been proposed as a highly sensitive test system for detecting carcinogens. To test the ability of K6/ODC mice to detect a known human carcinogen, the carcinogenicity of melphalan, a genotoxic carcinogen, was evaluated in this model. Groups of 15 female K6/ODC and wild-type control mice, 7 week old, were used in this study. Melphalan was systemically administered by gavage to K6/ODC mice at doses of 0, 1, 4, 16 mg/kg and to wild-type control mice at doses of 4, 16 mg/kg once a week for 20 weeks. Development of skin tumors was monitored weekly. Mean group body weight gain of melphalan-exposed mice was dose-dependently decreased in comparison to the vehicle control mice, for both strains (K6/ODC and wild-type mice). At the highest dose, all mice in both K6/ODC and wild-type control groups were dead at 8 week after treatment. However, at low doses groups, melphalan induced skin tumors were observed in a dose-response manner in K6/ODC mice. The incidence of skin tumors was 7% and 25% at doses of 1 and 4 mg/kg, respectively. The majority of tumors were squamous cell carcinomas. Tumors were not observed in both vehicle and wild-type control groups. Theses results demonstrate that the K6/ODC is a useful model for evaluating the human carcinogenic potential of genotoxic chemicals.

565 A NON-ANIMAL ALTERNATIVE CARCINOGENICITY TEST: THE *IN OVO* CARCINOGENICITY ASSAY (IOCA).

M. K. Reeder[1], M. J. Iatropoulos[2], G. M. Williams[2], T. L. Ripper[1], D. R. Cerven[1] and G. L. DeGeorge[1]. [1]*MB Research Laboratories, Spinnerstown, PA and* [2]*Pathology, New York Medical College, Valhalla, NY.*

We have developed a non-animal alternative method to the currently accepted Rodent Chronic Bioassay (RCB), which uses large numbers of rodents over a two year period. The IOCA is similar in concept to the Rodent Liver Focus (RLF) assay in which preneoplastic liver lesions are quantified as indicators of carcinogenicity. In the IOCA, a*via*n embryos are exposed to carcinogens which induce focal lesions in the embryonic liver. Since IOCA is performed in a*via*n embryos (pre-hatching), it directly addresses the three R's (reduction, refinement and replacement) of animal testing. The advantages of IOCA are numerous; 1) testing time is reduced from 2 years to 24 days, 2) no laboratory animals are required, 3) eggs are kept in small incubators, eliminating the need for costly animal facilities, and 4) dose amounts are small, reducing the amount of chemical needed and human exposure to potential carcinogens. Using IOCA, we have investigated the effects of several well-studied genotoxic and non-genotoxic carcinogens by measuring: (1) induction of preneoplastic lesions in the a*via*n embryonic liver; (2) increase in the size of hepatocellular nuclei *in ovo*; and (3) damage to a*via*n mitochondrial DNA (mtDNA). Our results demonstrate that the carcinogens, diethylnitrosamine (DEN), N-nitrosomorpholine (NNM), and aflatoxin B1 (AFB), induce preneoplastic lesions in a*via*n liver, manifested as hepatocellular foci and hepatic cord transformation, which correlate to rodent liver preneoplastic lesions. Moreover, a threshold treatment level for lesion development was found for both DEN and AFB. Finally, increased size of hepatocelluar nuclei and damage to liver mtDNA was observed at sub-threshold concentrations of carcinogens, indicating these changes may precede hepatocellular foci and hepatic cord transformation.

566 MATERNAL ATRAZINE (ATR) ALTERS HYPOTHALAMIC DOPAMINE (HYP-DA) AND SERUM PROLACTIN (SPRL) IN MALE PUPS.

C. Langdale[1], T. Stoker[2] and R. Cooper[2]. [1]*Cell Biology Department, NCSU School of Veterinary Medicine, Raleigh, NC and* [2]*Endocrinology Branch, RTD, NHEERL, ORD, USEPA, Research Triangle Park, NC.* Sponsor: R. Kavlock.

In rat and mouse, milk-borne PRL (MB-PRL) is important for normal development of the offspring's tuberoinfundibular (TIDA) neurons. The absence of MB PRL on postnatal days (PND) 1-5 leads to impaired TIDA activity and prepubertal hyperprolactinemia in the pups. We previously showed that ATR will block suckling-induced PRL release. Here, we characterized the effect of ATR on sPRL and HYP-DA and DOPAC in the male pups of Wistar rat dams by dosing the dam from PND 1-9 with ATR (0, 12.5, 25 or 50 mg/kg, twice daily). PRL was measured from PND 2-35 and HYPO DA and DOPAC determined on PND 20, 25, 30 and 35. On PND 12-30, PRL was significantly higher in the 12.5 mg/kg group (AUC 22.5 ng/ml control and 32.67 ng/ml for ATR), but not the 25 and 50 mg/kg groups. Posterior HYP, DA was significantly decreased on PND 30, while DOPAC was significantly decreased from PND 20-30 in the 12.5 mg/kg dose but the same as controls in the high dose. In contrast, anterior HYP DA was increased on PND 20 and 30 in the 12.5 mg/kg group and DOPAC was significantly increased in the 12.5 and 25 mg/kg dose on PND 20 and 30. DOPAC was significantly decreased in all three dose groups on PND 35. Thus atrazine produced a non-linear dose response for PRL, HYP DA and DOPAC. These results suggest that ATR either induced alterations in the developmental progression of HYP-DA and PRL regulation or compensatory changes in this system in response to increasing doses. (This abstract does not necessarily reflect EPA policy; Supported in part by NCSU/USEPA Coop Agreement).

567 ATRAZINE INHIBITION OF OVULATION IN IMMATURE RATS TREATED WITH PREGNANT MARE SERUM GONADOTROPIN (PMSG).

C. Eldridge[1], D. Wynn[1], C. Breckenridge[2] and J. Stevens[1]. *[1]Physiology-Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC and [2]Toxicology, Syngenta Crop Protection, Greensboro, NC.*

Treatment with the herbicide atrazine (ATR) has been shown to disrupt estrous cycling and to inhibit ovulation of adult Sprague-Dawley (SD) rats. Effects have been observed with short-term treatment of 2 days-1 week at maximum tolerated doses, and with lower doses after treatment up to 6 months. Although female rats normally begin ovulating at PND 36-40, it is long known that a single injection of PMSG on day 30 will induce a spontaneous LH surge on day 32 and ovulation on day 33. The present studies asked whether the PMSG model is suitable for assessing ATR effects on ovulation and whether these very young animals are more sensitive to ATR than adults. In Study 1, ATR was administered by gavage to groups of SD females on PND 30, 31 and 32. A single sc PMSG dose (4 IU) was given on PND 30. The presence of ova in the oviducts was assessed on PND 33. 76% of controls ovulated and groups treated with 1, 5, 10 and 50 mg/kg ATR produced similar results. Ovulation % was reduced at 100 and 500 mg/kg ATR, but not reduced at 300 mg/kg. The number of ova per ovulation was significantly reduced only at 500 mg/kg. Body weight gains were reduced at all ATR doses of 50 mg/kg and higher. In Study 2, groups were administered ATR by gavage (0, 10, 30, 100 mg/kg) from PND 22-32, and 4 IU PMSG was administered once on PND 30. The percent of animals ovulating in the 4 groups was nearly identical (range = 51-56%), and the mean number of ova per ovulation was unaltered. Body weight gain was retarded only in the 100 mg/kg group. Results suggest (1) the PMSG model could be suitable for testing ovulation blockade, but (2) the model may lack reliability, as many controls did not ovulate, (3) ATR had limited effect on ovulation at doses of 100 mg/kg and higher, (4) many ATR doses that suppressed BW gain did not suppress ovulation, (5) immature rat ovulation seems not to be more sensitive than the adult to ATR treatment, and is possibly more resistant.

568 ATRAZINE ALTERS STEROIDOGENESIS IN MALE WISTAR RATS.

W. Modic[1], J. Ferrell[2], C. Wood[2], J. Laskey[2], R. Cooper[2] and S. Laws[2]. *[1]Department Mol. Structural Bch., NCSU, Raleigh, NC and [2]RTD, NHEERL, ORD, USEPA, Research Triangle Park, NC.* Sponsor: R. Kavlock.

We have reported that atrazine (ATR, 200 mg/kg x 30 d) causes increased serum estrone (E) and estradiol (E2) in male wistar rats (Toxicol. Sciences. 2000, 58:50-59). This study evaluates the short-term effects of ATR on E, E2 and their precursors in the steroidogenic pathway. Sixty-day-old male Wistar rats were gavaged once per day with ATR (0, 50, 200 mg/kg) for 1, 4 or 21 days. Following the single dose, males were killed at 3, 6, or 24 hr. Males receiving multiple doses (4 or 21 days) were killed 3 hr following the last dose. Serum E, E2, testosterone (T), androstenedione (A), progesterone (P), and corticosterone (C) were measured. After one dose of ATR (200 mg/kg), P and C were increased at 3 hr. By 24 hr this dose elevated C and E. A and T were increased at 6 hrs for both doses. After 4 or 21 days of treatment with 200 mg/kg, C, E and E2 were elevated, but A and T remained at control levels. To determine the effect of ATR on adrenal steroidogenesis, ATR treated castrated males were examined. Despite reduced A and T levels in the castrated 200 mg/kg males, increased serum C, E, E2 mirrored previous results. Elevated P was also observed. No change in aromatase CYP19 mRNA was detected by real time RT-PCR in the testicular or hypothalamic tissues at any time point. These data suggest that elevated E and E2 were not strictly due to increased testicular steroidogenesis, altered aromatase mRNA, or substrate availability, but do not rule out a change in steroid metabolism or elimination. Although no CYP19 mRNA was detected in the adrenals, a stress-induced adrenal response may be partially responsible for the increase in steroids. However, *in vitro* data using a minced testes assay show an increase in T following a 4 hr exposure to ATR. Together, these data demonstrate that ATR can alter the steroidogenic pathway in male Wistar rats. This abstract does not necessarily reflect USEPA policy. Supported in part by the NCSU/NHEERL, USEPA Co-op Agreement.

569 CARBOFURAN-INDUCED ENDOCRINE DISRUPTION IN MALE RATS.

R. T. Goad[1], R. C. Gupta[1], J. T. Goad[1], B. H. Atieh[2] and R. B. Doss[1]. *[1]Toxicology, Murry State University, Hopkinsville, KY and [2]Occup. Safety & Hlth, Murray State University, Murray, KY.*

The objective of this investigation was to determine the acute toxic effects of the carbamate insecticide carbofuran on the levels of endocrine hormones in the serum of adult male Sprague-Dawley rats. Using chemiluminescent immunoassay, the hormones determined were progesterone, cortisol, estradiol, testosterone, tiidothyronine (T3), total thyroxine (total T4), and non-protein-bound thyroxine (free T4). Rats exposed to an acute dose of carbofuran (1.5 mg/kg, s.c.) showed the onset of cholinergic signs (salivation, chewing, and fine tremors) within 5-7 minutes. With increasing intensity, toxic signs of maximal severity (severe convulsions and fasiculations) were observed within 30-60 min, and lasted for about 2 to 3 hours. Time courses of hormones for 24 hrs revealed significant alterations in hormone levels during 0.5 to 3 hrs, with the exception of estradiol at 6 hrs. The levels of progesterone, cortisol, and estradiol were significantly increased (1279%, 202%, and 150%, respectively), while the levels of testosterone were decreased by 88%. No significant change occurred in thyroid hormones (T3, total T4, and free T4) at any time during the time course, despite the fact that body temperature was significantly low at 1 to 2 hrs. Carbofuran caused significant increases in glucose (189 to 242%), partly due to > 2-fold increase in cortisol that stimulates gluconeogenesis. In fact, the timings for increases in cortisol and glucose were transient and identical, i.e., 0.5 to 3 hrs. The results suggest that an acute exposure to carbofuran may cause endocrine disruption, which may consequently lead to serious reproductive problems.

570 *IN VITRO*/*IN VIVO* EVALUATION OF THE (ANTI)-ANDROGENIC ACTIVITY OF THE ANTIPROGESTIN RU486.

G. Charles, H. L. Kan, M. R. Schisler, B. B. Gollapudi and M. S. Marty. *The Dow Chemical Company, Midland, MI.*

These experiments are part of an on-going study to evaluate the concordance between *in vitro* and *in vivo* Endocrine Disrupter Tier I androgen screening assays. Given that progesterone (PROG) is known to bind the androgen receptor (AR), we examined the anti-androgenic potential of the anti-progestin, RU486 using both *in vitro* (AR transactivation and AR binding) and *in vivo* (Hershberger) assays. For the transactivation assay, AR(+) LNCaP prostate carcinoma cells were transfected with an inducible luciferase reporter construct with concatenated androgen-responsive elements (pGudLuc7ARE) and exposed for 24h to concentrations of RU486 and PROG ranging from 1 nM to 10 uM in the presence and absence of 1nM of the AR agonist, R-1881. PROG, but not RU486, produced partial agonist activity (30-45 fold induction) as compared to R-1881 (65-100 fold). However, RU486 produced significant anti-androgenic activity *in vitro* as evidenced by its 70 and 100% inhibition of agonist responses by PROG (10 nM) and R-1881 (1 nM), respectively. *In vitro* binding experiments utilizing the recombinant ligand-binding domain of the AR with a fluorescence polarization methodology indicated that RU486 exhibited an IC50 (approx. 24 nM), comparable to that of testosterone (approx.17 nM). For the assessment of anti-androgenic activity *in vivo*, castrated CD rats (8/group) were dosed for 10 days by gavage with RU486 (0, 1, 5, 10 and 25 mg/kg/day) and by subcutaneous injection with 0.4 mg/kg/day testosterone propionate (TP). Flutamide at 10 mg/kg/day, by gavage, was used as the positive control for anti-androgenicity. At 25 mg/kg/day, RU486 produced significant decreases in ventral prostate, seminal vesicle and Cowpers gland weights without significant effects on body or liver weights. Thus, RU486 showed good concordance between *in vitro* and *in vivo* assays, whereas previous screens with other chemicals (BaP and methoxychlor) demonstrated poor concordance. Overall, our results indicate the importance of including *in vivo* data in assessing endocrine activity of test materials.

571 PHOXIM STIMULATE STEROIDOGENESIS IN RAT GRANULOSA CELLS *IN VITRO*.

H. Chen[1, 2] and X. Wang[1]. *[1]Institution of Toxicology, Nanjing Medical University, Nanjing, Jiangsu Province, China and [2]Environmental Health Sciences, UCLA School of Public Health, Los Angeles, CA.* Sponsor: L. You.

BACKGROUND: Phoxim is a kind of organophosphorus pesticides and is commonly used in agriculture, especially in China. Former studies on the toxicity of this pesticide focused on the nervous system, however, our previous study indicated that phoxim can interrupt the steroidogenesis in whole rat model. The purpose of this study was to determine how phoxim affect the steroidogenesis in rat granulose cells (rGCs) *in vitro*. **METHODS:** rGCs was cultured in the serum-free medium and phoxim and/or FSH were added. Progesterone (P_4) and estradiol (E_2) concentration in the medium were determined by radioimmunoassay (RIA) at 12h and 24h of culture. The effects of phoxim on the mRNA expression of P450*scc* and 3β-HSD were evaluated by RT-PCR at 24h of culture. Aromatase activity assay was performed at 24h by evaluating the ability of transferring ^{3}H-1β-androstenedione to ^3H_2O. Also, the cellular cAMP concentration was detected by RIA after 24 h of culture. **RESULTS:** The synthesis of P_4 was stimulated by phoxim in rGC *in vitro*, however it was suppressed by high doses of phoxim (125 and 625 μmol/L) with the medium contained FSH (2 mg/L). The synthesis of E_2 in rGC *in vitro* was stimulated by phoxim no matter whether the medium contained FSH (2 mg/L) or not. Although mRNA expression of P450*scc* and 3β-HSD were induced by phoxim, it

was inhibited by high doses of phoxim (125 and 625 μmol/L) with the medium contained FSH (2 mg/L). Phoxim can stimulate cAMP production. No significant effect of aromatase activity was observed in phoxim treated groups. **CONCLUSION:** Phoxim can stimulate the synthesis of P_4 and E_2 in rGC *in vitro* which are related with the mRNA expression alteration of P450*scc* and 3β-HSD. The effects of phoxim on mRNA expressions of these two emzymes may be caused through cAMP/PKA pathway. Aromatase activity is not involved in the stimulation of E_2 synthesis in rGC *in vitro*.

572 PROCESS OF VALIDATION OF THE LUMI-CELL™ ER RECOMBINANT BIOASSAY FOR RAPID EVALUATION OF POTENTIAL ESTROGENIC ENDOCRINE DISRUPTOR CHEMICALS.

J. D. Gordon[1], A. C. Chu[1], M. D. Chu[2], C. L. Taylor[1], M. S. Denison[3] and G. C. Clark[1]. *[1]Xenobiotic Detection Systems, Inc., Durham, NC, [2]Alta Analytical Perspectives, Wilmington, NC and [3]Department of Environmental Toxicology, University of California, Davis, Davis, CA.*

Endocrine disruptor chemicals (EDCs) are a class of compounds, which have tremendous adverse effects on human and wild life populations. The association between the exposure to, and bioaccumulation in the food chain, of EDCs has raised concern worldwide. Identification of ECDs requires a relevant bioassay, which can both detect these chemicals, as well as provide a relevant estimate of their endocrine disrupting potency. Xenobiotic Detection System (XDS) Inc. developed the LUMI-CELL™ ER bioassay in order to detect EDCs using a high-throughput bioassay system. To detect EDCs, BG-1 cells were stably transfected with an estrogen-responsive luciferase reporter gene plasmid (pGudLuc7ere). The resulting cell line responds to estrogenic chemicals in a time-, dose dependent- and chemical-specific manner with the induction of luciferase gene expression. The LUMI-CELL™ ER bioassay system produced by XDS has tested over 110 chemicals, 53 of these chemicals were recommended by ICCVAM for validation of ER binding and transcriptional activation. Of the 53 chemicals tested, which were recommended by ICCVAM, all of the 28 compounds having historical data for a positive response demonstrated estrogenic activity. Out of the 110 chemicals tested by LUMI-CELL™ ER bioassay system, 69 demonstrated estrogenic activity, while 41 showed no activity. Of the 57 chemicals tested, which were not included in the ICCVAM requirements for validation, 30 were found to possess estrogenic activity, while 27 showed no activity. This data clearly demonstrates that LUMI-CELL™ ER high-throughput bioassay system produced by XDS is a fast, reliable, and relatively inexpensive method for detection of EDCs, meeting many of the requirements mandated by the EPA and ICCVAMs Tier I (screening) requirements for EDC detection assays. Supported by NIEHS SBIR grant ES10533-03, and Superfund Basic Research Grant ES04699.

573 *IN VIVO* AND *IN VITRO* ANTI-ANDROGENIC EFFECTS OF DE-71, A COMMERCIAL POLYBROMINATED DIPHENYL ETHER (PBDE) MIXTURE.

T. E. Stoker, R. L. Cooper, C. S. Lambright and L. E. Gray. *EB, RTD, NHEERL, USEPA, Research Triangle Park, NC.*

PBDEs are synthesized in large quantities as flame retardants for commercial products. The detection of PBDEs in tissues in wildlife species and in human milk and plasma samples has raised concerns about possible health effects. Recently, we showed that DE-71 delayed preputial separation (PPS) and suppressed the growth of androgen-dependent tissues in the Wistar rat following peri-pubertal exposure. These effects occurred concurrently with hypothyroidism and hepatomegaly and suggested that DE-71 may be either inducing steroid hormone metabolism or acting as an androgen receptor (AR) antagonist. To elucidate the anti-androgenic effects of this mixture, we evaluated DE-71 in several *in vivo* and *in vitro* assays. In a pubertal exposure study, we observed a delay in PPS with 30, 60 and 120 mg/kg/day of DE-71 (3, 4 and 5 days) and a suppression of ventral prostate (VP) and seminal vesicle growth. In another study, in which adult male rats were exposed to DE-71 for three days, luteinizing hormone, testosterone, androstendione and estrone concentrations were increased following exposure to 60 mg/kg/day. DE-71 and DE-100 (2, 2', 4, 4', 6-pentaBDE), one of the congeners in this mixture, both inhibited AR binding in a competition assay using rat VP cytosol (both with IC50s approximately 5 uM). In addition, both DE-71 and DE-100 inhibited DHT-induced transcriptional activation in MDA-kb2 cells. DE-71 was also positive for anti-androgenic activity in a weanling rat Hershberger assay. In conclusion, DE-71 and -100 have anti-androgenic activity, which appears to be mediated *via* AR antagonism. This alteration of androgen function may be the mechanism by which DE-71 delayed PPS and suppressed the growth of the androgen dependent tissues in the previous study. Additional studies are in progress to determine the specific (competitive verses noncompetitive) nature of the chemicals in the rat AR binding assays and to repeat the Hershberger assay. (Abstract does not necessarily reflect EPA policy.)

574 EFFECTS OF *IN UTERO* EXPOSURE OF DIETHYLSTILBESTROL AND DIBUTYL PHTHALATE ON THE TESTIS DESCENDENT IN RAT OFFSPRINGS.

T. Kim, J. Shin, S. Lee, H. Moon, I. Kang, I. Kim and S. Han. *Endocrine Toxicology Division, National Inst of Toxicol Res, Seoul, South Korea.* Sponsor: I. Yu.

The aims of present study were to compare the effects of *in utero* exposure to DES and DBP on the development of reproductive organs and to investigate the specific mechanisms of these abnormalities in the male reproductive system. During gestation days 10-19, pregnant Sparague-Dawley rats were administered oraly with corn oil, DES (25, 50, or 100 microgram/kg/day) or DBP (250, 500, or 700 mg/kg/day). Animals were killed at 31 or 42 days of age and blood was collected for serum testosterone analysis. Testes and accessory organs (epididymides, seminal vesicles, ventral prostate, LABC, Cowper's glands) were weighed and examined histologically. Steroid hormone receptor (AR and ER) and SF-1 expression levels were also examined in the testes, respectively. At 31 days of age, effects of DBP on the male reproductive tract abnormalities (hypospadias, cryptorchidism) were dose-dependent. DBP treatment significantly decreased the weights of testes and accessory sex organs compared to those of control. In the DBP group, the weights of the testes, epididymides ventral prostate, seminal vesicle, and Cowper's glands were significantly decreased. DBP significantly delayed the testes descent in a dose-dependent manner, whereas DES slightly delayed the testes descent only at the high dose group (100 microg/kg/day). DBP also significantly increased the expression of ER in the testes but DES did not change the expression of AR, ER in the testis compared to those of control. At 42 days of age, the animals of DBP-treated group did not show any different change of testicular and accessory sex organ weights. DBP and DES markedly decreased serum testosterone levels. In addition, DES significantly increased the expression of ER in the testes respectively. In contrast, DBP did not effect the expression of AR, ER in the testis. These results demonstrate that during gestation days 10-19 exposure to DES and DBP causes permanent changes in the endocrine system resulting in abnormal development of male reproductive tract.

575 RAT SPERMATID AND OVARIAN PRIMARY FOLLICLE COUNTS FOLLOWING *IN UTERO* EXPOSURE TO LOW DOSE BISPHENOL A.

C. Talsness, J. Nowak, K. Grote, S. Kuriyama and I. Chahoud. *Department of Toxicology, Charite University Medical School, Campus Benjamin Franklin, Institute of Clinical Pharmacology and Toxicology, Berlin, Germany.*

Conflicting studies exist whether bisphenol A (BPA), a compound with *in vitro* estrogenic and anti-androgenic activity, interferes with the developing reproductive system. We evaluated possible effects on gamete numbers in the testis and ovary following *in utero* exposure to a low dose of BPA. Gravid Sprague-Dawley rats were treated per gavage with either 2 μg/kg/d BPA, 2 μg/kg/d 17-αethinyl estradiol (EE) or cornstarch (one control group for each treatment group) on gestation days 6 - 21. Adult female offspring were sacrificed in estrus at approximately four months of age and adult male offspring were killed on PND 170. Paraffin-embedded ovaries were cut in 6μm thick serial sections, stained with hematoxylin and eosin and the number of primary follicles were evaluated in five sections, 240 μm apart, taken from the mid portion of the ovary. After removal of the tunica albuginea, the testes were minced and homogenized in 10 ml 0.9 % NaCl containing 0.5 % Triton X-100. After dilution, the number of homogenization-resistant spermatids was counted with a hemocytometer. The preliminary results suggest that there is a decrease in primordial follicle numbers in the ovaries (n=10) exposed to BPA and an increase in primordial follicle numbers in the ovaries (n=8) exposed to EE. A statistically significant decrease in spermatid number was observed following BPA exposure ($244 \pm 29 \times 10^6$ *vs* $207 \pm 33 \times 10^6$). The number of ovarian primary follicles may be sensitive to prenatal exposure to endocrine active compounds. *In utero* exposure to BPA results in decreased spermatid numbers at adulthood in the rat at a dose which may be pertinent to human exposure levels. The study was funded by the Federal Agency for Education and Research (07HORO1/7).

576 REGION-SPECIFIC GLOBAL GENE EXPRESSION ANALYSIS IN THE MICRODISSECTED HYPOTHALAMIC MEDIAL PREOPTIC AREA OF RAT NEONATES INJECTED WITH ESTRADIOL BENZOATE OR FLUTAMIDE.

M. Shibutani, K. Lee, H. Takagi, N. Kato, S. Takigami and M. Hirose. *Division Pathol., NIHS, Tokyo, Japan.* Sponsor: M. Ema.

To identify genes linked to disruption of brain sexual differentiation, region-specific gene expression profiles in the microdissected medial preoptic area (MPOA) were examined in rat neonates exposed to estrogenic and anti-androgenic drugs utilizing methacarn that we have recently found as a versatile fixation tool for mRNA

expression analysis in the paraffin-embedded tissue specimens. For this purpose, a preliminary validation study on the microarray analysis was performed using the rat liver. With 50 ng of total RNA, up to 0.5 million-fold of antisense RNAs (aRNAs) could be amplified by two-round (2x) *in vitro* transcription. With the amplified aRNAs, expression profile was compared with those in 1x or 2x-amplified aRNAs from unfixed tissue, and high fidelity was confirmed by a correlation coefficient of 0.91 with 1x-amplified aRNAs from unfixed tissue. In the next experiment, neonatal rats at birth were treated either s.c. with estradiol benzoate (EB; 10 μg/pup) or flutamide (FA; 250 μg/pup), and gene expression profiles in the MPOA were examined 24 hour later. Among 8, 000 probes in the microarray, genes up-regulated twice or more by EB or FA as compared to the control levels were 26 and 6 for males, and 299 and 9 for females, respectively. Genes down-regulated twice or less were 1 and 6 for males, and 125 and 29 for females, respectively. Among them, cyclin G1 showed chemical and sex unrelated up-regulation. By EB, 11 of down-regulated genes in females were up-regulated in males. By FA, each one gene showed opposite expression pattern between males and females. In females, 7 genes were down-regulated both by EB and FA, and 3 genes including GnRH were up-regulated by EB and down-regulated by FA. In summary, we have now established a global expression analysis method in paraffin-embedded histologically defined area by employing methacarn fixation, and genes showing altered expression by EB or FA with sex-dependent manner in the MPOA were identified as candidates to play a role in central endocrine disruption.

577 EFFECT OF DDT ON TESTOSTERONE AND AROMATASE ACTIVITY VIA ESTROGEN RECEPTOR IN LEYDIG CELL.

K. Lee[1, 2] and H. Jeong[1, 2]. *[1]Pharmacy, Chosun University, Kwangju, Kwangju, South Korea and [2]Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea.*

Various pesticides known or suspected to interfere with steroid hormone function were screened for effects in leydig cells on catalytic activity and mRNA expression of aromatase. Dichlorodiphenyltrichloroethane (DDT) is a widespread environmental pollutant. In this study, we investigated the effect of DDT on testosterone production through aromatase activity and its molecular mechanism in testicular leydig cell, R2C by using radioimmunoassay (RIA). As the results, the potent leydig cell activator LH increased testosterone production compared to the control. DDT exposure significantly decreased testosterone production in R2C cell. In addition, DDT was found to increase aromatase gene expression and activity in R2C cell in a dose dependent manner. In order to assess whether the suppressive effects of DDT on LH-inducible testosterone (T) production might be influenced by the ER, ICI 182.780 was used, and it was found that these inhibitory effects of DDT were antagonized by ICI 182.780, implying that the estrogen receptor (ER) mediates the suppressive effects of DDT. Furthermore, the inducible effects of DDT on aromatase gene expression might be influenced by the ER, ICI 182.780 was used, and it was found that these enhancing effects of DDT were antagonized by ICI 182.780, implying that the ER mediates the inducible effects of DDT. Our results indicated that DDT inhibition of luteinizing hormone (LH)-inducible T production in R2C cell is mediated through aromatase. The current study suggests the possibility that DDT might act as a modulator aromatase gene transcription.

578 CATECHOL ESTROGEN-INDUCED DNA DAMAGE IN MCF-7 CELLS.

M. B. van Duursen, T. Sanderson and M. van den Berg. *Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands.*

Epidemiologic evidence suggests that alterations in estrogen metabolism are associated with breast cancer risk. In breast tissue, estradiol is metabolized to the catechol estrogens (CEs) 2-OHE2 and 4-OHE2 by CYP1A1 and CYP1B1, respectively. A major inactivation step for the CEs is conversion to their inactive methoxy derivatives by catechol-O-methyltransferase (COMT). We have previously shown that several dioxin-like compounds can cause a concentration-dependent increase of CE levels but a decrease in the 4-OHE2/2-OHE2-ratio in MCF-7 and MCF-10A cells. In addition, we have shown that phytochemicals with a catechol structure, like quercetin, have the ability to inhibit methylation of CEs by COMT in cytosol from healthy human breast tissue. In this study, we investigated the effects of altered estrogen metabolism on DNA damage in MCF-7 cells using the comet assay. MCF-7 cells were incubated with 4-OHE2 and 2-OHE2 (total CE concentration held constant at 1 or 10 μM) in ratios 0, 0.2, 0.5, 1, 2 or 3. COMT activity was inhibited by the selective inhibitor Ro 41-0960. After a 5h incubation, the cells were lysed and electrophoresed. DNA was stained with ethidium bromide and analysed under a fluorescence microscope. Tail moment (% tail DNA x tail length) as measure of DNA damage was calculated using the PC-image-analysis program Casp. The solvent vehicles, Ro 41-0960 or CEs alone caused no increase in DNA damage compared with control cells. However, when COMT was inhibited by Ro 41-0960, we found a linear correlation between the increase in the 4-OHE2/2-OHE2-ratio and the increase of DNA damage. At 10 μM, there was significantly more DNA damage than at 1 μM CE at each ratio. Quercetin appeared to act like a COMT inhibitor resulting in increased CE-induced damage. These data show that 4-OHE2, but not 2-OHE2, is a potential DNA damaging compound in a concentration-dependent manner. Further, COMT activity appears to be an important factor in determining the genotoxic potential of the CEs and inhibition of COMT might play a role in the etiology of breast cancer.

579 ACTIVATION OF HUMAN ESTROGEN RECEPTORS IN HEPG2 CELLS BY GENISTEIN AND ITS CONJUGATED METABOLITES.

S. Borghoff, H. D. Parkinson, S. M. Ross, K. Gaido and M. Sochaski. *CIIT Centers for Health Research, Research Triangle Park, NC.*

Genistein is a phytoestrogen present in soy and virtually all rodent diets. It has been shown to activate both estrogen receptor (ER) α and β *in vitro*. Genistein has also been reported to elicit estrogenic effects in developing rodents. Following oral administration, genistein is found in plasma predominantly conjugated to glucuronic acid (GA) and to a lesser degree to sulphate (S). However, fetal plasma levels of genistein-S and genistein-GA are similar to each other following administration of genistein to pregnant rats. Because these conjugated forms are the major metabolites of genistein *in vivo*, these metabolites should not be overlooked as possible contributors to estrogenic effects of genistein. The objective of this study was to determine if conjugates of genistein activate ERα or ERβ as does free genistein. HepG2 human hepatoma cells were transfected with various concentrations of receptor plasmids encoding human ERα or ERβ, and complement 3-luciferase and pCMV β-galactosidase plasmids. Transfected cells were treated with various concentrations of either estradiol, genistein, genistein-GA (isolated from bile of genistein treated rats), genistein-S (chemically synthesized) or dimethylsulfoxide (control vehicle). Following 24-hr incubation, cell lysates were assayed for luciferase activity (receptor activation) and ?-galactosidase activity (transfection control). Estradiol and genistein activated both ER α and β, however, neither genistein-GA nor genistein-S activated either receptor or antagonized the ability of estradiol to activate these receptors. These data demonstrate that conjugation eliminates the estrogenic activity of genistein and that it is important to determine the ratio of free genistein to conjugated forms when estimating estrogenic potency. (This study was funded by the American Chemistry Council.)

580 INTERACTION ANALYSIS OF SYNTHETIC CHEMICALS AND PHYTOESTROGENS *IN VITRO*.

E. W. Carney[1], G. D. Charles[1], C. Gennings[2], B. B. Gollapudi[1] and T. R. Zacharewski[3]. *[1]The Dow Chemical Company, Midland, MI, [2]Biostatistics, Virginia Commonwealth University, Richmond, VA and [3]Biochemistry & National Food Safety & Toxicology Center, Michigan State University, East Lansing, MI.*

Humans are potentially exposed to many different estrogenic chemicals including synthetic chemicals (SCs), typically at very low levels, and phytoestrogens (PEs), often present in diets at high levels. In this study we assessed interactions between SCs and PEs using an estrogen receptor-alpha reporter gene assay. Individual concentration-response curves were first generated for each of six SCs [methoxychlor, o, p-DDT, bisphenol A, beta-hexachlorocyclohexane, 2, 3-bis (4-hydroxyphenyl)-propionitrile), octylphenol] and for a PE preparation (genistein:daidzein, 2:1). A fixed-ratio mixture of all six SCs was then devised with the proportion of each chemical in the mixture based on its no-effect concentration. The SC mixture was tested at total concentrations of 0.02, 0.2, 1.0, 2.0, or 3.0 uM, with 2.0 uM being the total dose at which each individual chemical in the mixture was at its own response threshold. We then asked whether or not the threshold and slope of the PE concentration-response curve (0, 0.01, 0.025, 0.05, 0.1, 0.15, 0.5 uM) were shifted when the PEs were combined with various concentrations of the SC mixture. In general, the SC mixture significantly increased response thresholds relative to the PEs alone, an effect most likely due to the lower potency of the SCs and their competition with the more potent PEs. Once these thresholds were exceeded, slopes of the PE+SC mixture responses were significantly steeper than that of the PEs alone. Finally, analysis of the SC mixture without PEs indicated an antagonistic interaction between the SC mixture components at the ratio tested. These results highlight the dose-dependency of chemical interactions and also exemplify a novel experimental and statistical approach for assessing complex chemical mixtures at low concentrations. Funded by the American Chemistry Council, Long-Range Research Initiative.

581 COMPARATIVE ACTIVATION OF ESTROGEN RECEPTOR α (ERα) AND ERα/SP1 IN BREAST CANCER CELLS BY XENOESTROGENS.

F. Wu[1] and S. Safe[2]. [1]*Biochemistry & Biophysics, Texas A&M University, College Station, TX and* [2]*Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.*

17β-Estradiol (E2) and diethylstilbestrol (DES) are potent estrogenic compounds *in vivo* and activate estrogen-responsive genes and constructs containing estrogen responsive elements (EREs) in transfection experiments in cell culture. The estrogenic activity of several synthetic "xenoestrogenic" compounds were evaluated in transient transfection assays in breast cancer cells transfected with constructs containing three tandem EREs ($pERE_3$) or GC-rich motifs ($pSp1_3$) that are activated by ERα and ERα/Sp1 respectively. Relatively high concentrations (10-50 μM) of nonylphenol, 2, 2-bis(p-hydroxyphenol)-1, 1, 1-trichloroethane (HPTE), octylphenol, endosulfan and 2', 3', 4', 5'-tetrachlorobiphenol-2-ol induced transactivation in cells transfected with $pERE_3$, whereas maximal induction was observed for 1 or 10 nM E2 and DES. Ten nM E2 and higher concentrations (up to 75 μM) of the xenoestrogenic compounds also induced 4- to 5-fold increase in reporter gene activity in MCF-7 cells transfected with $pSp1_3$, whereas only minimal induction (<50%) was observed in cells treated with 10 nM and higher concentrations of DES. Thus, E2 and DES differentially activated ERα/Sp1. A series of mutant ERα constructs containing selective deletions and mutations were also used in these assay and the results show that DES, E2, antiestrogens and xenoestrogens differentially induced transactivation in MCF-7 cells and other breast cancer cell lines transfected with $pSp1_3$. Thus, ER-dependent activation of $pSp1_3$ and $pERE_3$ by E2, DES and xenoestrogens in breast cancer cells is ligand structure-dependent, suggesting that these compounds will induce unique *in vivo* biology. (Supported by NIEHS E209106 and ES04176)

582 LACK OF SYNERGISTIC OR ANTAGONISTIC EFFECTS OF A MIXTURE OF PHYTOESTROGENS ON CELL PROLIFERATION OF MCF-7 HUMAN BREAST CANCER CELLS (E-SCREEN).

J. van Meeuwen[1], A. Piersma[2], M. van den Berg[1] and J. Sanderson[1]. [1]*IRAS, University Utrecht, utrecht, Netherlands and* [2]*RIVM, Bilthoven, Netherlands.*

A normal human diet is known to contain a large number of phytochemicals, of which a minority has shown estrogenic or anti-estrogenic effects *in vitro* as well as *in vivo*. It has been suggested that these phytoestrogens may act synergistically or antagonistically in combination with endogenous estrogens, such as 17β-estradiol (E_2). To investigate potential synergism and antagonism, several phytoestrogens were tested individually, in a mixture and as combinations of this mixture with E_2 for cell proliferative effects on MCF7-bus cells (kindly provided by A. Soto). Cell numbers were determined using MTT reduction as a measure of mitochondrial activity. E_2 caused 50% of maximal cell proliferation at 7pM (EC_{50}). Genistein and naringenin produced full concentration-response curves, with EC_{50}'s of 0.06μM and 0.8μM respectively. For catechin, coumestrol and quercetin maximal cell proliferation was not reached at concentrations up to 0.1mM, but for coumestrol and quercetin the concentrations 10 μM respectively 0.1mM caused the same proliferative response as the EC_{50} of E_2. For catechin the highest tested concentration (0.1mM) caused the same proliferative response as the EC_{30} of E_2. Epicatechin did not cause cell proliferation. A phytoestrogen mixture (phytomix) was prepared reflecting measurements of concentrations found in serum from humans consuming a regular diet (genistein=500nM; naringenin=100nM; coumestrol=10pM; quercetin=50nM; catechin=70nM; epicatechin=250nM). Various dilutions (ranging from 0.03 to 10 times the phytomix concentration) produced a full concentration-response curve with an EC_{50} at 0.16 times phytomix. Binary combinations of the phytomix and E_2 in various ratios, which assuming additivity would result in a proliferative response equal to the EC_{50} of E_2 alone, were tested. These combinations showed no departure from additivity. In conclusion, the tested phytoestrogens appeared to act as full agonists for the estrogen receptor and interacted among each other and with E_2 in an additive manner.

583 CELLULAR UPTAKE OF DAIDZIN AND GENISTIN BY MCF-7-ERE CELLS VIA GLUCOSE TRANSPORTER.

I. Kim[1], Y. Sheen[2] and H. Kwon[1]. [1]*Department of Food and Nutrition, college of Human Ecology, Seoul National University, Seoul, South Korea and* [2]*College of Pharmacy, Ewha Womans University, Seoul, South Korea.* Sponsor: Y. Cha.

It has been thought that the metabolism of phytoestrogen glycosides to aglycone is necessary to exert their estrogenic activity due to their poor absorption. However, our previous study suggested estrogenic activity of glycoside is similar to that of aglycone in cultured MCF-7-ERE cells, human mammary cancer cells transfected with reporter genes. The mammary cancer cells appeared to take up glycosides, since the *in vitro* estrogenic activities of the glycosides apparently were as high as the aglycons. In order to identify the transporter which is responsible for cellular uptake of the glycosides, glucose transporter GLUT blocker cytochalasin B(CytoB) and sodium/glucose cotransporter SGLT blocker phlorizin(PLZ) were employed. Estrogenic activities of glycoside daidzin and genistin were inhibited in a dose-dependent manner by the treatment with 1×10^{-7}~2×10^{-5}M of cytochalasin B. Phlrorizin inhibited also, but failed to show dose dependency. On the contrary, CytoB and PLZ stimulated estrogenic activities of aglycone daidzein and genistein. These results suggest glycosides daidzin and genistin could be transported into the cell in their intact glycoside form *via* GLUT.

584 INTERACTION ANALYSIS OF SYNTHETIC CHEMICALS AND PHYTOESTROGENS *IN VIVO*.

S. D. Seidel[1], D. R. Boverhof[2], E. W. Carney[1], G. D. Charles[1], C. Gennings[3], B. B. Gollapudi[1] and T. R. Zacharewski[2]. [1]*The Dow Chemical Company, Midland, MI,* [2]*Biochemistry & Molecular Biology, National Food Safety & Toxicology Center, Michigan State University, East Lansing, MI and* [3]*Biostatistics, Virginia Commonwealth University, Richmond, VA.*

Humans are potentially exposed to many different estrogenic chemicals including synthetic chemicals (SCs), typically at very low levels, and phytoestrogens (PEs), often present in the diet at high levels. In this study we assessed interactions between SCs and PEs using the immature rat uterotrophic assay (UA) coupled with expression analysis of estrogen-responsive genes. All test compounds were administered by gavage once daily for 3 days. 24 h after the last dose, uteri were harvested, wet weights recorded and tissues were snap frozen for real-time RT-PCR analysis. Individual dose-response curves in the UA were first generated for each of six SCs [methoxychlor, o, p-DDT, octylphenol, bisphenol A, beta-hexachlorocyclohexane, 2, 3-bis(4-hydroxyphenyl)-propionitrile)] and for a PE preparation (genistein:daidzein, 2:1) with ethynylestradiol (EE) (10 ug/kg/day) as a positive control. A fixed-ratio mixture of all six SCs was then devised with each chemical in the mixture present at its individual response threshold dose (i.e., a dose causing a 20% increase in uterine wet weight). We then compared the dose-response for the PE preparation alone (total doses of 0, 10, 30 or 120 mg/kg/day) with that of the PEs combined with the SC mixture. Statistical analysis of the UA data indicated that the PEs and SCs, at the tested dose levels, produced an additive response in the uterotrophic assay. Expression of two genes induced by EE, complement component 3 and intestinal calcium-binding protein, also appeared to increase in a dose-responsive manner following treatment with the PEs alone and with the PE+SC mixtures. Studies to further evaluate the estrogenic effects of mixtures of PEs with low levels of SCs are currently in progress. Funded by the American Chemistry Council, Long-Range Research Initiative.

585 *IN VIVO* INTERACTIONS OF THE ENDOCRINE DISRUPTOR ETHINYL ESTRADIOL WITH THYROID HORMONE ACTION.

A. Tindall[1, 3], I. D. Morris[2], H. Isaacs[3], B. Pownall[3], D. Pickford[4], T. Hutchinson[4], R. Schultz[5] and L. Tattersfield[5]. [1]*Biological Sciences, Manchester University, Manchester, United Kingdom,* [2]*Hull York Medical School, York, United Kingdom,* [3]*Biology, York University, York, United Kingdom,* [4]*Environmental laboratory, AstraZeneca, Brixham, United Kingdom and* [5]*Ecological Sciences, Syngenta, Bracknell, United Kingdom.* Sponsor: I. kimber.

There is evidence that xenobiotics with estrogenic activity may interact with other endocrine systems. A detailed understanding of these effects may enable more informed evaluation of environmental exposure risk. This study aims to characterise the interactions between environmental estrogens and the thyroid axis. Our model species is Xenopus tropicalis, a sentinel species for environmental thyroid disruption as it is aquatic and the thyroid axis controls metamorphosis which is well studied. Iodothyronine type III deiodinase deactivates thyroid hormones and is regulated by prolactin, which in turn is a physiological/toxicological target for estrogen. X.tropicalis larvae (120/treatment) were continuously exposed to: 100microl/L methanol control with or without 100ng/L 17-alpha ethinyl estradiol (EE2). Exposure began at stage 45-48 and larvae were sampled at stage 53-56 and 61-64. Thyroxine (T4) was measured in larval bodies by RIA at stage 61-64. Whole body expression levels of DI mRNA were quantified by real time PCR in stage 54 larvae. When compared to control EE2 treated larvae had increased snout-vent length at stage 55 ($P<0.05$) and decreased total length at stage 62 ($P<0.05$). EE2 accelerated developmental rate between stages 45-48 and 53-56 (log rank $P<0.0001$), but not between stages 45-48 and 61-64. EE2 treatment increased variability in T4 levels ($P<0.005$) and increased DI mRNA expression ($P<0.005$). In this experiment EE2 caused subtle disruption of thyroid hormone action. Developmental rate and 5DI expression increased up to stage 53-56, indicating interaction between the oestro-

gen and thyroid axes. This is supported by increased variability in T4 levels at stage 61-64 and may be due to modulation of prolactin. Future work will determine whether persistent disruption of the endocrine system occurs which affects later development.

586 DOSE-DEPENDENT GLOBAL GENE EXPRESSION ANALYSIS IN THE MICRODISSECTED HYPOTHALAMIC MEDIAL PREOPTIC AREA OF RAT NEONATES EXPOSED PERINATALLY TO ETHINYLESTRADIOL.

K. Lee, M. Shibutani, H. Takagi, N. Kato, S. Takigami and M. Hirose. *Division Pathol, NIHS, Tokyo, Japan.* Sponsor: M. Ema.

To identify genes linked to disruption of brain sexual differentiation by exogenous xenoestrogens, a region-specific global gene expression analysis was performed in the medial preoptic area (MPOA) microdissected from hypothalamus of rat neonates following maternal exposure to ethinylestradiol (EE; 0, 0.01, 0.1 and 0.5 ppm in diet) from gestational day 15. At postnatal day 2, brains of offspring (3 males and 3 females per group) were fixed with methacarn and paraffin-embedded. Total RNA (50 ng) extracted from microdissected MPOA was subjected to two-round *in vitro* transcription, hybridized with Affymetrix GeneChip Rat Genome U34A Array containing 8000 probes, and analyzed the expression data by GeneSpring software ver. 5 (Silicon Genetics). In the MPOA, about 3600 genes showed presence call in both sexes. Among these genes, a total of 5% showed sex difference in the expression (> 2-fold) in untreated controls, with 62 and 124 genes showing up-regulation in males and females, respectively. At 0.5 ppm, a total of 270 and 130 genes were up- or down-regulated (> 2-fold) in males and females, respectively as compared with corresponding controls. Among these genes, dose-dependent up- or down-regulation was revealed in 14 and 146 genes in males, and 6 and 44 genes in females, respectively. Among them, following genes showed strong dose-dependent down-regulation: ribosomal protein S7, *S*-adenosylmethionine decarboxylase, tyrosine hydroxylase, neurotimin, sodium channel III, GTP binding protein and synaptotagmin in males, and amyloid precursor-like protein 2 and cyclin G in females. At 0.5 ppm, 10 down-regulated genes including GTP binding protein and synaptotagmin observed in male were up-regulated in females. On the other hand, 11 up-regulated genes at 0.5 ppm in males were down-regulated in females. The results suggest that genes showing dose-dependent alteration in the expression with apparent sex difference in response to perinatal exposure to EE can be linked to events in the central endocrine disruption.

587 COMPARISON OF 17β-ESTRADIOL- AND GENISTEIN-INDUCED CELL GROWTH AND PROLIFERATION IN THE RODENT UTERUS.

G. Orphanides, H. Tinwell, F. Lim, D. Moore, I. Pate, I. Kimber, J. Ashby and J. G. Moggs. *Syngenta Central Toxicology Laboratory, Alderley Park, Cheshire, United Kingdom.*

The xenoestrogen group of endocrine disruptors have the potential to cause reproductive and developmental effects through stimulation or disruption of estrogen receptor signalling. Phytoestrogens have attracted attention due to opportunities for dietary exposure (e.g. soy) and their higher affinity for estrogen receptor (ER) β, which has been proposed to antagonise the effects of ERα. It is, therefore, unclear whether phytoestrogens elicit their estrogenic effects *via* a mechanism analogous to that of natural estrogens. Using transcript profiling, we have compared the molecular mechanisms through which the phytoestrogen, genistein (GEN), and the natural estrogen, 17b-estradiol (E2), induce growth and maturation of the immature mouse uterus. Mice (19-20 days) received a single subcutaneous injection of either E2 (400 μg/kg) or GEN (250 mg/kg), sufficient to induce a sustained and equivalent increase in uterine weight. Control mice received vehicle (arachis oil) alone. Gene expression levels, uterine weights and histological parameters were analysed 1, 2, 4, 8, 24, 48, and 72hr after exposure. Hierarchical clustering of co-regulated genes, combined with functional classification, revealed that E2 and GEN induce analogous, multi-stage transcriptional programs that drive uterine growth. This involves the sequential induction of genes involved in transcriptional regulation/signal transduction, protein biosynthesis, cell proliferation and epithelial cell differentiation. Our data provide novel insights into the molecular mechanisms that accompany estrogen-regulated uterine growth and, moreover, suggest that GEN is capable of modulating the full spectrum of biological processes associated with the action of E2 in the uterus. Furthermore, we have identified a novel class of estrogen-regulated transcription factors, induced by both chemicals, that appear to be direct targets of ERs and whose function may be linked to the timing of cell cycle progression and proliferation.

588 ETHYNYL ESTRADIOL ELICITED TEMPORAL AND DOSE-DEPENDENT HEPATIC GENE EXPRESSION PATTERNS IN IMMATURE, OVARIECTOMIZED MICE.

D. R. Boverhof[1], K. C. Fertuck[1], L. D. Burgoon[1], J. E. Eckel[2], C. Gennings[2] and T. R. Zacharewski[1]. *[1]Department of Biochemistry & Molecular Biology, Institute for Environmental Toxicology, and, Department of Pharmacology & Toxicology, National Food Safety & Toxicology Center Michigan State University, East Lansing, MI and [2]Department of Biostatistics, Virginia Commonwealth University, Richmond, VA.*

Temporal- and dose-dependent changes in hepatic gene expression were examined in immature ovariectomized C57BL/6 mice gavaged with ethynyl estradiol (EE). For temporal analysis, mice were gavaged every 24 hrs for 3 days with 100 μg/kg EE or vehicle and liver samples were collected after 2, 4, 8, 12, 24 and 72 hrs. Gene expression was monitored using custom cDNA microarrays containing 3068 cDNA clones of which 393 exhibited a significant change at one or more time points as determined using a model-based t-statistic filtering approach. Functional gene annotation extracted from public databases provided an association between temporal gene expression changes and physiological processes such as growth and proliferation, cytoskeletal and extracellular matrix responses, microtubule based processes, oxidative metabolism and stress, and lipid metabolism and transport. In the 24 hr dose-response study, hepatic samples were collected from mice treated with 0, 0.1, 1, 10, 100 or 250 μg/kg EE. Thirty-nine of the 79 genes identified as differentially regulated at 24 hr in the time course study exhibited a dose response relationship with an average ED50 value of 47±3.5 μg/kg. Comparative analysis indicated that many of the identified temporal and dose-dependent hepatic responses are similar to estrogen-induced uterine responses reported in the literature and in a companion study using the same animals. Results from these studies confirm that the liver is a highly estrogen responsive tissue that exhibits a number of common responses shared with the uterus as well as distinct estrogen mediated profiles. These data will further aid in the elucidation of the mechanisms of action of estrogens in the liver as well as in other classical and non-classical estrogen responsive tissues. Supported by ES011271

589 ELUCIDATING THE MOLECULAR PATHWAY FOR REGULATION OF THE STEROIDOGENIC ACUTE REGULATORY PROTEIN BY BETA-SITOSTEROL.

J. Kocerha[1], K. J. Kroll[2] and N. D. Denslow[1, 2]. *[1]Biochemistry and Molecular Biology, University of Florida, Gainesville, FL and [2]Biotechnology Program, University of Florida, Gainesville, FL.*

The steroidogenic pathway is targeted by some environmental contaminants, resulting in reduced levels of steroid hormone synthesis. Alterations in steroid levels can lead to severe reproductive and physiological abnormalities. The Steroidogenic Acute Regulatory Protein (StAR Protein) is a major rate-limiting step in steroid production and its activity and expression is inhibited by certain toxin exposures. StAR Protein transports cholesterol across the mitochondrial membrane, allowing cholesterol to be metabolized into the various steroids. Using largemouth bass (LMB) as a model, we have begun to examine the molecular mechanism(s) involved in the regulation of StAR by normal endogenous compounds like cAMP, as well as by beta-sitosterol, a common contaminant found in paper mill effluent. We have cloned the entire coding region of LMB StAR and used this sequence to develop a real-time PCR assay for quantitation of changes in StAR mRNA levels in ovarian tissue cultures. We performed dose response experiments with 0.25, 0.5, 0.75, 1 and 4 mM dbcAMP and show increased mRNA production that saturates between 1-4mM dbcAMP. Preliminary results show that cultured ovarian follicles with diameter size ranges of 0.48-0.6mm show about a 3-fold induction with 1mM dbcAMP. These results suggest that LMB and mammalian StAR may be similarly regulated. Preliminary ovarian follicle culture data shows that beta-sitosterol down-regulates StAR after 8 hours by 3 to 5 fold. To further analyze the regulation of LMB StAR, a 3 kb portion of the promoter was also cloned. Sequence upstream of the transcriptional start site has been analyzed for consensus sites. Putative binding sites for ER, AHR/ARNT, SF-1, AP-1, C/EBP and GATA transcription factors have been identified. These preliminary results will be used to help elucidate the transcriptional regulation of LMB StAR by beta-sitosterol.

590 FUNCTIONAL CHARACTERIZATION OF ARYL HYDROCARBON RECEPTOR (AHR) LOCALIZATION AND DEGRADATION IN ZEBRAFISH.

J. Wentworth, R. Buzzeo and R. S. Pollenz. *Biology, University of South Florida, Tampa, FL.*

The basic-helix-loop-helix/PAS (bHLH/PAS) family of proteins is a group of transcription factors that regulate key pathways in response to stress and in normal development. The aryl hydrocarbon receptor (AHR) is a member of this pathway.

Recently, Danio rerio (zebrafish) has become an important model system in the study of the signal transduction pathway and complement results in mammalian models. However, studies of the AHR protein have been limited by the lack of antibody reagents and thus, little are known condensing the localization and degradation of the zebrafish AHR (zfAHR). In this report, we describe the production and characterization of specific polyclonal antibodies to the zfAHR2 protein and the analysis of AHR-mediated signal transduction in the zebrafish liver cell line (ZFL). The results show that the zfAHR2 is degraded *via* the 26S proteasome following exposure of cells to Beta-napthoflavone (BNF). Interestingly, the time course is slower and the magnitude of zfAHR2 degradation is not as great as for mammalian AHR. Studies also show the zfAHR2 is rapidly degraded in a ligand independent manner by exposure of cells to geldanamycin (GA) to levels consistent with mammalian AHR. Finally, immunohistochemical staining of the ZFL cells suggest that the unliganded AHR resides in both the cytoplasm and nucleus and appears to undergo active nucleocytoplasmic shuttling in the absence of ligand. These results suggest that there is conservation of function between fish and mammals with respect to ligand dependant and independent degradation of the AHR and that the zfAHR2 is degraded *via* the 26S proteasome. Sponsored by NIEHS grants ES10991 and ES08980.

591 ZEBRAFISH AHR REPRESSOR: CLONING, REGULATORY INTERACTIONS AND INDUCIBILITY BY TCDD.

B. R. Evans[1, 2], S. I. Karchner[1] and M. E. Hahn[1]. *[1]Biology, Woods Hole Oceanographic Institution, Woods Hole, MA and [2]Biology, Boston Unviersity, Boston, MA.*

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and may have roles in the cell cycle and development. Several fish species, including the zebrafish (Danio rerio), possess at least two distinct AHR genes versus a single gene in mammals. Recently, an AHR repressor (AHRR) has been identified in mammals and fish. AHRR and AHR form a negative feedback loop in which AHR activation up-regulates expression of AHRR, which then inhibits transactivation by AHR. This study investigated AHRR structure, function, and regulation in zebrafish, a powerful model in developmental toxicology. We cloned a zebrafish AHRR (zfAHRR) cDNA sequence that encodes a protein of 550 residues and shows high sequence identity to AHRRs from mice, humans, and killifish. The zfAHRR gene maps to linkage group 24, which has regions of conserved synteny with human chromosome 5, the location of the human AHRR gene. The mapping data and phylogenetic analysis show that zfAHRR is an ortholog of the mammalian AHRRs. A single AHRR sequence was also identified in the genome of pufferfish (Fugu rubripes), suggesting that AHRR, unlike AHR, exists as a single gene in fish. In ZF-L zebrafish liver cells dosed with TCDD, real-time RT-PCR showed that expression of AHRR was induced approximately 15-fold, while CYP1A increased by 95-fold and AHR2 by 3.4-fold. In transient transfection assays performed in COS-7 cells, zfAHRR repressed both the constitutive and TCDD-inducible ability of the zebrafish AHR2 to activate expression of a reporter gene under control of AHR response elements. Increasing the amount of transfected ARNT expression construct failed to rescue zfAHR2 activity, suggesting that AHRR inhibition of AHR signaling does not occur solely through sequestration of ARNT. These results indicate that zebrafish possess an AHRR ortholog that acts in a negative feedback loop to repress the AHR signaling pathway downstream of ARNT heterodimerization. [NIH ES06272 and NIH ES07831]

592 ANALYSIS OF LIGAND-DEPENDANT AND INDEPENDENT DEGRADATION OF THE HUMAN ARYL HYDROCARBON RECEPTOR (HAHR) IN HUMAN CELL CULTURE LINES.

C. Buggy and R. S. Pollenz. *Biology, University of South Florida, Tampa, FL.*

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that is member of the basic-helix-loop-helix PAS family of proteins. In rodent model systems, the AHR is rapidly degraded following ligand binding *via* the 26S proteasome resulting in attenuation of AHR-mediated gene regulation. The domains required for degradation and the specific amino acids involved in ubiquitination have not been identified. Since the degradation of the AHR in human is relatively limited, several novel human cell culture lines were characterized following exposure to 2, 3, 7, 8-tetrachlorodibezo-p-dioxin (TCDD) and also to geldanamycin (GA). The cell lines included: kidney adenocarcinoma (A-498), brain carcinoma (DU-145), lung hepatocellular carcinoma (LS-180), normal lung (MRC-9), lung adenosquamous carcinoma (NCI-H596) and breast carcinoma (MCF-7). Each cell line exhibited expression of both the hAHR and hARNT proteins as determined by Western blotting. Interestingly, all cells showed degradation of the hAHR by 70-90 percent following TCDD exposure yet exhibited different time courses. Unlike rodent cells that show maximal degradation between 1-3 hours, LS-180, MRC-9 and NC1-H596 cells show maximal degradation during 4-8 hours. The degradation of the hAHR following exposure to GA also showed differences among the various cell lines. A-498, DU-145, NCI-H596, and MRC-9 cells exhibited >90 percent reduction in the hAHR within 2 hrs of GA exposure. In contrast, GA exposure to LS-180 and MCF-7 cells resulted in < 25 percent reduction of the hAHR after 2 hrs. These results show that the hAHR is degraded in a ligand dependant and independent manner and importantly, identify several novel human cell lines that exhibit reduction in the time course and magnitude of degradation. Future studies will be designed to exploit these cells and to gain information about the enzymes required for the ubiquitination of the AHR and the domains required for this event. Supported by NIEHS grants ES10401

593 TCDD-MEDIATED ACTIVATION OF THE AROMATIC HYDROCARBON RECEPTOR DISPLACES p300 FROM E2F-DEPENDENT PROMOTERS.

J. L. Marlowe and A. Puga. *Department of Environmental Health, University of Cincinnati, Cincinnati, OH.*

2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous environmental pollutant and confirmed carcinogen. The molecular basis for the biological effects of TCDD is largely unknown. TCDD mediates the transcription of the CYP1 family of P450 monooxygenases *via* activation of the AHR (aromatic hydrocarbon receptor), but this does not adequately explain the diversity of TCDD effects. Transactivation by the AHR can explain gene induction, but not gene repression, which constitutes a major effect of dioxin exposure that has not been characterized at the molecular level. We have shown that complexes formed between the AHR, the retinoblastoma protein RB, and the transcription factor E2F at E2F-regulated promoters result in repression of S-phase-specific genes, and induce cell cycle arrest. Expression of a reporter gene driven by an E2F-dependent promoter shows that TCDD treatment leads to repression of these genes. Relative expression of E2F-dependent genes is largely dependent on the presence of several co-activators and co-repressors, including p300/CBP, BRG-1, and HDAC. As the AHR is known to interact with several co-regulators of transcription, we hypothesized that AHR activation may disrupt the activity of such co-regulators at E2F-regulated promoters. TCDD did not affect the activity of the co-repressors BRG-1 or HDAC in transfection assays. However, TCDD was able to reverse the effect of p300 on an E2F-dependent reporter gene. We used chromatin immunoprecipitation assays to analyze this effect at *in vivo*, E2F-regulated promoters. Such assays show that AHR and p300 can be detected at the Cyp1a1 promoter in TCDD-treated cells. However, while p300 associates with E2F-dependent promoters (Dhfr, cyclin E, cdk2) in DMSO-treated cells, TCDD treatment results in the loss of p300 from these promoters. Furthermore, the AHR associates with the promoters of these genes in TCDD-treated cells, but not in DMSO-treated cells. These results suggest a potential mechanism by which TCDD exposure causes gene repression. *(Supported by NIH R01 ES06273).*

594 ALTERED CELL CYCLE REGULATION IN AH RECEPTOR-NULL MOUSE EMBRYO FIBROBLASTS.

X. Chang and A. Puga. *Environmental Health, University of Cincinnati, Cincinnati, OH.*

2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) causes diverse toxic effects in animals and humans. Most effects of TCDD result from activation of the cytosolic aryl hydrocarbon receptor (AHR) to a transactivator of Phase I and II drug-metabolizing enzymes. In addition to mediating target gene transcription, AHR causes cell cycle arrest *via* interacting with the retinoblastoma protein (RB) and potentiating repression of E2F-dependent transcription. To investigate the role of AHR in cell proliferation and differentiation, we developed a tetracycline receptor-regulated Tet-Off system to regulate the expression of mouse AHR and of its deletion mutant D323-494, which lacks the RB-interacting motif "LXCXE" and part of the PAS-B domain. Mouse embryonic fibroblasts (MEFs) derived from AHR null mice were infected with a retrovirus vector carrying the tetracycline receptor gene (tetR). Stable colonies with the highest tetR expression were selected and infected with retroviral vectors carrying either wild type or D323-494 AHR variants, followed by antibiotic selection to obtain double stable cell lines. These cell lines showed complete inhibition of AHR expression after 24-36 hours of treatment with 10 ug/ml doxycycline. In addition, induction of CYP1A1 by TCDD in wild type expressing cells was also suppressed by doxycycline, whereas CYP1A1 expression was constitutively high in D323-494 expressing cells. Doxcycline treatment in both cell lines decreased cell proliferation and removal of doxycycline reversed the effects, suggesting that AHR is critically involved in cell cycle regulation. ——- Supported by NIH grant ES06273 and by a grant from Phillip Morris USA

595 COMPARATIVE STUDY OF MOUSE AND HUMAN AH RECEPTORS.

P. Ramadoss, J. R. Petrulis and G. H. Perdew. *Center for Molecular Toxicology and Carcinogenesis, Department of Veterinary Science, The Pennsylvania State University, University Park, PA.*

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates the toxic response induced by TCDD and related compounds. Mouse models are used to study the toxicity of TCDD and estimate risk to humans. Differences between the mouse AhR (mAhR) and human AhR (hAhR) may be important, as these differences could result in altered responses to TCDD exposure. In this study, mAhR and hAhR were compared in terms of their ability to be modulated by the Hepatitis B virus X-associated protein (XAP2), as well as their relative ligand affinities. Fluorescence microscopy studies demonstrated that mAhR localizes predominantly in the nucleus in COS-1 cells, whereas hAhR is mostly cytoplasmic. Co-expressed XAP2 can redistribute and sequester mAhR to the cytoplasm, but appears to have no effect on hAhR's localization. Co-expressed XAP2 also enhances mAhR protein levels, but does not significantly affect hAhR levels; this may be explained in part by XAP2s ability to sequester mAhR to the cytoplasm. An XAP2 construct with an exogenous NLS (XAP2-NLS) was used to target XAP2 to the nucleus. XAP2-NLS can drag hAhR into the nucleus in both ligand-bound and ligand-free states, but not mAhR. This indicates that XAP2 is present in the hAhR complex that translocates to the nucleus. Immunoprecipitation experiments showed that the hAhR complex may contain less XAP2 compared to mAhR, and this could, perhaps, explain the differences seen between mAhR's and hAhR's behaviour in cells. Photoaffinity labeling experiments done *in vitro* and in COS-1 cells showed that the hAhR has a lower affinity for a photoaffinity ligand (2-Azido, 3 [125I]-Iodo 7, 8-dibenzo-para-dioxin) as compared to mAhR. These studies show that there are considerable differences between mAhR and hAhR, and this could result in variations in response to TCDD exposure. This work was supported by NIEHS grant ES04869.

596 EPIREGULIN: A POTENTIAL TARGET GENE REGULATED BY AHR.

R. D. Patel and G. H. Perdew. *Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State Univeristy, Univeristy Park, PA.*

TCDD is known to cause a large number of toxic effects, mediated largely by its binding to the AhR. It is believed that AhR is involved in most of the TCDD induced biological effects like carcinogenesis and teratogenesis through untimely and disproportionate expression of genes. However, the exact mechanism through which TCDD leads to these effects is not known. We have discovered a novel target gene, epiregulin, regulated by TCDD-activated AhR. Epiregulin is a recently discovered growth regulator which belongs to the EGF family and has been shown to stimulate DNA synthesis of primary rat hepatocytes. It is one of the most potent mitogens for hepatocytes and has an additive effect with hepatocyte growth factor and TGF-alpha. On conducting microarray experiments with TCDD treated and untreated temperature sensitive SV-40 virus immortalized mouse hepatocytes, we found that TCDD upregulates epiregulin gene expression. The promoter region of epiregulin has a dioxin responsive element (DRE), 56 nucleotides upstream of the transcription start site, along with three Sp1 binding sites. We performed luciferase reporter assays using NIH/3T3 cells and a vector generated by inserting the first 125 base pairs of the epiregulin rat promoter into pGL3-basic vector. Our results indicate a four-fold induction on TCDD treatment. When a single base of the DRE was mutated, this TCDD mediated induction was lost, establishing the involvement of AhR-ARNT heterodimer. In contrast to this, about 3 fold induction was obtained even when all the Sp1 binding sites were mutated. Our results indicate that epiregulin expression is induced on TCDD exposure through the AhR pathway. Future studies will aim at establishing the significance of epiregulin in TCDD mediated effects such as tumor promotion. (Supported by NIEHS grant ES04869)

597 MODULATION OF ARYL HYDROCARBON RECEPTOR FUNCTION BY XAP2 AND P23.

B. D. Hollingshead[1] and G. H. Perdew[2]. *[1]Graduate Program in Biochemistry, Microbiology, and Molecular Biology, Penn State University, University Park, PA and [2]Department of Veterinary Science and Center for Molecular Toxicology and Carcinogenesis, Penn State University, University Park, PA.*

The AhR is a ligand activated transcription factor that mediates a variety of toxic responses upon exposure to the environmental pollutant TCDD. The unliganded AhR exists as a heteromeric complex consisting of an hsp90 dimer, the immunophilin-like molecule XAP2, and the hsp90 associated protein p23. Different studies have shown that XAP2 can enhance and repress AhR function, and *in vitro* p23 has been observed to enhance DNA binding of the AhR/ARNT heterodimer. Previously, in an attempt to further understand the role of XAP2 in regulating mouse AhR function a point mutation was introduced in tyrosine 408 changing the residue to alanine. In COS-1 cells this mutation eliminated AhR binding to endogenous levels of XAP2 and showed reduced binding to coexpressed XAP2. Additionally, in AhR regulated luciferase assays this point mutation resulted in a receptor that displayed higher transcriptional activity compared to the non-mutant, and was unable to be repressed with the coexpression of XAP2. Finally, introduction of the Y408A mutation into an AhR-YFP construct showed that that unlike the non-mutant receptor the Y408A mutation renders the AhR incapable of the being redistributed to the cytoplasm when XAP2 is coexpressed. Recent experiments performed using this mutant receptor reveal that increasing transient expression of XAP2 in COS-1 cells reduces the amount of p23 present in the unliganded AhR complex, while having little or no effect on p23 binding in the Y408A mutant receptor complex. Additionally, in the absence of AhR expression p23 association with hsp90 does not appear to be altered by increasing amounts of XAP2. Since p23 is currently believed to be a positive acting factor in AhR function, its dissociation from the unliganded receptor complex in the presence of increased levels of XAP2 provides a mechanistic explanation of how XAP2 expression in COS-1 cells results in repressed AhR transcriptional activity.

598 MODULATION OF ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR ACTIVITY & PHOSPHORYLATION STATUS BY PKCε.

I. A. Murray[1], M. S. Denison[2] and G. H. Perdew[1]. *[1]Vet. Science, Pennsylvania State University, University Park, PA and [2]Environmental Toxicology, Uuniversity of California, Davis, CA.*

Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT), a member of the basic Helix-Loop-Helix/PAS transcription factor family, has been widely reported as the obligate dimerization partner required for the transcriptional activities of both the Aryl Hydrocarbon Receptor (AhR) and Hypoxia-Inducible Factor-1alpha (HIF-1α). Transfection of hepa1c1c7 cells with a constitutively active mutant of Protein Kinase C ε (PKCε) enhanced TCDD-dependent induction of an AhR-dependent reporter gene above that observed with wild type PKCε, no enhancement was with PKCα. Transient transfection/reporter assays in Cos-1 cells demonstrated that ARNT is transcriptionally active in the context of a homodimer; stimulating E-box mediated transcription (1.8-fold). Furthermore, the activity of ARNT is potentiated (4-fold) by phosphorylation through co-transfection with the novel calcium-independent PKCε and further enhanced (20-fold) with a constitutively active mutant of PKCε, an effect not observed with PKCα. We report through immunoprecipitation studies with exogenously expressed human ARNT (hARNT) that the phosphorylation status of hARNT increases (17-fold) in the presence of active PKCε relative to control. PKCα induces a moderate (6-fold) enhancement of phosphorylation status. Coimmunoprecipitation studies reveal that hARNT physically interacts with endogenous PKCε. Using phospho-amino acid analysis, we demonstrate that phosphorylation of hARNT occurs exclusively at serine residues. 2D tryptic phospho-peptide mapping has revealed at least three hARNT tryptic peptides, which are predominantly phosphorylated in response to activated PKCε exposure. Phospho-peptide mapping in conjunction with MADLI-TOF mass spectrometry and radiometric Edman sequencing will allow the identification of the precise serine residues modified in response to PKCε. These data suggest an important role for phosphorylation in the modulation of ARNT activity and that PKCε is involved in this regulation.

599 A POSSIBLE ROLE FOR THE MAP KINASES IN DIOXIN-INDUCED ARYL HYDROCARBON RECEPTOR PHOSPHORYLATION.

Z. Tan, A. Puga and Y. Xia. *Department of Environmental Health, University of Cincinnati, Cincinnati, OH.*

2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure causes the induction of mitogen-activated protein (MAP) kinases involved in aryl hydrocarbon receptor (AHR) activation and up-regulation of gene expression. Post-translational modification of AHR is known to modulate its activities as a transcription factor, but a direct connection between specific protein modifications, such as phosphorylation, and receptor activation has yet to be established. The objective of the present studies was to characterize the phosphorylation status of the Ah receptor upon ligand activation and to determine the involvement of MAP kinases in the activation process. We find that TCDD treatment of mouse hepatoma Hepa-1 cells causes a rapid induction of AHR phosphorylation at tyrosine and threonine residues. Two-dimensional analyses of the AHR protein immunoprecipitated from control and TCDD-treated cells show isoelectric point changes corresponding to charge differences consistent with receptor phosphorylation caused by TCDD exposure. To determine whether the Extracellular signal Regulated Kinase (ERK) MAP kinase directly catalyzes AHR phosphorylation, we measured AHR phosphorylation by active-ERK in an *in vitro* kinase assay. We find that ERK is able to phosphorylate

AHR. MALDI-TOF analyses of the phosphorylated AHR reveal two potential ERK phosphorylation sites in the PAS domain of the receptor, in a sequence evolutionarily conserved. Our studies suggest that AHR phosphorylation by MAP kinases is a likely complement to TCDD regulation of receptor activity. *(Supported by NIH ES06273, NIH ES10807, NIH 11798, ES NIH P30-ES06096 and grants from the Philip Morris University.S.A).*

600 ACTIVATION OF THE AH RECEPTOR CAN PROMOTE BENZO(A)PYRENE-7, 8-DIHYDRODIOL INDUCED APOPTOSIS IN THE ABSENCE OF MAP KINASE ERK1/2 ACTIVITY.

S. Chen, T. Operana, J. Bonzo, N. Nguyen and R. H. Tukey. *Pharmacology, University of California, San Diego, La Jolla, CA.*

Exposure of hepatoma cells to B[a]P-7, 8-dihydrodiol activates the Ah receptor (AhR) resulting in oxidative metabolism to B[a]P-7, 8-dihydrodiol epoxide (BPDE-2) and cell death. We have demonstrated that inhibition of MAP kinase signaling through ERK1/2 and p38 by BPDE-2 blocks cellular apoptosis. In contrast, inhibition of MEK1/2 by U0126 synergizes apoptosis when cells are treated with B[a]P-7, 8-dihydrodiol. To elucidate the role of U0126 in B[a]P-7, 8-dihydrodiol induced apoptosis, studies were carried out to examine the functional properties of the AhR. U0126 was shown to activate the AhR, leading to nuclear translocation and induction of *CYP1A1* transcription. However, in comparison to TCDD activation of the AhR, we observed dramatic differences in the cellular properties of the AhR. As previously observed by others, TCDD leads to a dose and time dependent reduction in the abundance of total cellular AhR, a property that is linked to ubiquitination. In contrast, AhR activation by U0126 is followed by a significant increase in total cellular AhR, which is also apparent by enhanced accumulation of nuclear AhR. The increase in cellular AhR is dependent upon protein synthesis, since treatment of cells with cycloheximide blocks U0126 accumulation of total cellular AhR with no apparent effect on nuclear translocation. Thus, AhR accumulation results from an imbalance of synthesis and degradation. Since the inhibition of MAP kinases correlates with a reduction in BPDE-2 induced apoptosis, these results indicate that B[a]P-7, 8-dihyrodiol induced apoptosis in the absence of functional MAP kinases ERK1/2 is linked to AhR activation (Supported by USPHS grant ES10337).

601 A NOVEL MECHANISM FOR REGULATION OF CHOLESTEROL BIOSYNTHESIS.

Q. Tan[1], S. Ke[1], M. A. Gallo[2] and Y. Tian[1]. *[1]Vet. Physiology and Pharmacology, Texas A&M University, College Station, TX and [2]Environmental & Community Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

Aryl hydrocarbon receptor (AhR) complex consists of the ligand-binding subunit and AhR nuclear translocator (ARNT) which is also known as the hypoxia inducible factor 1β. The AhR complex has been well characterized for its roles in mediating toxic effects of dioxin and related environmental contaminants. However, these evolutionarily conserved proteins also play important roles in normal physiological responses that are beginning to be understood. Recent studies from our laboratory revealed a potential novel mechanism for regulation of cholesterol biosynthesis: activation of AhR by prototype agonist 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) suppressed the HMG-CoA reductase mRNA levels in mouse liver cells (Hepa1c1c7) in a time and dose-dependent manner as determined by real-time RT-PCR. In mice (C3H/HeNCr, 3 wk old, 14-24 g), TCDD treatment (20 and 100 μg) caused suppression of the serum cholesterol levels. Using chromatin immunoprecipitation assay, we found that binding of the sterol response element binding protein 1a (SREBP1a) to the HMG-CoA reductase promoter region was markedly inhibited by TCDD treatment (10 nM, 2 hr) in Hepa1c1c7 cells. Furthermore, in collaboration with J. A, Gustafsson (Karolinska Institute, Sweden), we found that ARNT interacts with sterol response element binding protein (SREBP1a) in two hybrid assays. These results suggest a novel mechanism for regulation of cholesterol biosynthesis by AhR and its agonists such as TCDD and indole-3-carbinol (I3C), the former is an environmental contaminant and the later is a plant compound, which has documented beneficial effects, such as the hypocholesterolemic effects. Supported in part by NIEHS grants ES09859, ES09106 and a grant from the American Heart Association (0355131Y).

602 MODULATION OF ARYL HYDROCARBON RECEPTOR-REGULATED GENES BY TUMOR NECROSIS FACTOR-α AND LIPOPOLYSACCHARIDES BY AHR-DEPENDENT MECHANISMS.

N. Gharavi and A. O. El-Kadi. *Faculty of Pharmacy, University of Alberta, Edmonton, AB, Canada.*

Little is known about the mechanisms involved in the modulation of aryl hydrocarbon receptor (AHR)-regulated genes during pathophysiological conditions such as inflammation. In the present study the effect of TNF-α and lipopolysaccharides (LPS) on the constitutive and inducible expression of the AHR-regulated genes: cytochrome P450 1a1 (cyp1a1), glutathione S-transferase Ya (GST Ya), and NAD(P)H:quinone oxidoreductase (QOR) were determined. Murine hepatoma Hepa 1c1c7 (WT), AHR-deficient (C12) and AHR nuclear translocator protein (ARNT)-deficient (C4) cells were incubated with recombinant murine TNF-α (1, 5 and 10 ng/ml) or LPS (1 and 5μg/ml) with or without β-naphthoflavone (βNF, 10μM). We found that TNF-α and LPS had no apparent cellular toxicity effects on WT, C12 and C4 cells at all concentrations tested. In WT cells, in the absence of βNF, TNF-α and LPS dose-dependently inhibited the constitutive activities and mRNA levels of cyp1a1, GST Ya and QOR. QOR activity was significantly induced by TNF-α and LPS in C12 and C4 cells respectively, while other enzymes remained unchanged. Treatment of WT cells with βNF alone resulted in significant increase in the cyp1a1, GST Ya and QOR activities, as well as their mRNAs levels. When WT cells were treated with TNF-α or LPS with βNF, the βNF-mediated induction of cyp1a1, GST Ya and QOR at their activities and mRNA levels were significantly reduced in a dose-dependent manner. There were no changes in C12 and C4 cells. In addition, a significant increase in reactive oxygen species (ROS) was observed in WT, C12 and C4 cells with TNF-α and LPS treatment. These results show that both TNF-α and LPS strongly repress the constitutive expression and inducibility of cyp1a1, GST Ya and QOR in WT but not in AHR- or ARNT-deficient cells. Therefore, down-regulation of AHR-regulated genes by inflammation are dependent on the presence of both heterodimeric transcription factors, AHR and ARNT. Furthermore, ROS may directly or indirectly be involved in down-regulation of AHR-regulated genes.

603 AFFYMETRIX MICROARRAY AND REAL-TIME PCR ANALYSIS OF BENZO(A)PYRENE INDUCE CHANGES IN GENE EXPRESSION IN RAT LIVER.

A. University. N'jai, A. Jelaso, C. Ide and J. Means. *Chemistry/Environmental Institute, Western Michigan University, Kalamazoo, MI.*

Benzo(a)pyrene (BaP), an environmental contaminant produced from combustion, is associated with a variety of adverse health effects, including cancer. In order to elucidate the BaP-induced health effects prior to cell transformation, we used real-time PCR and Affymetrix microarray analysis to measure gene expression changes associated with early molecular mechanisms occurring in response to BaP dietary exposures. Preliminary results with our affymetrix genechips show regulation of several gene families. For instance, after two weeks, exposure to 0.01mg/g-diet increased expression (>=3-fold) of 266 genes and decreased expression of 66 genes vs. controls, at 0.1mg/g-diet increased expression of 53 genes and decreased expression of 79 genes. After 3-months, a dose-dependent trend occurred in genes that were up-regulated, 28 genes were increased (>=3-fold) in rats exposed at 0.01mg/g-diet vs. controls, 64 genes in rats at 0.1mg/g-diet and 102 genes in rats at 1.0mg/g-diet. Similarly, a large number of genes were down-regulated after exposure to BaP for 3 months (185 genes decreased at 0.01mg/g-diet; 30 in rats exposed to 0.1mg/g-diet; 76 in rats exposed to 1.0mg/g-diet). Our affymetrix data revealed changes in gene expression of several biochemical pathways such as cyp450 detoxification, redox, metabolism, steroid hormone, transcription regulators and so on. We used our affymetrix data as a screen to select genes for further quantitative analysis using real-time PCR. Gene candidates selected for real-time PCR include Cyp1a1, Cyp1a2, glutathione-s-transferase, glutathione peroxidase, NADPH cytochrome p450 reductase, Zinc finger protein, protein Kinase C, ras-related protein p23, c-myc and aldehyde dehydogenase. The real-time PCR results were used to both validate and to provide quantitative estimate of our Affymetrix genechip data. Also, analysis of BaP metabolites in rat liver samples were carried using an LC/MS method developed in our lab.

604 COMPARATIVE ANALYSIS OF DIOXIN REGULATORY ELEMENTS IN HUMAN, MOUSE AND RAT GENOMIC SEQUENCES.

Y. Sun[1], D. R. Boverhof[1], M. R. Fielden[2] and T. R. Zacharewski[1]. *[1]Biochemistry & Molecular Biology, National Food Safety & Toxicology Center, Institute for Environmental Toxicology, Michigan State University, East Lansing, MI and [2]Iconix Pharmaceuticals, Mountain View, CA.*

Dioxin is a highly toxic compound produced as a by-product of modern industry and causes a wide range of health problems. Most, if not all, of the effects elicited by dioxin and related compounds are mediated *via* ligand activation of the aryl hydrocarbon receptor which forms a heterodimer with the AhR nuclear translocator. This complex modulates gene expression through specific binding to dioxin response elements (DREs) in the regulatory regions of responsive genes. The purpose of this study is to identify conserved DREs through the analysis of available human, mouse and rat genomic sequences, corresponding to nucleotide RefSeq accessions (17882, 11697, and 3896 promoters respectively), obtained from the UCSC Genome Browser (http://genome.ucsc.edu/). Thirteen bona fide DRE sequences, each included the 5bp core sequence (GCGTG) and the 7bp flanking sequences,

were used to establish a position weight matrix. Human, mouse and rat genomic sequences were scanned for the presence of DREs in both upstream and transcribed regions. Identified DREs were disproportionately distributed in the upstream regulatory regions, close to transcription start site, in all three species. Conserved DREs were identified in 365 out of 8872 orthologous human and mouse genes, 140 out of 3379 orthologous human and rat genes, 133 out of 2840 orthologous rat and mouse genes, and 48 out of 2437 orthologous genes across the three species. These findings corroborate many previously published reports, including the well characterized dioxin responsive genes Cyp1a1 and Cyp1b1. This in silico approach to identifying DREs will complement ongoing microarray studies of dioxin-mediated alterations in gene expression and suggests that dioxin-mediated effects on gene expression may not be well conserved across species, which could have significant implications towards human risk assessment. This work was supported by National Institute of Health Grant ES012245

605 TRANSCRIPTIONAL PROFILES FOLLOWING LIGAND-ACTIVATED AHR SIGNALING IN THE DEVELOPING KIDNEY: A ROLE FOR WT1 AND IGF SIGNALING.

M. Falahatpisheh[1, 2], C. D. Johnson[1, 2] and K. S. Ramos[1, 2]. [1]*Biochemistry and Molecular Biology, University of Louisville, Louisville, KY and* [2]*Center for Genetics and Molecular Medicine, University of Louisville, Louisville, KY.*

Ligand-activated aryl hydrocarbon receptor (Ahr) signaling induces accumulation of (–)KTS splice variants of the Wilms' tumor suppressor gene (*Wt-1*) and inhibits nephrogenesis. The present studies were conducted to define global patterns of gene expression following ligand-activated Ahr signaling during the course of kidney morphogenesis *in vitro*. In these experiments, organ cultures of E11.5 metanephric blastema from C57BL/6J mice were treated for four consecutive days with 3 uM benzo(a)pyrene (BaP), a hydrocarbon ligand of the Ahr. Genes involved in cellular differentiation, proliferation, apoptosis, cell cycle progression, stress signaling, matrix remodeling and transcriptional control were identified as Ahr targets during the course of disrupted nephrogenesis. Many of these genes are involved in nephrogenesis (glial derived neurotrophic factor, frizzled receptor, insulin-like growth factor (*Igf*), insulin-like growth factor binding protein (IgfBP), syndecan, and laminin), while others, such as *Sry*, oncostatin M, pinin, GATA-3, CCAAT/enhancer-binding protein, *Sox-18*, *Nrf-2*, FBG-MuSV, *Gro1*, cyclin G and fibulin are novel targets of developmental dysregulation. Because the Igf signaling pathway is pivotal in renal organogenesis *via* activation of mitogen activated protein kinase (MAPK) signaling, further studies were conducted to examine protein kinase cascades during the course of nephrogenesis. Activation of Ahr signaling altered the expression of extracellular signal-regulated protein kinase. Immunostaining for PCNA, TUNEL and Apaf showed massive hyperplasia coupled to increased apoptosis in distinct regions of the developing kidney. Collectively, these results suggest that altered *Wt1* splicing following ligand-activated Ahr regulates differentiation and apoptosis of mesenchymal cells through the Igf signaling pathway. (Supported by NIH grants ES04917 and ES09106). –

606 MECHANISMS OF CARDIOVASCULAR TOXICITY BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN AND RELATED POLYHALOGENATED AROMATIC HYDROCARBONS.

M. K. Walker. *College of Pharmacy, University of New Mexico, Albuquerque, NM.*

Previously, the cardiovascular system has not been considered to be a primary target of toxicity induced by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and other structurally related polyhalogenated aromatic hydrocarbons (PHAHs), particularly in mammalian species. However, considerable research in the past 5-10 years has demonstrated that TCDD and related PHAHs exhibit significant impacts on both the developing and adult cardiovascular system and these effects are apparent across vertebrate classes, including piscine, a*via*n, and mammalian species. Furthermore, occupational exposure of humans to TCDD and related PHAHs has been linked to an increased risk of mortality from ischemic heart disease, demonstrating that humans are not impervious to the cardiovascular risk posed by TCDD/PHAH exposure. This symposium will cover the recent advances in understanding the mechanisms underlying TCDD/PHAH-induced cardiovascular toxicity. The speakers will present data on the effects of TCDD/PHAHs on both cardiac and vascular development and function, providing unique comparisons across vertebrate classes.

607 TCDD CARDIOTOXICITY IN DEVELOPING ZEBRAFISH.

W. Heideman, D. K. Sieprawska and R. E. Peterson. *Molecular and Environmental Toxicology and School of Pharmacy, University of Wisconsin, Madison, WI.*

2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) causes cardiac defects in developing fish, birds, and mammals. While it is well known that TCDD is an aryl hydrocarbon receptor (AHR) agonist, and that AHR activation mediates toxic effects of TCDD, we know little about the mechanism by which AHR causes cardiotoxicity, or the role that AHR plays in normal heart development. The zebrafish (*Danio rerio*) has emerged as an attractive vertebrate model for studying cardiovascular development. In addition to ease of observation, zebrafish do not require a functional circulatory system during the first week of life. This allows the study of cardiac defects that would be immediately lethal in other organisms. TCDD exposure (1ppb) immediately after fertilization does not appear to affect basic heart morphogenesis: atrium, ventricle, bulbus arteriosis and sinus venosus are all formed in TCDD treated fish. Thereafter, TCDD exposure produces reductions in heart rate, cardiac output, and heart size. The change in heart morphology caused by TCDD is characterized by a pattern in which the atrium and ventricle are stretched out with altered looping, and the sinus venosus fails to migrate dorsally as the heart matures. TCDD treated fish have hearts with thin walls and reduced myocardium. Morpholino knockdown of zfAHR2 expression indicates that the cardiotoxic effects of TCDD are mediated by zfAHR2. The earliest signs of carditoxicity are observed before the formation of pericardial and yolk sac edema. Furthermore, while osmotic support with mannitol can relieve the edema produced by TCDD, mannitol does not block the cardiotoxicity. These results indicate that the cardiotoxic effects of TCDD in zebrafish larvae are not secondary to edema formation. The ability to manipulate the AHR/ARNT pathway in developing zebrafish through transgenic, morpholino, and genomic approaches provides an excellent opportunity for studying both AHR-mediated TCDD cardiotoxicity and the role of the AHR/ARNT pathway in normal heart development. (Supported by UW Sea Grant)

608 FETAL DIOXIN EXPOSURE INHIBITS CORONARY VASCULOGENESIS. A POTENTIAL RISK FACTOR FOR ISCHEMIC HEART DISEASE.

M. K. Walker. *College of Pharmacy, University of New Mexico, Albuquerque, NM.*

Epidemiology studies show a strong association between exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and structurally related polyhalogenated aromatic hydrocarbon and an increased risk of mortality from ischemic heart disease (IHD); however, the potential contribution of fetal exposure to these chemicals to IHD risk is unknown. IHD mortality inversely correlates with the ability to induce vascular endothelial growth factor-A (VEGF-A) and stimulate coronary collateral growth. Since coronary collateral growth recapitulates fetal coronary development, we studied the effects of fetal TCDD exposure on (1) cardiac VEGF-A expression and endothelial signaling, (2) coronary vascular development, and (3) myocardial ischemia post natally. In the a*via*n embryo, TCDD reduces coronary vascular outgrowth by 50% and this reduction is associated with a 50 and 65% decrease in cardiac VEGF-A mRNA and protein, respectively, and reduced activation of VEGF receptor 2. The cause-and-effect relationship between reduced VEGF-A and reduced coronary vasculogenesis is revealed by the ability of exogenous VEGF-A and hypoxia-stimulated endogenous VEGF-A to rescue the reduced vascularization, and that the rescue is blocked by VEGF-A neutralizing antibody. Further, VEGF-A neutralizing antibody alone reduces coronary vasculogenesis in control hearts by 50%, but fails to effect coronary vascular outgrowth from TCDD hearts, suggesting that the VEGF-A component of coronary vasculogenesis is impaired significantly by TCDD. In the murine fetus, TCDD reduces cardiac VEGF-A mRNA by 50% and leads to reduced coronary capillary density on postnatal day 21. In addition, fetal TCDD exposure increases the sensitivity to stress-induced myocardial ischemia after birth. While the mechanism by which TCDD reduces cardiac VEGF-A and alters VEGF-A signaling, leading to reduced coronary vascular development, remains to be fully elucidated, these studies suggest that TCDD exposure early in life may represent a previously unidentified risk factor for IHD. Supported by NIH R01 ES09804.

609 PROINFLAMMATORY MECHANISMS OF PCB TOXICITY IN THE VASCULAR ENDOTHELIUM.

B. Hennig[1], M. Toborek[1] and L. W. Robertson[2]. [1]*University of Kentucky, Lexington, KY and* [2]*University of Iowa, Iowa City, IA.*

There is now increasing evidence that exposure to polyhalogenated aromatic hydrocarbons can contribute to the development of inflammatory diseases such as atherosclerosis. Activation, chronic inflammation and dysfunction of the vascular endothelium are critical events in the initiation and acceleration of atherosclerotic lesion formation. Because of its constant exposure to environmental contaminants and other blood components, including prooxidants, the vascular endothelium is extremely vulnerable to chemical insult, as well as to necrotic and apoptotic injury. Our data demonstrate that coplanar polychlorinated biphenyls (PCBs), and possibly other aryl hydrocarbon receptor (AhR) ligands and environmental contaminants, can compromise normal functions of vascular endothelial cells by activating oxidative stress-sensitive signaling pathways and subsequent proinflammatory

events critical in atherosclerosis and cardiovascular toxicity. Our studies indicate that an increase in cellular oxidative stress and an imbalance in antioxidant status are critical events in PCB-mediated induction of inflammatory genes and endothelial cell dysfunction. Furthermore, we found that specific dietary fats (e.g, linoleic acid, the parent unsaturated fatty acid of the omega-6 family) can further increase endothelial dysfunction induced by selected PCBs, probably by contributing to oxidative stress and the production of toxic lipid metabolites such as leukotoxins. We also observed that antioxidant nutrients (such as vitamin E and dietary flavonoids) can protect against endothelial cell damage mediated by PCBs or polyunsaturated dietary fats by interfering with oxidative stress-sensitive and proinflammatory signaling pathways. Even though the concept that nutrition may modify or ameliorate the toxicity of environmental chemicals is provocative and warrants further study, the implications for human health could be significant. Supported in part by grants from NIH/NIEHS (ES 07380), and the Kentucky Agricultural Experimental Station.

610 EXPRESSION PROFILES OF CULTURED VASCULAR SMOOTH MUSCLE CELLS AND AORTA ARE WIDELY DIFFERENT, BUT SHOW COMMON RESPONSES TO DIOXIN EXPOSURE.

A. Puga, M. Sartor, M. Huang, J. Kerzee, Y. Wei, C. R. Tomlinson and M. Medvedovic. *Department of Environmental Health, University of Cincinnati, Cincinnati, OH.*

Environmental toxicants may play a role in the onset or progression of cardiovascular disease, the main cause of mortality and morbidity in industrialized countries. Many environmental agents, such as dioxin, may be risk factors because they alter gene expression and exacerbate an underlying disease through deregulation of critical genes. Expression profiling of vascular tissues may provide predictive information particularly useful during early stages of heart disease. Often, however, direct experimentation is unfeasible for logistic or ethical reasons. In these cases, extrapolation from cultured cells is necessary, although there is no assurance that cultured cells and live tissues share common global responses. We compared gene expression profiles of C57BL/6 mice aortas and cultured vascular smooth muscle cells from the same mice to determine if gene responses to dioxin exposure in the one would predict responses in the other. Using microarrays with sequences from 13, 400 genes, aorta tissues and vSMCs were found to differ in the expression of more than 4, 500 genes, many showing differences greater than 1000-fold. We used GenMAPP (Gene Microarray Pathway Profiler), a program to analyze microarray data on profiles representing biological pathways, to integrate Gene Ontology Project annotations and organize the data on the basis of known biological processes. The resulting GenMAPPs showed that many of the genes differentially expressed belonged to the same biological process or metabolic pathway and were coordinately deregulated. Notwithstanding, a subset of 20-30 genes were equally altered by dioxin exposure in both systems. Genes in this subset encode phase I and phase II detoxification enzymes as well as signal transduction kinases and phosphatases and proteins involved in cell cycle and DNA damage repair. We conclude that vSMCS can be used as aorta surrogates for the study of early responses to dioxin exposure provided that the analyses focus on this subset of predictive genes.

611 CARDIOVASCULAR CHANGES FOLLOWING CHRONIC RODENT EXPOSURE TO DIOXIN-LIKE COMPOUNDS.

M. P. Jokinen[4], N. J. Walker[2], D. M. Sells[5], A. E. Brix[2] and A. Nyska[2]. *[1]ETP, NIEHS, Research Triangle Park, NC, [2]NIEHS, Research Triangle Park, NC, [3]EPL, Research Triangle Park, NC, [4]Pathology Associates - A Charles River Company, Durham, NC and [5]Battelle Columbus, Columbus, OH.*

Polyhalogenated aromatic hydrocarbons (PHAHs) comprise a large class of compounds including polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), and polybrominated diphenyl ethers (PBDEs). Certain PCDDs, PCDFs and co-planar PCBs, have the ability to bind to the aryl hydrocarbon (Ah) receptor and exhibit biological actions similar to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and are commonly referred to as dioxin-like compounds (DLCs). Recent epidemiologic studies have linked dioxin exposure to increased mortality caused by ischemic heart disease (IHD). The Department of Health and Human Services, National Toxicology Program has recently conducted multiple 2-year lifetime rat bioassays to evaluate the chronic toxicity and carcinogenicity of dioxin-like compounds, structurally-related PCBs, and mixtures of these compounds. Chronic administration of dioxin-like compounds to female rats was associated with treatment-related increases in the incidence of cardiomyopathy, a degenerative myocardial change seen commonly in rats, and of chronic active arteritis. Chronic active arteritis occurred primarily in the mesentery and pancreas, although other tissues, including rectum, liver, heart, ovary, uterus, or glandular stomach in the PCB 126 (3, 3', 4, 4', 5-PCB) study, and liver or ovary in the TCDD study were affected in a few dosed animals. These studies indicate that the cardiovascular system is a target for dioxin exposure in the adult rat, exacerbating the incidence and severity of spontaneous cardiomyopathy and arteriopathy.

612 NEW DEVELOPMENTS IN OXIDATIVE PHOSPHOLIPID SIGNALING IN APOPTOSIS AND PHAGOCYTIC REGULATION OF INFLAMMATORY RESPONSE.

V. Kagan[2] and D. Jones[1]. *[1]Department of Biochemistry, Emory University, Atlanta, GA and [2]Environment and Occupational Health, University of Pittsburgh, Pittsburgh, PA.*

Apoptosis eliminates unwanted or irreparably damaged cells by orderly phagocytosis in the absence of inflammatory responses. Oxidative stress is one of the most common factors that induce apoptosis. In addition, apoptosis itself is often accompanied by the generation of reactive oxygen species (ROS) and oxidative stress, resulting from departure of cytochrome c (cyt c) from mitochondria and attendant disruption of electron transport with enhanced production of one-electron reduced oxygen intermediates. Until recently, it was not known whether this apoptosis-associated oxidative stress is a meaningless but unavoidable side effect or an important component of the final common pathway for apoptosis. Findings from several laboratories implicated ROS production and oxidative stress in the execution of apoptotic program *via* activation of two essential mechanisms: mitochondrial permeability transition pore and caspases. The latest discoveries indicate that oxidative modifications of two types of phospholipids are critically involved in the execution of apoptotic program. In mitochondria, oxidation of cardiolipin loosens its association with cyt c and facilitates release of the latter into the cytosol, the central event in intrinsic apoptosis. In the cytosol, cyt c plays a redox-dependent catalytic role in selective oxidation of phosphatidylserine (PS) a signaling molecule of the pathway culminating in recognition of apoptotic cells by phagocytes. PS-dependent signaling involves externalization of PS on the outer leaflet of plasma membrane, its interactions with specialized adapter molecules, and tethering to specific receptor(s) on the surface of phagocytes. Cyt c-catalyzed PS oxidation in the cytosolic leaflet of plasma membrane is essential for both its externalization and recognition by macrophages. These exciting new developments in oxidative control of apoptosis, clearance of apoptotic cells, and regulation of inflammatory response will be discussed by leading researchers of the field.

613 REACTIVE OXYGEN SPECIES IN ACTIVATION AND EXECUTION OF APOPTOSIS.

D. Jones. *Department of Biochemistry, Emory University, Atlanta, GA.*

Early studies revealed that thiol antioxidants such as N-acetylcysteine are effective inhibitors of apoptosis in many systems, suggesting that oxidative reactions serve a central function in this conserved pathway. However, other studies provided evidence that oxidative events are not essential for initiation of apoptosis. This presentation will describe accumulating mechanistic data on apoptosis signaling which show that key oxidative events occur at both activation and execution steps. An increase in reactive oxygen species can be detected in many systems in early in activation of apoptosis. Oxidation of thioredoxin allows activation of apoptosis signal-regulating kinase 1 (ASK-1). Oxidants activate the mitochondrial permeability transition in mitochondria with associated release of cytochrome c and other apoptogenic factors. Over-expression of antioxidant systems in mitochondria, including mitochondrial thioredoxin, glutathione reductase and superoxide dismutase, result in protection against apoptosis. Opposing models of apoptosis activation by lipid-derived oxidation products and direct oxidation of critical apoptosis-signaling proteins will be presented. Evidence will also be presented to describe a switch in electron transport, which occurs upon release of cytochrome c from mitochondria. This switch results in inhibition of normal respiration, increased ROS generation and activation of downstream oxidation reactions, which function in execution of apoptosis. A comparison of possible mechanisms indicates that lipid oxidation processes at least provide a functional redundancy to protein oxidation in signaling the terminal phases of apoptosis and may, in fact, be the key components signaling the final phagocytic elimination of apoptotic bodies.

614 LIPOCALINS AND APOPTOSIS.

J. P. Kehrer. *Pharmacology and Toxicology, The University of Texas at Austin, Austin, TX.*

In recent years, studies on the cancer preventing and treating activities of aspirin, indomethacin and related cyclooxygenase (COX) inhibitors have indicated that the anticancer/apoptosis-inducing activity of these agents is in many systems independent of COX. Our work on the mechanism(s) by which inhibitors of lipoxygenase (LOX) enzymes induce apoptosis has similarly shown independence from LOX inhibition. Specifically, a number of LOX inhibitors, as well as MK886, a structural analog of indomethacin that inhibits the activity of 5-LOX by binding to the 5-

LOX-activating protein (FLAP), have strong pro-apoptotic activity that is not mediated through the inhibition of FLAP, LOX or COX. Changes in polyunsaturated fatty acid protein binding, possibly leading to fatty acid oxidation may be involved. An interesting and diverse family of lipid binding proteins are the lipocalins. Their biological functions are not fully understood. However, one member of this family, murine 24p3, binds fatty acids and retinoids, enhances the activity of the c-AMP-dependent protein kinase, delivers iron to the cytoplasm, and plays a crucial role in IL3-deprivation-induced apoptosis. This latter activity suggested that apoptosis-inducing xenobiotics, particularly those affecting lipid pathways, might also function *via* 24p3. We have found that 24p3 is profoundly induced by MK886, as well as related compounds. The extent of apoptosis closely parallels this induction. MK886 and related apoptosis-inducing xenobiotics also upregulate the human analog of 24p3, neutral gelatinase associated lipocalin (NGAL). Importantly, over- and under-expressing 24p3/NGAL levels by transfection and antisense strategies leads to changes in levels of apoptosis. These data suggest 24p3/NGAL represent a new upstream signaling pathway for apoptosis regulation/initiation. (Supported by CA83701 and NIEHS Center Grant ES07784.)

615 PHOSPHATIDYLSERINE OXIDATION DURING INTRINSIC AND EXTRINSIC APOPTOSIS: CATALYTIC AND SIGNALING MECHANISMS.

V. E. Kagan. *Environmental and occupational Health, University of Pittsburgh, Pittsburgh, PA.*

Release of cytochrome c from mitochondria is central to successful progression and completion of intrinsic apoptotic program. Evidence will be presented for a new role for cytochrome c in the cytosol as a competent redox catalyst of phosphatidylserine (PS) peroxidation on the inner leaflet of plasma membrane. Experimental data will be shown to illustrate an important role of cytochrome c-catalyzed PS peroxidation in trans-membrane export of PS and its externalization on the outer surface of apoptotic cells. Because externalized PS acts as a unique eat-me-signal for phagocyte clearance of apoptotic cells, participation of cytochrome c in apoptosis is not limited to its activation of caspases but includes PS signaling cascades. Catalytic redox mechanisms of cytochrome c, based on its specific interactions with anionic PS leading to activation of its peroxidase activity and responsible for selective PS oxidation, will be discussed. Because extrinsic apoptotic pathways in type II cells include disruption of mitochondria and release of cytochrome c, we hypothesize that this additional cytochrome c-dependent loop is utilized not only for enhanced caspase-8 activation but it is also essential for PS-dependent signaling. Finally, interruption of cytochrome c-catalyzed PS oxidation by different antioxidant enzymes and free radical scavengers will be considered in lieu of their potential effects on PS-dependent signaling pathways, clearance of apoptotic cells by phagocytes. Supported by NIH HL 70755.

616 BRIDGING PROTEINS IN LIPID DIRECTED PHAGOCYTOSIS.

A. Schroit and K. Balasubramanian. *Cancer Biology, The University of Texas, M. D. Anderson Cancer Center, Houston, TX.* Sponsor: V. Kagan.

Many studies have established the unifying concept that plasma membrane phospholipids are asymmetrically distributed across membrane bilayers. The majority of the aminophospholipids reside in the cell's inner membrane leaflet and most of the choline-containing phospholipids are in the outer membrane leaflet. If cells fail to maintain this asymmetry, phosphatidylserine (PS) appears at the cell surface and marks the cell for disposal. Analysis of proteins recovered from PS-containing liposomes injected into mice showed that beta-2-glycoprotien 1 (β2GP1), a normal plasma protein, bound to the vesicles. This raised the possibility that B2GP1 participates in the recognition and phagocytosis of PS-expressing apoptoic cells to phagocytes. *In vitro* experiments showed that β2GP1 dramatically enhanced the uptake of PS-containing vesicles through a specific macrophage receptor that binds the bridging protein only after first binding to its lipid ligand. Additional *in vivo* experiments indicated that cells undergoing Fas-induced apoptosis bind endogenous β2GP1. These data support the concept that β2GP1 marks apoptotic and dying cells for clearnace by the reticuloendothelial system.

617 PROGRAMMED CELL CLEARANCE: STUDIES ON THE MECHANISM AND IMPORTANCE OF PHOSPHATIDYLSERINE EXPOSURE AND PLASMA MEMBRANE BLEBBING DURING FAS-TRIGGERED APOPTOSIS.

B. Fadeel. *Institute of Environmental Medicine, Division of Toxicology, Karolinska Institutet, Stockholm, Sweden.* Sponsor: V. Kagan.

Apoptosis is essential during development and for the maintenance of tissue homeostasis in the adult organism. Considerable efforts have been made in recent years to elucidate the molecular mechanisms that govern this mode of cellular demise; however, the process of programmed cell clearance, i.e. the subsequent recognition and removal of apoptotic corpses by neighboring phagocytes, has received less attention. The current presentation will address studies on the mechanism of programmed cell clearance in a model of death receptor (Fas/APO-1/CD95)-induced apoptosis. Fas-triggering results in the selective oxidation and externalization of phosphatidyserine (PS) on the surface of the apoptotic cell, and oxidized as well as non-oxidized species of PS mediate recognition and engulfment of effete cells by macrophages. Moreover, recent studies indicate that plasma membrane blebbing (zeiosis) and the concomitant aggregation of PS molecules on the cell surface is an important determinant of phagocytic clearance of Fas-triggered cells. The co-expression on the cell surface of other "eat me" signals, such as annexin I, and the opsonization of apoptotic cells by soluble factors, including milk-fat globule epidermal growth factor-8 (MFG-E8), may also serve to facilitate macrophage clearance. Macrophages, in turn, employ an array of receptors that mediate tethering and ingestion of dying cells. Taken together, these specific interactions between phagocyte and prey ensure that inflammation and tissue scarring due to secondary necrosis and lysis of neglected apoptotic cells is limited.

618 PUBLIC TOXICOGENOMIC DATABASE RESOURCES AND THEIR ROLE IN THE TOXICOLOGY COMMUNITY.

W. B. Mattes[1] and S. D. pettit[2]. *[1]Pfizer, Kalamazoo, MI and [2]Health and Environmental Sciences Institute, ILSI, Washington, DC.*

Over the last several years, the volume of microarray data generated in studies of toxicology has been steadily increasing. Likewise, the expectations of the toxicology community with respect to the information buried in this volume of data has also been increasing, and with those expectations, an appreciation for more sophisticated approaches to data housing, sharing, and analysis. Thus, public microarray databases are now considering the need to include appropriate biological context (i.e., linked toxicology data) in conjunction with array data. However, the ultimate scientific value and utility of public toxicogenomic databases, such as those developed by HESI-EBI and NIEHS-NCT will depend upon a clear assessment of how the community (both public and private sector) hopes to utilize these resources. This workshop will include presentations about the status of, challenges in, and expectations for current public toxicogenomic database development efforts by leading developers. Discussions will cover issues around whether these databases are or will meet the needs and interests of the toxicology community.

619 DEVELOPMENT OF TOXICOGENOMIC GENE EXPRESSION DATA INFRASTRUCTURE.

S. Sansone, S. Contrino, M. Shojatalab, N. Abeygunawardena, University. Sarkans, G. Garcia Lara, H. Parkinson, P. Rocca-Serra and A. Brazma. *Microarray Informatics, EMBL-EBI, Cambridge, United Kingdom.* Sponsor: S. Pettit.

The ArrayExpress infrastructure[1] for microarray gene expression data has been established at the EMBL-EBI. ArrayExpress provides the community with easy access to high quality gene expression data and associated metadata (e.g. histopathology, clinical chemistry) in a standard format, serves as repository for data in publications, and facilitates the sharing of array designs and experimental protocols. The meaningful exchange of information is supported by the use of standard contextual information (MIAME and **MIAME/Toxicology**)[2], a standard data exchange format (MAGE-ML)[2] and a team of curators. ArrayExpress infrastructure consists of the database itself, a high-throughput data submission route *via* ftp in MAGE-ML format or *via* a web-based annotation/submission tool MIAMExpress, an online database query interface, the web-based analysis tool Expression Profiler and query optimised data warehouse. Following an agreement with ILSI HESI[3], the infrastructure has undergone further development according to the specifics of toxicogenomics experiments. To integrated gene expression data with conventional toxicology metadata, a toxicogenomics-specific version of the annotation/submission tool has been developed. **Toxicology-MIAMExpress** enforces the use of controlled vocabularies and complies with MIAME/Toxicology, an effort initiated by the EBI, NIEHS-NCT and ILSI-HESI to fulfill the need of capturing and exchanging toxicogenomics and similarly pharmacogenomics, chemogenomics experiments. The use of common terminologies, the consistent annotation and a standard data exchange format bewteen ArrayExpress and Chemical Effects in Biological Systems (CEBS), the toxicogenomics knowledgebase at NCT, are the first steps towards the establishment of an international infrastructure for toxicogenomics data. 1=Brazma *et al.* ArrayExpress a Public Repository for Microarray Gene Expression Data at the EBI. Nucleic Acids Res. 31(1):68-71(2003) 2=MIAME and MIAME/Toxicology: http://www.mged.org 3=Toxicogenomic project: http://www.ebi.ac.uk/microarray/Projects/ilsi/index.html

620 THE CHEMICAL EFFECTS IN BIOLOGICAL SYSTEMS KNOWLEDGE BASE.

M. Waters[1], P. Bushel[1], W. Eastin[1], S. Gustafson[2], P. Hurban[3], A. Merrick[1], G. Nehls[1], J. Selkirk[1], S. Stasiewicz[1], N. Stegman[1], K. Tomer[1], H. Wan[1], B. Weis[1], J. Yost[2], S. Xirasagar[2] and R. Tennant[1]. *[1]NIEHS, Research Triangle Park, NC, [2]SAIC, Germantown, MD and [3]Paradigm Genetics, Research Triangle Park, NC.*

The Chemical Effects in Biological Systems (CEBS) knowledge base is under development as a public information resource combining datasets from transcriptomics, proteomics, metabonomics, and conventional toxicology for environmental exposures. CEBS will facilitate data integration across multiple sources, centers, and technology platforms. CEBS is designed to meet the information needs of systems toxicology, involving study of chemical or stressor perturbations, monitoring changes in molecular expression, and iteratively integrating biological response data to describe the functioning organism. International database content guidelines, minimal information about a microarray experiment, MIAME and MIAME/Toxicology, and microarray gene expression markup language, MAGE-ML, are used to assemble and exchange high quality datasets with the goal of creating a system of predictive toxicology. Toxicogenomics experiments performed using validated NCT and NTP methodologies are captured in their entirety *via* a unique extended implementation of the MAGE-OM object model. Dictionaries and metadata will introduce and guide interpretation of publicly available datasets using open source statistical analysis tools and scripted data workflows. CEBS creates the capability to relationally link toxicogenomics data to animal effects data so as to evaluate global changes in molecular expression as a function of dose, time, and target cell type. By analogy to GenBank, CEBS will support global sequence-based query using probe sequence of differentially expressed genes or analytically determined proteins. CEBS also supports conventional query for compound/ structure/class, toxic/pathologic effects, gene annotation, gene groups, pathways and phenotypes. CEBS links to the NTP Toxicology Database Management System and the NTP Studies Database leveraging these OracleTM systems in experimental design and interpretation of phenotypes. CEBS will serve the scientific community in both discovery and hypothesis-driven research.

621 dbZach - AN INTRALABORATY TOXICOGENOMIC SUPPORTIVE DATABASE.

T. Zacharewski. *Biochemistry & Molecular Biology, National Food Safety & Toxicology Center, and Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.*

In order to fully assess the adverse effects of subchronic and chronic exposure to drugs, chemicals, natural products and their mixtures, an integrated systematic understanding of the molecular, cellular and physiological effects is required within the context of a whole organism its genome, proteome and metabonome. Consequently, bioinformatics and global assessment technologies, including genomics, proteomics, and metabonomics, are being incorporated into existing mechanistic and safety assessment paradigms. These technologies generate vast quantities of data that must be properly indexed and integrated with traditional toxicological data in order to facilitate comprehensive assessments. Failure to consider all available data may lead to poor product development decisions, incorrect conclusions and inaccurate risk predictions resulting in significant health, social and economic consequences. Relational databases provide an effective solution for indexing large amounts of information and can unify disparate chemical, toxicological, pathological and omic databases. Although many existing databases support discovery research, their utility for toxicogenomic efforts is limited since issues specific to toxicology such as multiple dose levels, time course, replicate data sets and extrapolation between species are not adequately addressed. This presentation will describe dbZach (http://dbzach.fst.msu.edu), a MIAME compliant relational database running under Windows 2000/Oracle 9i that supports ongoing toxicogenomic studies and provides data management. It also facilitates data analysis, storage, retrieval and querying. dbZach consists of four core subsystems (i.e. Clones, Genes, Protocols, and Sample Annotation), four Experimental Subsystems (i.e. Microarray, Affymetrix, Protein, Real-Time PCR, and Toxicology), and two In Silico Subsystems (i.e. Pathway and Gene Regulation) as well as a number of data analysis tools. Examples of the utility of dbZach will also be discussed. Development of dbZach is supported by ES11271.

622 MICROARRAY DISCOVERY: INTEGRATING BIOLOGY WITH EXPRESSION.

J. Quackenbush[1]. *[1]The Institute for Genomic Research, Rockville, MD and [2]ILSI Health & Environmental Sciences Institute, Washington, DC.* Sponsor: W. Mattes.

Over the last several years, the volume of microarray data generated in studies of toxicology has been steadily increasing. Likewise, the expectations of the toxicology community with respect to the information buried in this volume of data has also been increasing, and with those expectations, an appreciation for more sophisticated approaches to data housing, sharing, and analysis. Thus, public microarray databases are now considering the need to include appropriate biological context (i.e., linked toxicology data) in conjunction with array data. However, the ultimate scientific value and utility of public toxicogenomic databases, such as those developed by HESI-EBI, NIEHS-NCT, Stanford, will depend upon a clear assessment of how the community (both public and private sector) hopes to utilize these resources. This workshop will include presentations about current public toxicogenomic database development efforts by leading developers and then discussion about such outstanding issues as: a) Which portions of the scientific community are mostly like to utilize a public toxicogenomic database resource and for what purposes? and b) What are the hurdles to data submission to these databases?

623 THE ROLE OF METHYLATION IN ARSENIC TOXICITY & RISK: THE ENIGMA CONTINUES.

M. Waalkes[2], B. D. Beck[1], D. Thomas[3], M. Kadiiska[2] and M. Del Razo[4]. *[1]Gradient Corporation, Cambridge, MA, [2]NIEHS, Research Triangle Park, NC, [3]USEPA, Research Triangle Park, NC and [4]Instituto Poletecnico Nacional, Mexico City, Mexico.*

Methylation of inorganic arsenic was originally considered to be solely a detoxification pathway. Recent studies have demonstrated that, *in vitro*, the trivalent mono- and di-methylated species of inorganic arsenic are both highly cytotoxic and genotoxic. However, the relationship of these findings to *in vivo* responses and to risk assessment remains an area of on-going investigation and debate. This workshop will address toxicological differences among different states of arsenic as a function of methylation status and valence, and will consider how the role of methylation in toxicity may vary according to endpoint, tissue type, exposure duration, and animal species. Recent investigations into the enzymology of arsenic methylation including the role of co-factors will be described. The importance of reactive oxygen species in cytotoxicity and genotoxicity of inorganic versus methylated arsenic, both *in vivo* and *in vitro*, will be addressed. The use of human biomonitoring data, specifically arsenic species in urine, to elucidate the role of methylation in toxicity and to inform the role of methylation differences in susceptibility to arsenic will be discussed. Pharmacokinetic and toxicological differences between methylated species of arsenic as generated in the body *via* metabolism versus the same species when ingested will be discussed. Finally, the significance of these recent developments will be considered in the context of risk assessment for arsenic; the implications for the shape of dose-response curve as well as inter and intra-species variability will be discussed.

624 ENZYMOLOGY OF ARSENIC METHYLATION.

D. J. Thomas. *USEPA, Res. Tri. Pk., NC.*

A remarkable aspect of arsenic metabolism in many species is its conversion from inorganic species into methylated species. Thus, individuals ingesting inorganic arsenic excrete in urine inorganic and methylated arsenicals containing trivalent or pentavalent arsenic. Cyt19, an S-adenosyl-L-methionine-dependent-arsenic(III) methyltransferase purified from rat liver converts inorganic arsenic into methylated arsenicals. The protein is encoded by a cyt19 gene orthologous to mouse and human genes. Although exogenous reductants (dithiothreitol or tris (2-carboxylethyl) phosphine) support catalysis by recombinant rat cyt19 (rrcyt19), endogenous reductants that support its activity are unknown. Glutathione (GSH), the most abundant endogenous reductant, does not support catalysis by rrcyt19. However, the endogenous reductants, thioredoxin, glutaredoxin, and dihydrolipoic acid, coupled with thioredoxin reductase or glutathione reductase and NADPH, support its activity. Glutaredoxin and dihydrolipoic acid support its function in the presence of GSH. Aurothioglucose, an inhibitor of thioredoxin reductase, decreases arsenic methylation by rrcyt19 in thioredoxin-supported reactions. Endogenous reductants in guinea pig liver cytosol, a poor source of arsenic methyltransferase activity, support its catalytic activity. Dependence of enzyme activity on reductants is consistent with its function as an arsenate reductase. A CX7R motif in rrcyt19 resembles a CX5R motif of the P-loop structure in known arsenate reductases; like these proteins, cyt19 activity is stimulated by the presence of an oxyanion, phosphate. Endogenous reductants may be required by rrcyt19 to catalyze the reduction of a methylarsonic (MAs(V)) intermediate to trivalency as a prerequisite for its conversion to a dimethylated product. Thus, cyt19 encodes a protein possessing both As(III) methyltransferase and arsenate reductase activities. Variation in cyt19 genotype may underlie phenotypic variation in the capacity to metabolize arsenic and interindividual differences in susceptibility to arsenic-induced diseases. (This abstract does not represent policies of the USEPA.)

625 ARSENIC METHYLATION AND OXIDANT INJURY BY ESR *IN VIVO* AND *IN VITRO*.

M. B. Kadiiska[1], S. Nesnow[2], J. Liu[3], M. Waalkes[3] and R. Mason[1]. *[1]NIEHS/NIH, Research Triangle Park, NC, [2]USEPA, Research Triangle Park, NC and [3]NCI at NIEHS, Research Triangle Park, NC.*

Arsenic is a serious environmental concern worldwide, because of the large number of known contaminated sites and millions of people at risk from drinking arsenic-contaminated water. Inorganic arsenic undergoes metabolic conversion from pentavalent arsenate (As^V) to trivalent arsenite (As^{III}) with subsequent methylation to generate organometallic forms of arsenic such as MMA^V, MMA^{III}, DMA^V, DMA^{III} and trimethylarsine oxide. Studies have shown that As^{III} metabolites MMA^{III} and DMA^{III} are quite toxic and cause extensive damage to DNA. It has been postulated that the *in vivo* toxicity and carcinogenicity result from the catalysis of free radical generation. Using electron spin resonance (ESR) in conjunction with the spin traps phenyl-N-tert-butylnitrone (PBN), α (4-pyridyl 1-oxide)- N-tert-butylnitrone (POBN) and 5, 5-dimethyl-1-pyrroline-N-oxide (DMPO) we investigated free radical production by sodium arsenite (As^{III}) and sodium arsenate (As^V) in a mouse model of acute poisoning. In addition, the role of free radicals in *in vitro* cytotoxicity of DMA^{III} using murine TRL 1215 liver cells was examined. And, in order to define the mode of action of DNA damage induced by DMA^{III}, this study investigated free radical generation by DMA^{III} in *in vitro* experiments with supercoiled ϕX174 DNA. Simultaneous administration of PBN and As^{III} to adult male 129/Sv mice resulted in the generation of free radical metabolites detected in the liver lipid extract by ESR. Free radical generation was subsequently observed in TRL 1215 liver cells subjected to an acute high dose of DMA^{III} exposure. Finally, ESR was used to identify the nature of the radical being trapped by DMPO in the DMA^{III}-DNA-damage studies. The complete system gave a characteristic spectrum of a DMPO-hydroxyl radical adduct. In conclusion, the present study provides the most direct ESR evidence for the generation of free radicals by both inorganic and biomethylated forms of arsenic *in vivo* and *in vitro*. The relationship of these metabolites to effects of arsenic in humans will be discussed. This abstract does not reflect EPA policy

626 USE OF HUMAN BIOMONITORING TO ASSESS ARSENIC METHYLATION.

L. M. Del Razo[1], O. L. Valenzuela[1], G. G. Garcia-Vargas[2] and E. S. Calderon-Aranda[1]. *[1]Toxicology, Cinvestav-IPN, Mexico City, Mexico and [2]UJED, Medical School, Gomez Palacio, Durango, Mexico.*

Metabolism of inorganic arsenic (As_i) in humans produces monomethylarsenic (MAs^V), dimethylarsenic(DMA^V), monomethylarsenic (MAs^{III}), and dimethylarsenic (DMA^{III}), which can be found in human urine. The concentration of arsenicals in urine is used as a biological indicator of As_i exposure, because excretion *via* kidney is the major route for elimination of most As species. As_i methylation is often evaluated by the relative distribution of urinary As species. Due to increased recognition of trivalent methylated and dimethylated arsenicals (MAs^{III} and DMA^{III}) as more cytotoxic and more genotoxic than As_i, we studied the relationship of trivalent methylated metabolites to skin signs of arsenicism in humans chronically exposed to As_i. A cross-sectional study was conducted in central Mexico (about 220 km NE of Mexico City). Seventy-six residents (ages 15-51) from an endemic As-area have been exposed to very high levels of As_i in drinking water (150 to 1, 350 ppb) for at least 10 years. The participants answered a questionnaire and were clinically examined. Fifty-five individuals presented skin signs of arsenicism, such as keratosis and hyper- or hypo-pigmentation. Participants provided drinking water and spot urine samples. Due to instability of methylated As species in urine, samples were immediately frozen in dry ice and trivalent As species were analyzed approximately 6 hrs after collection. Trivalent methylated arsenic species were present in almost all the urine samples (99 %), DMA^{III} being the major metabolite (51.2%), followed by DMA^V (22.6%), MAs^{III} (7.2%), MAs^V (2.7%) As_i^{III} (8.8%) and As_i^V (7.5%). Urinary MAs^{III} and DMA^{III} were directly correlated with As_i in water. Individuals with skin-lesions had higher concentration of MAs^{III} in urine than those without skin lesions. The main factor associated with arsenicism was cumulative As_i exposure. More studies are necessary to determine if the urinary excretion of MAs^{III} or $DMAs^{III}$ provides a new biomarker of the effects of chronic exposure to As_i. Study supported by Conacyt-Mexico (grant 38471-M).

627 ARSENIC METHYLATION: CONSIDERATIONS FOR RISK ASSESSMENT.

B. D. Beck and A. Schoen. *Gradient Corporation, Cambridge, MA.*

Recent studies have demonstrated significant cytotoxicity and genotoxicity *in vitro* of the trivalent forms of methylated arsenic species (specifically monomethyarsonic acid or MMA and dimethylarsinic acid or DMA), often orders of magnitude more potent than the corresponding pentavalent forms. These studies have raised doubts about earlier understanding of methylation being a detoxification pathway. However, an analysis of both *in vivo* and *in vitro* studies indicates that generalizations about the toxicological significance of arsenic methylation are not appropriate for several reasons. First, a cell's capacity to methylate arsenic does not correlate with resulting toxicity or ability to undergo transformation. Moreover, distinctions must be made between MMA and DMA that are generated internally *via* methylation of inorganic arsenic *versus* MMA and DMA, which are directly administered. In the latter case, MMA and DMA are excreted rapidly from the body nearly unchanged and demonstrate relatively low toxicity. High doses of DMA cause bladder cancer in rats, possibly through oxidative damage from DMA^{III} formation and subsequent generation of trimethyl arsine oxide (TMAO); these metabolites are believed to cause urothelial cell necrosis followed by regenerative cell proliferation and tumorigenesis; however, this response appears to be unique to the rat, with hamsters, mice and humans forming much less (if any) TMAO than rats. It may also be necessary to make distinctions between acute and chronic exposure to arsenic. More research is required to clarify the toxicological significance of arsenic methylation. In particular, a better understanding of the amount and persistence of trivalent metabolites present *in vivo* at critical target sites would help clarify the role of such forms in injury and disease. Risk assessment considerations about arsenic methylation, the role of trivalent forms, and dose response implications must be made on a case-by case basis with emphasis on species-specific and dose and duration-specific responses.

628 CONTRIBUTION OF NEUROBEHAVIORAL ASSESSMENT OF OFFSPRING TO HAZARD IDENTIFICATION AND CHARACTERIZATION.

D. L. Shuey[1] and L. D. Middaugh[2]. *[1]Preclinical Safety Assessment, Endo Pharmaceuticals Inc., Chadds Ford, PA and [2]Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC.*

Neurobehavioral assessment of offspring following maternal exposures during gestation and lactation have long been a routine part of preclinical safety assessment during pharmaceutical development (Peri/Postnatal Development Study). Similar studies have recently become more common for agricultural and industrial chemicals (USEPA Developmental Neurotoxicity Study). Recently, the Health and Environmental Sciences Institute of ILSI collected data from 174 studies to retrospectively evaluate the contribution of these assessments to hazard identification (i.e., definition of a NOEL) and characterization. A similar retrospective analysis of developmental neurotoxicity studies submitted for EPA review has also recently been updated. In June 2003, a workshop was held to review and evaluate current behavioral test methods. The outcomes of these activities will be presented to provide a basis for discussion of the overall contribution of these assessments to hazard identification and characterization, as well as study design and methodologic considerations for consistent and effective conduct and interpretation of these studies.

629 NEUROBEHAVIORAL ASSESSMENT: A SURVEY OF USE AND VALUE IN SAFETY ASSESSMENT STUDIES.

L. D. Middaugh. *Psychiatry and Behavioral Sciences/ CDAP, Medical University of South Carolina, Charleston, SC.* Sponsor: D. Shuey.

A survey designed to evaluate the contribution of F1 neurobehavioral testing to hazard identification and characterization in safety assessment studies was distributed to industrial laboratories in the United States, Europe, and Japan. The survey provided information about studies completed since 1990 on 174 compounds. General categories of compounds included pharmaceuticals (81%), agricultural (7%), industrial (1%), or were undefined (10%). Information collected included: 1) intended use of the test agent, 2) general study design and methodology, 3) types and characteristics of F1 behavioral evaluations, and 4) the frequency with which agents affected neurobehavioral parameters in comparison to other F0 and F1 generation parameters. F1 general toxicology parameters such as mortality, pre- and post-weaning body weight, and food intake were assessed in most studies and were affected more frequently than other parameters by the test agents. F1 behavioral parameters were assessed less consistently across studies, and were less frequently affected by the agents tested. Although affected by agents less often than general toxicology parameters, F1 behavioral parameters along with other parameters defined the no-observed-effect level (NOEL) in 17/113 (15%) studies, and solely defined the NOEL in 3/113 (2.6%) studies. Thus, the F1 behavioral parameters sometimes improved on the standard toxicological measures of hazard identification. While not detecting agent effects as readily as some measures, the F1 behavioral parameters provided information about agent effects on specialized functions of developing offspring that were not provided by other standard measures of toxicity. The survey results emphasize the need for further research into the methods of behavioral assessment as well as the mechanisms underlying the neurobehavioral alterations.

630 A RETROSPECTIVE ANALYSIS OF DEVELOPMENTAL NEUROTOXICITY STUDIES SUBMITTED TO THE USEPA.

S. L. Makris. *OPPTS/OPP/HED (7509C), USEPA, Washington, DC.*

Over the past decade, a number of developmental neurotoxicity (DNT) studies have been submitted to the USEPA in support of pesticide registration. These studies, conducted in rats, include detailed neurobehavioral and neuropathological assessments, following *in utero* and postnatal exposures. An analysis was conducted retrospectively on the DNT study data that have been received and reviewed by OPP staff scientists between 1991 and 2003, and of the influence of these data on regulatory decisions for pesticides. The DNT study has been found to be a sensitive study for characterizing hazard and dose-response in the young, particularly for chemicals with known neurotoxic potential. Primary contributions of the DNT study in pesticide risk assessment have been: 1) for some chemicals, the DNT study has been used as a source of endpoints and doses for the calculation of risk following various durations and routes of potential human exposure, and 2) the presence or absence of the DNT study in some pesticide databases has been a critical factor in determining the magnitude of the uncertainty factors applied in the risk assessment. The analysis of these DNT studies also included inter-laboratory comparisons of control data, and has identified a number of issues and problems in study conduct and data interpretation. (This abstract does not necessarily reflect the policy of the USEPA.)

631 BEHAVIORAL TEST METHODS WORKSHOP.

W. Slikker. *Division of Neurotoxicology, NCTR/FDA, Jefferson, AR.*

The overall goal of the Behavioral Test Methods Workshop, conducted June 20 and 21, 2003 as a satellite meeting just preceding the Teratology, Neurobehavioral Teratology and Behavioral Toxicology annual meeting in Philadelphia, was to improve the sensitivity and quality of behavioral testing thereby enhancing the utility of behavioral data as a reliable index of neurotoxicity in safety evaluation. Several objectives were determined including 1) understanding better the scientific rationale underlying high quality behavioral toxicity testing, 2) identifying the primary practical elements in the conduct of behavioral toxicity testing and analysis that optimize the development of high quality, scientifically creditble, and interpretable data, and 3) providing expert input from academia, industry and government to assist the Inter Agency Committee on Neurotoxicology (ICON) in finalizing its behaviroal test methods primer. After presentations by several experts on the fundamentals of behavioral assessment and statistical design, breakout groups were charged to address selected topics and to focus on several well-developed questions. The outcome of the breakout groups' discussions provided several recommendations including 1) the need for determining the purpose of behavioral screen or characterization, and factors that determine appropriate experimental design and data quality, 2) the need for appropriate technical training, logistical support, selection and validation of methods and equipment, and 3) need for appropriate statistical design and clarity of terminology. These and other important findings of the Workshop will be presented and discussed in terms of improving behavioral test methods.

632 INTERPLAY OF P53 AND P63 IN TRANSCRIPTIONAL RESPONSE AFTER CELL STRESS.

J. A. Pietenpol. *Biochemistry, Vanderbilt University, Nashville, TN.* Sponsor: J. Kramarik.

The p53 homologue, p63 shares structural similarity with p53 but exhibits functions that are quite distinct from those of p53. Six splice variants of p63 have been identified. They are similar to p53 in that they contain a central DNA binding domain and an oligomerization domain; however, differential transcriptional initiation results in proteins that contain a transactivation domain (TAp63) or lack the N-terminal transactivation domain (deltaNp63). Additionally, alternative splicing results in alpha, beta, and gamma transcripts that each display unique C-termini. deltaNp63 forms are considered to be a dominant negative form of the protein. Since they retain the ability to bind DNA, they may compete for DNA binding sites or they may bind the TA forms and render them inactive. In adult epithelium, we find that deltaNp63alpha is the predominantly expressed form of the protein. While p53 has a well-established role in tumor suppression, we find elevated levels of deltaNp63alpha in squamous cell carcinomas and it is hypothesized to have oncogenic properties. Conservation of the DNA binding domain between p53 and the p63 suggests that they bind a common subset of promoters. We have found this to be the case using chromatin IP-based assays to analyze p53 and p63 occupancy at target gene promoter sites in primary cultures of normal human keratinocytes and mammary epithelial cells under normal and stress conditions. We discovered that deltaNp63alpha acts as a transcriptional repressor, presumably to allow for maintenance of proliferation in these regenerative cells. Further, using several gene discovery approaches, we identified subsets of gene promoters that p53 can bind to after genotoxic stress, but to which deltaNp63alpha cannot and vice versa. Our results suggest that p53 and p63 coordinately regulate subsets of genes in a given cell type after stress; however, each p53 family member can regulate a distinct subset of genes as well. Determining mechanism(s) that dictate differential gene regulation by p53 family members will lend great insight to how cells reprogram gene expression in response to insult.

633 MAPPING THE REGULATORY SURFACES OF MEDIATOR WITH ARTIFICIAL TRANSCRIPTION FACTORS.

A. Mapp. *University of Michigan, Ann Arbor, MI.* Sponsor: J. Kramarik.

Regulated gene expression forms the basis of an organisms response to environmental stress. The metabolism of many xenobiotics, for example, relies upon the controlled production of cytochrome P450 oxidizing enzymes, and the proliferation of cells containing severely damaged DNA is checked by the selective up-regulation of the p53 tumor suppressor gene. Disruptions or faults in the regulatory network of gene expression are thus directly linked to a variety of human diseases and in many cases, the altered gene expression profiles are due to malfunctioning transcriptional regulators. As a result, the design and synthesis of artificial transcription factors, molecules that target specific genes and regulate their expression positively or negatively, has taken on renewed significance. Such factors will be powerful tools for defining the macromolecular interactions that dictate gene expression patterns and in the long term, serve as prototypes for the development of transcription-based therapeutics. Towards that end, we developed an approach for selecting molecules from synthetic combinatorial libraries that interact with individual transcriptional machinery proteins and employ these ligands in artificial transcriptional regulator construction. These regulators are then used to map key protein binding events that lead to a gene being turned on or off. The first generation ligands, short peptides, were used to identify binding sites within the Mediator complex that are used for gene up-regulation in S. cerevisiae. The ligands function as activators of transcription, and unexpectedly, their potency as activators is linked to the binding site location rather than binding affinity for the target protein. The binding site location and potency of these novel activators can be tuned by making small sequence changes, providing compelling evidence that assembly of the transcriptional machinery can be mediated by targeting a relatively small binding surface area. Using this information, organic molecules mimicking the activator characteristics were designed and indeed function as transcriptional activators *in vitro*.'

634 INHIBITION OF SMAD TRANSCRIPTION ACTIVITY AND ANTIPROLIFERATIVE FUNCTION BY CDK PHOSPHORYLATION.

F. Liu. *CABM and LCR, Rutgers University, Piscataway, NJ.* Sponsor: J. Kramarik.

Cell cycle progression from the G1 to S phase is governed by G1 cyclin-dependent kinases CDK4 and CDK2. Transforming growth factor-beta, a secreted polypeptide growth factor, exerts potent growth-inhibitory effects on many different cell types by causing cell cycle arrest at the G1 phase, which enables it to function as a potent tumor suppressor during the early stages of tumorigenesis. TGF-beta also regulates differentiation, cell adhesion, extracelluar matrix deposition, cell motility, cell death and other activities. Many environmental agents, such as reactive oxygen species hydrogen peroxide, and antioxidant treatments, also regulate TGF-beta expression, and their effects, at least in part, may be mediated by TGF-beta. TGF-beta binds to transmembrane serine/threonine kinase receptors. We and others have shown that Smad proteins can transduce the TGF-beta signal from the receptors to the nucleus. Smad proteins can be directly phosphorylated by activated TGF-beta receptor kinases. Following phosphorylation, Smad proteins form heteromeric complexes, accumulate in the nucleus, and regulate transcription of target genes. Smad proteins can mediate TGF-beta growth-inhibitory effects by regulating the expression of cell cycle components. For example, Smad proteins can upregulate the expression of CDK inhibitors p15 and p21 and downregulate the expression of the c-myc protooncogene. Through phosphorylation studies, we have recently discovered that CDK4 and CDK2 can phosphorylate Smad proteins. Except for the retinoblastoma (Rb) family members, Smad proteins are the only demonstrated substrates for CDK4. We have further shown that CDK phosphorylation of Smads inhibits their transcription activity and antiproliferative function. Since tumor cells often contain high levels of CDK4 and CDK2 activities, inactivation of Smad proteins by extensive CDK phosphorylation may provide an important mechanism for the resistance to the growth-inhibitory effect of TGF-beta in cancers.

635 REGULATION OF HUMAN DNA REPAIR GENE EXPRESSION AND ACTIVITY.

J. M. Ford, S. Adimoolam and M. Fitch. *Medicine & Genetics, Stanford University School of Medicine, Stanford, CA.* Sponsor: J. Kramarik.

The p53 tumor suppressor gene is involved in multiple aspects of cellular physiology, including DNA repair and the response to DNA damage. The function of the p53 gene is due to its role as a transcription factor regulating expression of genes involved in DNA damage response pathways and affecting apoptosis, cell cycle regulation and DNA repair. We have shown that wild-type p53 is required for proficient mammalian nucleotide excision repair (NER) of UV-irradiation induced DNA damage, and that p53 dysfunction or loss results in a DNA repair defective phenotype, specifically affecting the global genomic NER pathway. The dependence of global genomic NER, but not transcription-coupled NER, on p53 function suggests that the mechanism involves those NER enzymes specifically required for GGR. Using gene expression profiling techniques we identified the DNA damage recognition factors p48 (protein product of the DDB2 gene) and XPC as DNA damage inducible in a p53-dependent manner. Both genes contain consensus p53 responsive regulatory elements in their promoters domains. These promoter elements can activate p53-dependent transcription when placed in a promoter-reporter construct, and bind p53 protein following UV-irradiation, *in vivo*, as measured by chromatin immunoprecipitation assays. Both p48 and XPC proteins localize within minutes of UV-irradiation to sites of DNA damage *in vivo*, and this does not require the presence of wild-type p53. p53 protein itself does not co-localize to DNA damage sites in cells, but p48 activates the binding of XPC to these sites. Stable ectopic expression of a p48 cDNA results in complementation of the NER defect associated with p53 deficiency in human cells, demonstrating that efficient NER does not require the presence of wild-type p53 protein, but can be restored through expression of damage-inducible, p53-regulated downstream genes. Therefore, the DNA damage recognition of NER is highly regulated, by transcriptional and post-transcriptional means. Impaired NER due to loss of p53 may play a crucial role in the pathogenesis of human cancers.

636 USING DNA MICROARRAYS TO DEFINE TRANSCRIPTIONAL PATTERNS ASSOCIATED WITH AGING AND OXIDATIVE STRESS IN THE MOUSE HEART.

T. Prolla. *University of wisconsin, Madison, WI.* Sponsor: J. Kramaric.

To examine molecular events associated with aging and its retardation by caloric restriction (CR), we have employed high-density oligonucleotide arrays providing data on 9, 997 genes to define transcriptional patterns in cardiac muscle. Male B6C3F1 mice were either fed normally or subjected to CR. To investigate aging, 5 month (young adult) and 30 month-old normally fed mice were compared. To study CR, 30 month-old control and CR mice were compared. Aging was associated with specific transcriptional alterations that are prevented by CR. Specifically, aging was associated with a transcriptional pattern associated with a metabolic shift from fatty acid to carbohydrate metabolism, increased expression of extracellular matrix genes and reduced protein synthesis. CR was associated with a transcriptional pattern consistent with reduced oxidative damage and apoptosis. To investigate the transcriptional response to oxidative stress in the heart and how it changes with age, we examined the cardiac gene expression profiles of young (5-mo-old), middle-aged (15-mo-old), and old (25-mo-old) C57BL/6 mice treated with a single intraperitoneal injection of paraquat (50 mg/kg). Genes commonly induced in all age groups include those associated with stress, inflammatory, immune, and growth factor responses. Interestingly, only young mice displayed a significant increase in expression of all three isoforms of GADD45, a DNA damage-responsive gene. Additionally, the number of immediate early response genes (IEGs) found to be induced by paraquat was considerably higher in the younger animals. These results demonstrate that, at the transcriptional level, there is an age-related impairment of specific inducible pathways in the response to oxidative stress in the mouse heart.

637 REPROGRAMMING GENE EXPRESSION WITH OXIDATIVE STRESS AND STRESS HORMONES: A PARADOX OF ANTIOXIDANT RESPONSES.

Q. M. Chen. *Department of Pharmacology, University of Arizona, Tucson, AZ.*

Oxidative stress and mental stress have been considered as two different types of health hazards in the modern world. While oxidants have been shown to contribute to the toxicity of a large number of chemicals, aging and a number of diseases, the role of the stress hormone corticosterone remains controversial among various tissues. We have utilized several *in vitro* experimental systems to understand the mechanisms of oxidative stress. With normal human diploid fibroblasts (HDFs) in culture, oxidants induce a phenotype indistinguishable from replicatively senescent cells. However gene array analyses revealed only a few genes overlapping between H2O2 treated cells and replicatively senescent cells. Nevertheless antioxidant/redox genes were upregulated in both H2O2 treated HDFs (metallothioneins) and replicative senescent cells (thioredoxin reductase-1, peroxiredoxin-6). Induction of antioxidant genes was also observed with H2O2 treated cardiomyocytes [NAD(P)H:quinone oxidoreductase (NQO1)] or cardiac fibroblasts (NQO1, glutathione S-transferases α1, metallothionein1). While the expression of these antioxidant genes appear to be contradictory to observed deleterious effects of H2O2, corticosterone was found to be cytoprotective and inhibit apoptosis in cardiomyocytes. Gene array analyses found that corticosterone caused upregulation of metallothioneins, glutathione peroxidase-3 and glutathione S-transferase mu2. These data suggest that although expression of antioxidant genes can contribute to cytoprotection, the cellular outcome of oxidative stress is a balance of multiple factors.

638 EFFECTS OF TRANSSPECIES CARCINOGENS IN AVIAN EMBRYOS.

H. G. Enzmann[1], C. Goetze[1], K. Spicher[1] and H. Korr[2]. *[1]Preclinical Pharmacology and Toxicology, Federal Institute for Drugs and Medical Devices, Bonn, Germany and [2]Department of Anatomy and Cell Biology, RWTH University of Aachen, Aachen, Germany.*

A high number of chemicals are carcinogenic in the chronic rodent bioassay. Not all of them however may be considered transspecies carcinogens and are a cancer hazard to humans. The distinction between chemicals that are carcinogenic in rodents only and chemicals that are transspecies carcinogens requires non-rodent experiments. Long term carcinogenicity studies in non-rodents or even primates will usually not be possible for reasons of study duration, cost and ethical considerations. The use of a *via*n embryos is a rapid and inexpensive approach to study the effects of chemicals on non-rodent tissues. Turkey or quail eggs are injected with the test chemicals into the white of the egg prior to incubation. Control eggs are injected with an equivalent volume of the vehicle. After an incubation period of up to 24 days for turkey eggs and up to 21 days for quail eggs, the embryos are removed from the eggs and tissue samples are fixed in 4% phosphate buffered formaldehyde and processed for histological examination. For autoradiography 3H -thymidine is administered on the chorion allantois membrane 90 minutes before termination. For hepatocyte culture, in situ instillation of embryonic livers with collagenase is used before hepatocytes are plated on collagen-coated dishes. In liver samples, hepatocellular tumors, bile duct proliferations and hematological neoplasia are induced by the transspecies carcinogen diethlynitrosamineamine (DEN). In the kidney storage of PAS positive material is observed in tubular cells after exposure to ochratoxin A. Cell cultures from nitrosamine-treated livers (DEN, N-Nitrosomorpholine, NNK) exhibit persistent morphological alterations (megalocytosis). DNA synthesis in megalocytes is increased in situ and *in vitro*. We suggest that induction of tumors in a *via*n embryos by a chemical that is carcinogenic in rodents may be considered conclusive evidence of a transspecies carcinogen.

639 MODEL SYSTEMS FOR COMPARING THE ROLES OF AKR1A1 AND CPY1A1 IN THE METABOLIC ACTIVATION OF THE PROXIMATE CARCINOGEN BENZO(A)PYRENE-7, 8-DIOL.

H. Jiang, Y. Shen, A. Quinn, S. Gopishetty and T. M. Penning. *Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA.* Sponsor: S. Burchiel.

Benzo[a]pyrene (BP) is a representative polycyclic aromatic hydrocarbon (PAH) which is metabolically activated to exert its carcinogenic effects. One potential pathway of BP activation involves aldo-keto reductases (AKRs), which convert potent proximate carcinogen trans-dihydrodiols to their corresponding reactive o-quinones. Another pathway of activation involves CYP1A1 and CYP1B1 which oxidize trans-dihydrodiols to form reactive diol-epoxides. However, the relative contribution of these competing pathways is unknown. To address this issue, model *in vitro* and transfection systems *in vivo* were established. *In vitro* results verified that BP-7, 8-diol was oxidized by both recombinant AKR1A1 and human bronchoalveolar cells (H358) transfected with AKR1A1 to form the reactive BP-7, 8-dione which was trapped by ten- to twenty-fold mercaptoethanol as a stable BP-7, 8-dione thioether conjugate. To examine the competing roles of AKR1A1 and CYP1A1, CYP1A1 expression was induced with 2, 3, 7, 8-TCDD in parental bronchoaveolar cells (H358 and H441) and breast carcinoma MCF7 cells, and in cells transfected with AKR1A1. 10 nM 2, 3, 7, 8-TCDD was the optimal concentration for CYP1A1 induction in parental and transfected H358 and MCF7 cells

over 3-24 h, but no CYP1A1 induction was observed in H441 cells. These models provide the basis for comparison of trans-dihydrodiol activation *in vivo via* the AKR1A1 and CYP1A1 pathways. (Supported by RO1-CA-092537/awarded to T.M.P.)

640 ASSESSMENT OF THE ROLE OF CYP2E1-MEDIATED METABOLISM OF URETHANE ON THE EXPRESSION OF P53, PCNA, AND KI-67 USING CYP2E1-NULL AND WILD-TYPE MICE.

University. Hoffler[1, 2], D. Dixon[2] and B. I. Ghanayem[1, 2]. [1]*Meharry Medical College, Nashville, TN and* [2]*NIEHS/NIH, Research Triangle Park, NC.*

Urethane is a known multi-site carcinogen capable of inducing tumors in various organs and animal species. Recent work in this laboratory demonstrated that cytochrome P450 2E1 (CYP2E1) is the principal enzyme responsible for urethane metabolism. Earlier reports showed no immunoreactive cells for p53 in pulmonary proliferative lesions of mice treated with urethane. However, positive immunoreactions for proliferating cell nuclear antigen (PCNA) were observed and as the lesions progressed from hyperplasias to carcinomas, PCNA labeling indices also increased. In an attempt to evaluate the relationship between CYP2E1-mediated urethane metabolism and the pathogenesis of urethane-induced tumors, the expression of PCNA, Ki-67, and p53 were assessed using CYP2E1-null (KO) and wild-type (WT) mice. Mice were administered urethane *via* gavage at 0, 1, 10, or 100 mg/kg/day, 5 days/week for 6 weeks. At the end of the dosing regimen mice were euthanized and tissues were collected, fixed and processed for immunohistochemical staining. Present work supports earlier findings in that no immunoreactive cells for p53 were detected in the liver or lung of either genotype of mice. Further, no significant increase in PCNA expression was detected in the lung or liver of either genotype of mice. In contrast, however, a 4-fold increase in the expression of Ki-67 was exhibited in the livers of WT mice administered 100 mg urethane/kg in comparison to their vehicle-treated wild-type controls. Similarly, an approximate 2.5 and 4-fold increase in Ki-67 expression were observed in the alveolar regions and bronchioles (including terminal bronchi) of high-dosed WT mice, respectively. In contrast, KO mice administered 100 mg urethane/kg exhibited no significant alterations in Ki-67 expression in lungs compared to lungs of vehicle-treated KO mice. In conclusion, this study demonstrates that CYP2E1-mediated metabolism is a prerequisite for the induction of Ki-67 in the liver and lungs of urethane-treated mice.

641 INCREASED DNA METHYLATION IN THE HOXA5 PROMOTER REGION CORRELATES WITH DECREASED EXPRESSION OF THE GENE DURING TUMOR PROMOTION.

R. E. Watson[1], G. M. Curtin[2], G. M. Hellmann[2], D. J. Doolittle[2] and J. I. Goodman[1]. [1]*Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI and* [2]*Regulatory Toxicology, R. J. Reynolds Tobacco Co., Winston-Salem, NC.*

Methylation of cytosine residues of DNA constitutes an epigenetic mechanism for regulating gene transcription, with increased methylation of promoter regions often leading to decreased gene expression. HoxA5 has characteristics of a tumor suppressor gene, with HOXA5 binding to the promoter of the p53 tumor suppressor gene to increase its expression. Thus, a decrease in HoxA5 might contribute to carcinogenesis by decreasing the expression of p53. In this study, we used a SENCAR mouse skin initiation/promotion model to examine changes in gene expression and DNA methylation during tumor promotion. Skin was initiated with 75 ug 7, 12-dimethylbenz[a]anthracene and promoted with 27 or 36 mg cigarette smoke condensate (CSC) for 9 wks. Gene array analysis using a BD Biosciences Clontech Atlas™ mouse 1.2 nylon array indicated an average decreased expression for HoxA5 of 61% and 66% following treatment with 27 or 36 mg CSC, respectively. To determine if increased methylation plays a role in the decreased HoxA5 expression, we used the enzymatic regional methylation assay to assess the average methylation level in the promoter region. DNA was bisulfite modified and PCR amplified with primers containing dam sites (GATC), and incubated with [14C-methyl] S-adenosyl-L-methionine (SAM) and dam methyltransferase (internal control) to standardize DNA quantity. DNA was then incubated with [3H-methyl] SAM and SssI methylase (methylates C's 5' to G's) to quantify methylation status. A high 3H/14C ratio compared to control is indicative of hypermethylation. HoxA5 in animals promoted with 27 or 36 mg CSC was hypermethylated compared to initiated-only controls. The 3H/14C ratio in controls versus promoted with 27 or 36 mg CSC was 12.4±0.34, 48.2±6.9 and 24.2±6.1, respectively. These data indicate that DNA methylation may contribute to the decreased expression of HoxA5, which might then contribute to tumorigenesis.

642 IDENTIFICATION OF MOLECULAR PATHWAYS THAT MAY PROMOTE CELL GROWTH AND PROLIFERATION IN RESPONSE TO NONGENOTOXIC CARCINOGENS.

V. Bombail[1], R. Currie[1], J. Oliver[1], A. Morsi[2], I. Kimber[1], K. Chipman[2] and G. Orphanides[1]. [1]*Syngenta Central Toxicology Laboratory, Alderley Park, Cheshire, United Kingdom and* [2]*School of Biosciences, University of Birmingham, Birmingham, United Kingdom.*

A diverse group of industrial chemicals cause liver tumors in rodents without damaging DNA directly. Species differences in response to these nongenotoxic carcinogens question the relevance of such rodent tumors for human risk assessment. It is believed that these chemicals induce carcinogenesis through regulation of cell growth, proliferation and apoptosis. Assessment of the likely risk to humans posed by these chemicals will be facilitated by identification of the molecular pathways through which these compounds control cell proliferation. We have attempted to identify these pathways using global gene expression profiling. Mice were dosed with the model rodent nongenotoxic carcinogen and peroxisome proliferator diethylhexylphthalate (DEHP; 1150 mg/kg/day) or corn oil (control) by gavage for three days. Gene expression levels were measured 1, 2, 4, 8, 24, 48 and 72 hr following first exposure using Affymetrix MG-U74v2A GeneChips. 1, 257 genes showed statistically significant alterations in expression level between control and DEHP groups. Gene ontology analysis revealed significant alterations in gene expression in genes involved in peroxisomal function, the acute phase response, xenobiotic responses, prostaglandin metabolism, the regulation of apoptosis, and cholesterol biosynthesis. Analysis of components of signal transduction pathways revealed coordinated changes in the expression of genes of the TNF-α, IL-1, IL-6, EGF and TGF-β pathways. In addition, DEHP induced the expression of a family of protease inhibitors implicated in cell proliferation and of a novel protein that may regulate cell cycle progression in hepatocytes. These data shed light on the mechanisms by which nongenotoxic carcinogens control hepatocyte growth and proliferation and will facilitate assessment of the likely risk posed by these chemicals to humans.

643 AHR REGULATION OF C-MYC IN HUMAN BREAST CANCERS.

X. Yang, T. J. Murray, D. Liu and D. H. Sherr. *Enviormental Health, Boston University School of Public Health, Boston, MA.*

It has long been suggested that ubiquitous environmental chemicals, such as PAH, contribute to human breast cancer. The preferential targeting of breast tissue by orally administered PAH in rodent breast cancer models supports this contention. Most of the biologic activity of PAH and related dioxins is mediated by the AhR. Previously, we demonstrated that the AhR and CYP1B1, an AhR-regulated gene, are dramatically up-regulated in rodent and human breast tumors. Here, we investigated the possible consequences of this apparent constitutive AhR activation. In specific, we tested if constitutively active AhR in a human breast cancer cell line, regulates c-myc, an important breast cancer oncogene which contains six AhR binding sites (AhREs) in its promoter. Luciferase reporter vectors containing the c-myc promoter with its six AhREs was constructed. Variants with mutations in NF-kB and/or AhR binding sites were generated by site-directed mutagenesis. These constructs were transfected into Hs578T cells and subsequently assayed for luciferase activity in the presence or absence of TCDD, a strong AhR agonist. The results indicate that: 1) there is a significant baseline level of wildtype c-myc promoter driven reporter activity in these tumor cells which was not affected by inclusion of TCDD, 2) the baseline reporter activity was not affected by deletion of the NF-kB site, 3) while mutation of single AhRE sites had no effect on baseline reporter activity, mutation of all six sites resulted in a five fold increase in reporter activity; a similar increase in reporter activity was seen when the wildtype reporter construct was co-transfected with an AhR repressor plasmid, 4) c-myc-specific real time PCR indicated that AhR repressor transfection increased background levels of endogenous c-myc mRNA. These results suggest that the AhR represses c-myc transcription and that AhR up-regulation in tumor cells may represent a failed growth feedback mechanism.

644 2, 3, 7, 8-TETRACHOLODIBENZO-P-DIOXIN (TCDD) INDUCES MMP EXPRESSION AND INVASION IN A2058 MELANOMA CELLS.

L. A. White[1], K. Murphy[1], A. Akintobi[1] and C. Villano[2]. [1]*Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ and* [2]*Joint Graduate Program in Toxicology, Rutgers University, New Brunswick, NJ.*

The incidence of metastatic melanoma is increasing in industrialized nations, and we propose that environmental contaminants, such as the polycyclic and halogenated aromatic hydrocarbons (PAH/HAH), stimulate melanoma progression by altering matrix metalloproteinase (MMP) expression and activity. MMP activity is necessary for cell migration through matrix barriers, and expression of these en-

zymes correlates with aggressive and invasive tumors. Our data in melanoma cells demonstrate that though both non-invasive and invasive melanoma lines are responsive to TCDD, only the invasive cells (A2058) express MMPs following TCDD exposure. In addition, our data show that TCDD exposure results in a concomitant down-regulation of PAI-2 (plasminogen activator inhibitor-2), an inhibitor of matrix remodeling. Finally, *in vitro* invasion assays demonstrate that TCDD treatment results in an increase in invasion of the melanoma cells through a basement membrane barrier. These data suggest that TCDD directly increases melanoma invasion by stimulating degradation of the protein barriers between cells and tissues. In order to determine the mechanism of TCDD-induced MMP-1 expression in these cells, we performed transient transfection of 4.4 kb of the promoter linked to a reporter gene (luciferase; pGL3). Data from these experiments demonstrate that TCDD-induced MMP expression in melanoma cells is mediated, at least in part, through activation of the sequences in the enahncer region of MMP-1. A deletional series was used to identify a TCDD-responsive region of the MMP-1 promoter. Interestingly, the identified region contains no sequences homologous to the consensus binding site (xenobiotic responsive element: XRE) for the known receptor for TCDD, the Aryl hydrocarbon receptor (AhR). As no consensus XREs sequences have been identified in this distal region, the results of these experiments may reveal novel TCDD-responsive cis-acting element(s).

645 HEPATOMA MITOCHONDRIA RESIST THE MITOCHONDRIAL PERMEABILITY TRANSITION: POSSIBLE INVOLVEMENT OF HEAT SHOCK PROTEIN-25 (HSP25).

E. Bustamante, L. He and J. J. Lemasters. *Cell and Developmental Biology, University of North Carolina School of Medicine, Chapel Hill, NC.*

Opening of mitochondrial permeability transition (MPT) pores plays a causative role in cell death by committing cells to either apoptosis or necrosis, and increased expression of HSP25 after heat shock is associated with a capacity to resist the MPT and cell killing. We investigated the MPT in mitochondria isolated from three lines of rat hepatoma cells (AS-30D, N1-S1, and RH7777) and from rat liver using a mitochondrial swelling assay at 540 nm and 25°C. Mitochondrial Ca^{2+} uptake and membrane potential were also monitored with Fluo-5N and tetramethylrhodamine methyl ester (TMRM), using a fluorescence plate reader at 25°C. Mitochondrial functional and structural integrity were assessed by oxygen consumption, electron microscopy, and confocal microscopy (after loading live cells at 37°C with TMRM, propidium iodide, or MitoTracker Red). In Mg^{2+}-free medium, 200 μM Ca^{2+} caused swelling of liver mitochondria within 10 min, but hepatoma mitochondria did not swell after 30 min even with over 500 μM Ca^{2+}, although the membrane-piercing peptide alamethicin did induce swelling. Lack of swelling in tumor mitochondria was not due to a defect of ruthenium red-sensitive Ca^{2+} uptake, which occurred at a similar rate as liver mitochondria, or to differences of mitochondrial membrane potential. Mitochondria from neonatal liver and regenerating liver behaved similarly to normal liver. The uptake of Ca^{2+} by hepatoma mitochondria caused temporary mitochondrial depolarization as measured by TMRM fluorescence and mitochondria subsequently repolarized, strongly suggesting that the mitochondrial membranes were physiologically intact. Immunoblot analysis revealed HSP25 in hepatoma mitochondria, whereas HSP25 was not detectable in liver mitochondria. Resistance of hepatoma mitochondria to induction of the MPT may contribute to the relative insensitivity of tumor cells to pro-apoptotic stimuli. Expression of HSP25 in mitochondria may play a role in the resistance to the MPT of hepatoma mitochondria.

646 POSSIBLE ROLE FOR CHEMOTHERAPY IN THE UPREGULATION OF MITOCHONDRIAL BIOGENESIS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS.

J. S. Carew, Y. Zhou, M. J. Keating and P. Huang. *UT M.D. Anderson Cancer Center, Houston, TX.* Sponsor: D. McConkey.

Chronic lymphocytic leukemia (CLL) is the leading form of adult leukemia in the Western hemisphere. In spite of its prevalence, many of the biochemical and molecular features of this disease remain unclear. Previous studies by our group established that CLL cells from patients with prior chemotherapy had significantly higher basal levels of reactive oxygen species (ROS) and mtDNA mutation frequencies than cells from untreated patients. These findings led us to speculate that chemotherapy may also contribute to other mitochondrial (mito) aberrations. Considering that oxidative stress has been proposed to activate mito biogenesis, we hypothesized that treatment with certain anticancer agents may be associated with increased cellular mito mass. To test this hypothesis, we obtained clinical specimens from CLL patients with and without prior chemotherapy. Previously treated patients primarily received a regimen of fludarabine, cyclophosphamide, and rituxan. Basal superoxide (O2-) generation of primary CLL cells was assessed using hydroethidine. As expected, cells from previously treated patients generated higher levels of basal O2- than those from untreated patients. Cellular mito mass was determined by flow cytometry using Mitotracker Green. The mito mass of CLL cells from patients with prior chemotherapy was significantly higher ($p=0.0313$) than that of cells from untreated patients. Electron microscopy showed that CLL cells contained significantly higher numbers of mitochondria per cell than normal B lymphocytes, suggesting that increased mito biogenesis may play a key role in oncogenesis or disease progression. Analysis of a direct relationship between O2- generation and mito mass revealed a weak correlation. However, certain cells with especially high levels of O2- also had a very high mito mass, indicating that ROS may contribute to the increased mito mass observed in cells from treated patients. Taken together, the data suggest that treatment with anticancer agents is associated with elevated mito mass, possibly due to increased mito biogenesis.

647 EFFECTS OF RAT GENDER AND STRAIN ON ELUCIDATING LIVER TOXICITY.

B. W. Higgs, A. L. Castle, W. Zeng, M. W. Porter and D. L. Mendrick. *Toxicogenomics, Gene Logic Inc., Gaithersburg, MD.*

We examined the effects of gender and strain on our ability to use a reference database built mostly on male Sprague-Dawley rats. The differences in baseline gene expression for these factors were compared to see how they might affect our capabilities in building predictive models and in elucidating mechanism of toxicity. To address the effects of these two factors, we examined those genes that show significant changes between genders and between strains. The study design consisted of 59 vehicle-treated rat liver samples. Four groups of animals were compared: Male Sprague-Dawley (MSD), female Sprague-Dawley (FSD), male Wistar (MWS), and female Wistar (FWS). The expression intensities were generated with the Affymetrix RGU34 GeneChip® microarray using standard protocols. Differences between baseline expression across gender and strain and the resulting impact of both predictive models and mechanistic analysis were examined. The results show that the effects due to gender are much greater than that of strain, as many more genes were differentially regulated due to gender, including some known to be mediators of the toxic response. Examples of such genes showing high gender specificity include sulfotransferase, hydroxyacid oxidase 3, and carbonic anhydrase 3. These genes show significant modulation in signal intensities between male and female rats. Interestingly, the strain effects are greater in female rats than in male rats, when taken in context of liver toxicity markers identified by Gene Logic. Results also show that removal of the few most gender-specific genes from the toxicity marker set greatly improves the predictive accuracy. These results give us confidence in the ability to both predict a general toxic response and identify the mechanism of toxicity (MOT) across strain much better than gender. However, better overall success in prediction and MOT can be achieved if one discounts the most gender-specific genes. These strategies support our ability to accurately predict female-and Wistar-derived data.

648 LIVER EFFECTS AT THE GENE EXPRESSION LEVEL OF FOOD-FASTING, WATER DEPRIVATION, AND ANESTHETIC AGENT ADMINISTRATION IN UNTREATED RATS.

M. W. Porter, C. G. Chang, M. W. Orr and D. L. Mendrick. *Toxicogenomics, Gene Logic Inc., Gaithersburg, MD.*

Animal-based gene expression experiments reveal a large amount of information relating to the toxic mechanism of compounds. In addition, gene expression has been shown to be a more sensitive predictor of toxicity when measured at the whole transcriptome level than when measured by classical assays. The ability of gene expression-based methods to correctly characterize a toxic response is also dependent on its ability to separate toxicity from other experimental confounding factors such as food-fasting, water deprivation, and anesthetic agent administration within the organ of interest. We recently performed a study where male Sprague-Dawley rats were purposely fasted, deprived of water, and administered one of three anesthetic agents (Carbon Dioxide, Pentobarbital, and Halothane) to determine the gene expression-level changes in the liver. Food-fasting induced a relatively large number of changes that were localized to several metabolic pathways; most notably, Sterol Biosynthesis and Fatty Acid Metabolism (KEGG). Gene expression was also modified by the individual anesthetic agents across time. For example, GADD45-alpha shows no significant changes in gene dysregulation within 30 min of pentobarbital treatment, but is significantly upregulated at 60 min of exposure. In contrast, methallothionein is upregulated by a 5 min exposure and remains upregulated throughout the 60 min exposure period. These results illustrate that in-life standard operating procedures are critical for evaluating gene expression responses and demonstrate that animal husbandry processes and anesthesia selection can influence the expression of genes that are often modulated by cellular perturbations sometimes associated with adverse effects. In addition, the results suggest that the separation of a true test compound-induced toxic response from experiment-derived factors is essential before gene-level predictive and mechanistic assessments can be pursued.

649 CONCORDANCE OF TOXICOGENOMIC PREDICTIONS AND MECHANISTIC ANALYSIS FOR COMPOUNDS TESTED IN BOTH RAT LIVER AND PRIMARY RAT HEPATOCYTES.

M. S. Orr, B. W. Higgs, K. R. Johnson and D. L. Mendrick. *Toxicogenomics, Gene Logic Inc., Gaithersburg, MD.*

Even with an assortment of cell based assays available today, early detection of compounds capable of inducing liver toxicity is still a significant problem. A cell based assay that was able to identify multiple types of liver toxicity and provide richer information regarding the mechanisms of toxicity would improve upon current technologies. Comparisons between toxicogenomic data derived from rat liver or primary rat hepatocytes were evaluated to determine the feasibility of using the combination of rat hepatoyctes and microarray analysis for detecting liver toxicity. Predictive models used in this study were developed based on gene expression information from the ToxExpress® database that is derived from rat liver or primary rat hepatocytes analyzed with the Affymetrix RGU34 GeneChip® set following treatment with a large number of compounds. For the *in vivo* to *in vitro* comparisons, 42 compounds that had been used in both systems were evaluated. A concordance of 74% was observed for the 42 compounds examined, as 31/42 compounds studied were correctly identified as either human/rat liver toxicants or non-toxic agent by the respective predictive models. Notably, the rat hepatocyte models were able to identify a pharmacologically diverse set of compounds capable of inducing steatosis and/or hepatitis such as Valproic acid, Amiodarone, Tetracycline, and Diclofenac, agents that are difficult to detect by conventional techniques. In addition, a comparative analysis of microarray data from rat liver and hepatocytes treated with Clofibrate indicated a very similar response in beta oxidation genes such as Ratacoa1, Cpt1a, Cpt2, Crot, and Ech1 and peroxisome proliferation genes such as Pex11a, Hsd17b4, Decr2, and Abcd3, suggesting a similar biologic response in both biological systems. These results provide evidence that primary rat hepatocyte system in conjunction with microarray analysis is a valuable *in vitro* assay tool for detecting liver toxicants and it also provides precious mechanistic information regarding the observed toxicity.

650 EVALUATION OF THE BIOLOGICAL VARIATION IN GENE EXPRESSION PROFILES IN CULTURED PRIMARY RAT HEPATOCYTES.

C. Wang[1], S. M. Hussain[2], V. Chan[2] and J. M. Frazier[3]. *[1]Microarray Core, Cedars-Sinai Medical Center, UCLA, Los Angeles, CA, [2]ManTech Environ Technology, Inc., Dayton, OH and [3]AFRL/Wright-Patterson AFB, Dayton, OH.*

Cultured primary rat hepatocytes are valuable tools for toxicological research. They are used widely for testing toxicological and pharmacological effects of chemicals and drugs. Although it is known that variation may exist in data generated in animal models, the biological variation exhibited by time-course gene expression profiles has not been fully evaluated. Hepatocytes were harvested from male Fisher 344 rats, plated in 6-well plates and allowed to recover for 24 h. To evaluate biological variations in mRNA expression profiles, total RNA was extracted from cultured primary hepatocytes derived from four different rats over a 24 h period (0, 2, 5, 8, 14 and 26 h) following the recovery period. Gene expression profiles were obtained using the Affymetrix RatTox U34 array. To evaluate the intra-animal reproducibility, gene expression data were obtained for 2 replicate samples at all time points for each rat. Our results indicated that: (1) mRNA levels were above the detection limit (present or marginal calls) for 367 out of 1031 probe sets across all time points in all four animals and 58% (212/367) of these probe sets exhibited statistically significant differences among the four rats (one-way ANOVA, $p < 0.05$), indicative of biological variation in gene expression; (2) for 250 out of 367 detectable probe sets (68%), the mRNA expression levels exhibited statistically significant time-dependent pattern in all four rats using one-way ANOVA ($p < 0.05$); (3) on the other hand, the gene expression for most genes (801 out of 1031 probe sets) were constant across all time points in all four rats; (4) no significant differences in gene expression were found at any time points in replicate samples (ANOVA, $P > 0.05$). Overall, these results strongly suggest that replicate samples of rat hepatocytes for gene expression profiling are highly consistent, however, inter-animal variability is a confounding factor and must be carefully evaluated to make sense of microarray gene expression studies.

651 CLASSIFYING CHEMICAL INDUCED LIVER PATHOLOGY USING GENE EXPRESSION PROFILING *IN VITRO* IN PRIMARY HEPATOCYTES.

S. Baumhueter, L. Brady, G. Day, S. Dunlea, B. Eynon, J. Ferng, S. Fujimoto, R. Idury, K. Jarnagin, K. Kolaja, M. Lee, R. Nair, C. Pearson, A. Roter and A. Tolley. *Iconix Pharmaceuticals, Mountain View, CA.*

We have investigated rat primary hepatocytes as an alternative to *in vivo* studies for the characterization of the toxic liability of various environmental chemicals, therapeutics and toxicants. We have chosen to use global gene expression profiling in this *in vitro* system to identify sets of transcripts (signatures) whose regulation is closely correlated with compound group effects, either mechanism of activity or toxicity, from treated rat livers *in vivo*. Our results show that transcript sets derived from expression profiles *in vitro* and characteristic of Peroxisome proliferator receptor alpha activating compounds can be used to correctly identify the corresponding gene expression profiles generated from liver tissue of rats treated with compounds of the same activity class. This *in vitro* gene set is highly specific in that it recognizes 23 *in vivo* profiles from a database containing over 1500 gene expression profiles. Additionally we have used this *in vitro* system to derive transcript sets that are correlated with a chemicals propensity of inducing cholestasis *in vivo*.

652 CROSS-SPECIES ANALYSIS OF PHENOBARBITAL-INDUCED GENE EXPRESSION CHANGES IN DOG AND RAT.

W. B. Mattes, M. S. Orr and D. L. Mendrick. *Toxicogenomics, Gene Logic Inc., Gaithersburg, MD.*

A fundamental concept in toxicological hazard identification is the evaluation of findings in several different species and the extrapolation of these findings to humans. Adverse findings common to several species would suggest a hazard for humans. On the other hand, a finding unique to one non-human species must be evaluated in the context of whether the underlying mechanism is relevant to humans. Genomic information and technology offers the hope of providing a molecular and cellular basis for evaluating cross-species mechanisms of toxicity. With the advent of microarrays capable of querying canine mRNA such comparisons between dog and rat, the two most common test species, become possible. In a preliminary test of such a comparison, the gene expression responses to phenobarbital treatment were examined in livers from male and female dogs treated for 7 days, and in male rats treated for 2 days. Liver RNA from individual animals was analyzed with Affymetrix microarrays and gene expression changes meeting statistical cutoffs were evaluated. Probeset sequences on the canine array were annotated based upon public domain canine sequences and homology to human and mouse sequences. Despite the obvious hurdles in establishing biologically equivalent treatments and unambiguous homologies between genes across species, clear similarities in gene expression changes were seen. On an individual gene basis, induction of several cytochrome P450 mRNAs, notably those in the Cyp3A family, were seen in both species. When the regulated gene sets were analyzed using Gene Ontology analysis, concordance across species was found in the induction of genes involved in electron transport. Hierarchical cluster analysis clearly identified regulated gene sets within each species, and in the case of the dog, could differentiate males and females. These results point to the value and potential of gene expression analysis for comparing contrasting the mechanisms of toxicity for a compound in several species.

653 USE OF EXPRESS PROFILING™ TO CHARACTERIZE GENE EXPRESSION BIOMARKER SETS FOR LIVER TOXICITY.

J. Monforte[1], G. Vansant[1], P. Pezzoli[1], F. Ferre[1], S. Baumhueter[2], G. Day[2], S. Dunlea[2], B. Eynon[2], M. Fielden[2], S. Fujimoto[2], B. Ganter[2], R. Idury[2], K. Jarnigan[2], K. Kolaja[2], M. Lee[2], R. Nair[2], G. Natsoulis[2], S. Nicholson[2], C. Pearson[2], A. Roter[2] and A. Tolley[2]. *[1]Althea Technologies, Inc., San Diego, CA and [2]Iconix Pharmaceuticals, Inc., Mountain View, CA.*

There is a continuing need for improved methods to predict the toxicological liabilities of new drug candidates. In order to provide more informed methods for evaluating compounds during drug discovery and development, we have identified a large library of biomarkers for important toxicities and mechanisms of action. Gene expression profiles have been generated from tissues from rats treated with more than 580 drugs and environmental chemicals. Analysis of this extensive dataset has revealed specific gene expression patterns or signatures associated with specific mechanisms of toxicity. Relatively small sets of genes, typically 20-50, are adequate to diagnose many toxicities. A number of these gene signatures have been converted into high-throughput gene-expression assays using eXpress Profiling, a quantitative, highly-multiplexed rtPCR process. Using this format we have developed gene sets and assayed a number of specific toxicological endpoints, including DNA damage, bile duct hyperplasia, PPAR-alpha agonist activity and severe liver weight increase, and demonstrated the validity of this method for predicting these specific mechanisms of response in rats. Furthermore, the expression profiles show good correlation with phenotypic evidence. The expression signatures offer the advantage of early stage activity detection and may ultimately provide higher sensitivity than existing histopathological and pharmacological endpoints. The high throughput nature of the eXpress Profiling method offers a practical means for converting a process that we have validated *in vivo* into high throughput *in vitro* screening applications.

654 EARLY CHANGES IN HEPATIC GENE EXPRESSION FOLLOWING EXPOSURE TO THE RODENT CARCINOGEN METHYLEUGENOL.

J. T. Auman, R. D. Fannin, S. O. Sieber, M. L. Cunningham and R. S. Paules. *National Center for Toxicogenomics, NIEHS, Research Triangle Park, NC.*

Methyleugenol is a potent, nongenotoxic rodent carcinogen used widely as a fragrance and flavoring additive that caused liver tumors in both rats and mice in a 2-year exposure study. To determine if the indications of tumorigenic potential of methyleugenol could be detected earlier, we compared hepatic gene expression in male Fischer rats following exposure for 2 weeks with hepatocellular carcinomas obtained from the 2-year rat study. Methyleugenol was administered by gavage for 1 to 14 days, with hepatic gene expression measured at various times during the treatment regimen, as well as 6 months after the last dose. Hepatic gene expression of treated samples or tumors was measured with an Agilent rat 60-mer oligonucleotide microarray compared to time matched controls. We found that during the course of administration, methyleugenol elicited marked changes in hepatic gene expression especially of genes involved in xenobiotic detoxification, stress response and cholesterol homeostasis which were accompanied by changes in clinical chemistry parameters and histopathology indicating biliary and hepatic dysfunction. Following six months without exposure to methyleugenol after the initial daily dosing for 14 days as described above, differentially expressed genes were still detected, with several of the gene changes having been previously associated with hepatocellular carcinomas. We also detected gene changes in common between methyleugenol-induced hepatocellular carcinomas and those found during methyleugenol treatment and 6 months after the last dose. This study clearly demonstrates long-lasting changes in hepatic gene expression following exposure to methyleugenol, suggesting that the tumorigenic potential of a compound might be predicted before the emergence of neoplastic lesions.

655 IDENTIFICATION OF MOLECULAR TARGETS OF CURCUMIN IN RAT LIVER BY OLIGONUCLEOTIDE MICROARRAY.

V. Misra[1], R. Thimmulappa[1], K. Mai[1], L. L. Adams-Campbell[2] and S. Biswal[1]. *[1]Johns Hopkins University, Baltimore, MD and [2]Howard University, Washington DC, WA.*

Curcumin is an important component of turmeric, a rhizome of the plant Curcuma longa. It is commonly used as a spice, flavoring agent, food preservative, and coloring agent. The FDA has classified turmeric among substances as Generally Recognized as Safe (GRAS). *In vitro* and *in vivo* studies have demonstrated anti-inflammatory, antioxidant, antifungal, and anticarcinogenic properties of curcumin. Human phase I trials found no toxic effect of curcumin even at a dose as high as 8000 mg/day for 3 months. Deciphering the molecular targets of curcumin in liver will aid in its development as a chemopreventive agent. After feeding rats on 1% curcumin for 14 days, liver was excised and processed for RNA extraction. Gene expression study was done by oligonucleotide microarray analysis using Affymeterix Genechip Rat expression set 230A. The data was analyzed using MAS 5.0 and Data Mining Tool 3.0 (Affymetrix, CA). The results revealed the upregulation of genes coding for antioxidants and detoxification enzymes. Detoxification enzymes included UDP-glucuronosyltransferase, glutathione-S-transferase, aflatoxin B1 aldehyde reductase, epoxide hydrolase, NAD(P)H:menadione oxidoreductase. Among the antioxidants, glutathione related enzymes such as glutathione reductase, glutathione synthetase, and glutamate-cysteine ligase were elevated. In addition, heat shock proteins and several genes encoding for proteins associated with lipid metabolism were increased. Moreover, curcumin repressed certain genes associated with detoxification (CYP1A2, sulfotransferase), inflammation, fatty acid metabolism (carnitine palmitoyltransferase 1 alpha, hydroxysteroid dehydrogenase, 11 beta type 1) and cell cycle. In conclusion, the result provides a comprehensive list of molecular targets of curcumin in rat liver and underscores its beneficial effects. (Supported by Howard/Hopkins Partnership grant)

656 NORMAL HUMAN DENDRITIC CELLS: A TOOL TO STUDY ALLERGIC AND IMMUNOLOGICAL REACTIONS *IN VITRO*.

M. Klausner, S. Ayehunie, S. Lamore, K. Bellavance, R. Lappen, P. Hayden and J. Sheasgreen. *R & D, MatTek Corporation, Ashland, MA.*

Dendritic cells (DC) or Langerhans cells (LC) are immunological cells of the body which play a key role in allergic reactions and infectious diseases. However, widespread experimental use of DC has not occurred since previously-used methods of harvesting DC directly from tissue result in poor yields, short survival time, and rapid phenotypic changes. Currently, we have developed a new method of generating DC from CD34+ progenitor cells harvested from human umbilical cord blood. This method resulted in an average increase of 205 ± 86 fold (n = 15) in DC number versus previous methods. The DC express CD1a, HLA-DR, and co-stimulatory molecules (CD40 and CD80), and can be cultured for up to 36 days with no significant change in cell number or phenotype. Transmission electron microscopy showed the presence of Birbeck granules, a key ultrastructural marker of DC, over the duration of the culture period. The DC contain plasmacytoid (CD123+/cd11c-) and myeloid (CD11c+/cd123+) populations and can be frozen and recovered with a *via*bility of 80-90%. Exposure of the DC to external stimuli such as lipopolysaccharide (LPS), phorbol-12-myristate-13-acetate (PMA), and interferon-γ (IFN- γ), caused a reproducible (n = 4), high level of gene and protein responsiveness in terms of IL-12, macrophage inflammatory protein (MIP)-1α, MIP-3α, IL-6, and TNF-α expression. DC pulsed with the neo-antigen, keyhole-limpet hemocyanin (KLH), or the recall-antigen, tetanus toxoid, induced autologous T-cell proliferation as measured by 5-bromo-2-deoxyuridine (BrdU) incorporation. Functionally, the DC were also shown to: 1) express foreign genes following transfection, 2) migrate in response to chemotactic factors such as granulocyte/monocyte colony stimulating factor (GM-SCF) and MIP-3α, 3) become infected following exposure to HIV-1. Thus, these cells are likely to be useful in a broad variety of allergic and immunological studies.

657 DOSE RESPONSE ANALYSIS OF ALLERGEN-INDUCED GENE EXPRESSION CHANGES IN DENDRITIC CELLS.

L. A. Gildea[1], C. A. Ryan[1], B. C. Hulette[1], R. J. Dearman[2], I. Kimber[2] and F. Gerberick[1]. *[1]Procter & Gamble, Cincinnati, OH and [2]Syngenta CTL, Macclesfield, United Kingdom.*

Allergen uptake and processing by resident skin dendritic cells are critical steps in the induction of allergic contact dermatitis (ACD) and therefore are ideal events to model for study of the allergic immune response. Microarray analysis of human peripheral blood-derived dendritic cells (PBMC-DC), a surrogate for skin DC, has revealed many changes in gene expression following allergen treatment. Genes identified *via* this approach are potential targets for the development of an *in vitro* method for skin sensitization testing as well as provide a mechanistic understanding of the immune recognition phase of ACD. However, prior to final gene selection, analysis of their sensitivity, selectivity, and dynamic range must be evaluated. Therefore, we analyzed a select number of genes by real-time PCR analysis to determine their usefulness as markers for contact allergy and to reproduce previous microarray data using additional blood donors. PBMC-DC were treated for 24 hours with various doses of the contact allergen, dinitrobenzene sulfonic acid (DNBS), and RNA was extracted for use in real-time PCR reactions. Specific primers were designed for selected genes and mean relative fluorescence units (RFU) were calculated using individual well readings within a given PCR run and then converted to mean fold changes comparing mean RFU in control (vehicle-treated)samples versus mean RFU in treated samples. DNBS treatment induced changes in the expression of numerous genes associated with immune function, such as interleukin (IL)-8, CD86, IL-1 receptor antagonist, CD1E, interferon stimulated gene 20, and cathepsins H and L. Similar expression profiles were seen with other allergens, such as 3, 4-diethoxy-3-cyclobutene-1, 2-dione, NiSO4, oxazalone, and propyl gallate. Real-time PCR analysis of dose response studies has identified a number of additional genes whose role in immune regulation are unclear yet fit the selection criteria for sensitivity, dynamic range, and reproducibility and thus will be analyzed further for their specificity for contact allergy.

658 COMPARISON OF THE RESPONSE OF DENDRITIC CELLS DERIVED FROM CORD BLOOD CD34+ AND FROM CD14+ MONOCYTES TO THE CONTACT SENSITIZER NICKEL.

F. Boisleve[1], N. Aubert[2], J. Bernard[2], M. Pallardy[1] and S. Roemer[1]. *[1]Immuntoxicology, Inserm U461, Chatenay-Malabry, France and [2]laboratoire de therapie cellulaire, Institut Jean Godinot, Reims, France.*

Dendritic cells (DCs) consist of a heterogeneous population. Two distinct hematopoietic lineages, CD14+ monocytes and CD34+ stem cells, when cultured under desirable conditions, differentiate into DCs. These immature DCs can be activated *in vitro* by a variety of simple chemicals such as haptens and several metals. The purpose of this work was to compare three differents DCs models in order to know if they respond identically to such stimuli (1) CD34+ cells differentiated with GM-CSF, TNF-α and Flt-3L during 7 days (CD34+-DCs), (2) CD14+ cells differentiated with GM-CSF and IL-4 during 6 days (MoDCs) and (3) CD14+ cells differentiated with GM-CSF, TGF-? and IL-4 during 6 days (TGF-α+-MoDCs). We examined, *in vitro*, the effects of a metal, Nickel (Ni2+) known to be involved in contact allergy, on the expression of different markers: CD86, a co-stimulatory molecule, CD83 and CCR7 which is known to participate in the emigration of DCs from peripheral tissue to lymph nodes. Flow cytometric analysis revealed that immature DCs derived from monocytes expressed more CD86 than CD34+-DCs. When these cells were stimulated with Ni2+, CD86 was expressed at higher levels

on CD34+-DCs (13 fold) and on MoDCs (10 fold) than on TGF-α+-MoDCs (5 fold). CD83 was expressed at higher levels on immature TGF-α+-MoDCs (14.5 %) compared to immature CD34+-DCs (1.6 %) or immature MoDCs (5.3 %). Interestingly, Ni2+ induced a strong expression of CD83 in all three models [CD34+-DCs (48.6 %), MoDCs (93.6 %), TGF-α+-MoDCs (97.8 %)]. Moreover, CCR7 was also induced after Ni2+ stimulation and was more expressed on CD34+-DCs (34.4%) than on other models [TGF-α+-MoDCs (20.3%), MoDCs (9.1 %).].

659 INTRACELLULAR CYTOKINE STAINING PATTERNS OF ALLERGEN ACTIVATED LYMPH NODE CELLS (LNC).

N. Humphreys, R. Skinner, R. J. Dearman and I. Kimber. *Syngenta CTL, Macclesfield, United Kingdom.*

Topical exposure of BALB/c strain mice to the contact allergen 2, 4-dinitrochlorobenzene (DNCB), or to the respiratory allergen trimellitic anhydride (TMA) induces selective type 1 or type 2 cytokine secretion profiles, respectively. The contributions of CD4 and CD8 cells to these cytokine phenotypes have been investigated using flow cytometry for intracellular cytokine staining. Thirteen days following the initiation of exposure to chemical, auricular lymph nodes were excised, a single cell suspension prepared and cells cultured for 3h in the presence of mitogen and brefeldin A. Cells were stained with anti-CD4 or CD8 antibodies, fixed, permeabilized with saponin and stained with anti-cytokine antibodies. An increased frequency of interleukin (IL-4)+CD4+ LNC was recorded following TMA exposure compared with DNCB treatment (2.4% +/-0.5 and 0.5% +/-0.3, respectively, n=5 experiments). In all LNC populations less than 0.1% of CD8+ LNC expressed IL-4. Exposure to both TMA and DNCB resulted in marked increases in interferon (IFN)-γ+ CD8+ cells (47% +/-7.3 and 48.4% +/-9, respectively), compared with vehicle-treated controls (19.5% +/-3.9). In all populations, less than 5% CD4+ LNC expressed detectable IFN-γ. This pattern of cytokine staining was also observed for a further pair of contact and respiratory allergens; formalin and fluorescein isothiocyanate (FITC). Relatively low levels of IL-4+CD4+ LNC were recorded following treatment with formalin, whereas exposure to FITC resulted in increased frequency of these cells (0.9% +/-0.3 and 8.5% +/-0.6, respectively; mean and range of 2 experiments). Similar numbers of IFN-γ+ CD8+ cells were observed following topical application of either chemical (52% +/-18 and 65% +/- 11, respectively). Thus, topical exposure of mice to either class of chemical allergen results in increased numbers of IFN-γ+CD8+ cells, but treatment only with respiratory allergens is associated with an elevated frequency of IL-4+CD4+ cells. The vigorous IFN-γ production by contact allergen-activated LNC is likely to be regulated at the level of secretion.

660 THE SENSITIZING POTENTIAL OF PEANUT PROTEINS IN FOUR DIFFERENT MICE STRAINS.

L. M. Knippels[1], A. H. Penninks[1] and G. A. Bannon[2]. *[1]Experimental Immunology, TNO Nutrition and Food Research, Zeist, Netherlands and [2]Product Characterisation Center, Monsanto, St. Louis, MO.*

One aspect of the evaluation of genetically engineered crops prior to introduction to the public involves the determination of the potential allergenicity of the newly introduced protein(s). While present methods can identify known allergens or proteins with allergen characteristics, validated animal models to study the potential allergenicity of novel proteins are not yet available. In this study, four mice strains (Balb/C, C3H/HeJ, A/J, and C57/BL6) were compared for their ability to mount an IgE response to a peanut protein extract. Mice (n=5) were intraperitoneally (ip) sensitized with 0.1, 0.5 or 2.5 mg peanut-proteins with or without alum at days 0, 7 and 21. Serum samples obtained at day 0, 14 and 28 were tested for antigen specific IgE, IgG1 (both T-helper-2 mediated) and IgG2a (T-helper-1 mediated) antibody levels using a passive cutaneous anaphylaxis assay and enzyme-linked immunosorbent assay (ELISA). Clinical symptoms were also obtained by performing an active systemic anaphylaxis (ASA) test at day 45. Parenteral administration of peanut proteins, with or without alum, resulted in peanut protein-specific IgE, IgG1 and IgG2a antibody responses in all strains tested, although the responses were more pronounced upon co-administration with alum. In all strains the T-helper-2 mediated antibody responses (IgE and IgG1) were more vigorous compared to the IgG2a responses. The best antibody responses were observed in the C3H/HeJ mice followed by the A/J, Balb/C and C57/BL6 mice. In addition, a good correlation between the levels of specific IgE and the clinical symptoms, as determined by the ASA test, was observed in the C3H/HeJ, A/J and C57/BL6. Sera from peanut protein sensitised Balb/C mice elicited strong PCA reactions but little or no clinical reactions. From these studies it was concluded that the most sensitive strains were the C3H/HeJ and A/J mice. Whether these strains will also be able to distinguish between strong-, weak-, and non-allergenic proteins will be part of future research.

661 EVALUATION OF PROTEIN ALLERGENIC POTENTIAL : STUDIES IN MICE.

S. Stone, H. Caddick, R. J. Dearman and I. Kimber. *Syngenta CTL, Macclesfield, United Kingdom.*

There is a growing interest in the development of approaches for the characterization of the allergenic potential of proteins. Although most foreign proteins are immunogenic (able to induce IgG antibody responses), relatively few are important food allergens with the capacity to provoke IgE antibody production and immediate type hypersensitivity responses. The majority (90%) of food allergic reactions are due to ingestion of 8 main foodstuffs; eggs, cows milk, peanuts, nuts, shellfish, tree nuts, wheat and soybeans. We have examined the allergenic potential of a range of proteins as a function of the induction of IgE antibody responses following systemic (intraperitoneal; ip) exposure of BALB/c strain mice. Animals were exposed to proteins from the major allergenic food groups peanut, egg and cows milk (peanut agglutinin, ovalbumin [OVA] and bovine serum albumin [BSA]); to patatin, the major allergen in potato, a foodstuff with a relatively low allergenic potential, and to potato agglutinin, a material considered to lack allergenicity. Specific IgE antibody was measured by homologous passive cutaneous anaphylaxis assay and specific IgG antibody measured by enzyme-linked immunosorbant assay. Each of the proteins induced IgG antibody responses at all doses tested (0.2% to 10%), although there was some variation with respect to vigor of IgG responses. High titer IgE antibody was provoked by peanut agglutinin, OVA and BSA whereas patatin stimulated relatively low titer IgE antibody over the dose ranges examined. Potato agglutinin stimulated low titer IgE antibody at the highest dose (10%) only. Importantly, differences in IgE antibody production were observed against a background of equivalent immunogenicity (IgG antibody responses), with the vigor of the IgE response broadly reflecting the relative sensitizing potential of human allergens. These data suggest that the measurement of antibody (IgE) responses in BALB/c mice may allow discrimination between allergens and those materials that apparently lack significant allergenic potential.

662 PROGRESS IN THE EVALUATION OF AN IN-BRED RAT STRAIN ("ASTHMATIC RAT") FOR PREDICTING THE ALLERGIC POTENTIAL OF FOOD AND OTHER PROTEINS.

D. M. Hinton, M. Lorenzo, S. B. Harper and S. Francke-Carroll. *CFSAN, USFDA, Laurel, MD.*

Inbreeding of the Sprague-Dawley rat strain for dyspenic reactions to challenge with egg albumin (OVA) was first reported by Holme and Piechuta (Immunology, **42**, 1981) for evaluation of the efficacy of anti-asthmatic drugs. Using a protocol consisting of subcutaneous sensitization with one mg of protein in alum followed by i.p. challenge at two to three weeks, we evaluated respiratory and other physiologic changes as well as the histopathology after challenge to various food proteins, i.e. purified *ara*-H2 from peanuts, glycinin and beta-conglycinin from soy, tropomyosin (TROPO) from chicken muscle, and OVA. Also, we have compared the respiratory responses of the Asthmatic rat to the normal out-bred Sprague-Dawley strain using the Buxco Electronics (Troy, NY) non-invasive whole body plethysmograph after challenge with 10 μg of OVA. The respiratory responses are typically characterized by rapid or labored breathing 10 to 20 min after challenge. Asthmatic female rats are considered to be more sensitive than males responding maximally to one μg of OVA compared to 10 μg for male Asthmatic rats. Neither female nor male normal Sprague-Dawley rats responded to the 10 μg OVA challenge. Using a time/incremental dose protocol (one to ten μg) for the four proteins, greater responses were seen for OVA than with *ara*-H2, the soy proteins, and tropomyosin with female rats at the one μg dose. Tentative ranking at the low doses would be OVA>*ara*-H2>SOY>>TROPO. At the 10 ug dose, a tentative ranking would be *ara*-H2>OVA>SOY>>TROPO. The tropomyosin was chosen as a non-allergen protein control. No responses were seen at any of the doses tested with this protein. In conclusion, the Asthmatic rat is a promising animal model for evaluating the allergic potential of proteins.

663 INFLUENCE OF ENDOTOXIN ON IGE RESPONSES TO PROTEIN ALLERGENS.

R. J. Dearman and I. Kimber. *Syngenta CTL, Macclesfield, United Kingdom.*

Specific IgE antibody is the key biological mediator of immediate type hypersensitivity reactions including food allergy and asthma. It has been demonstrated previously that the endotoxin content of protein allergen can impact significantly on the quality of immune response provoked. Thus, intranasal (in) exposure of mice to the food allergen and asthmagen ovalbumin (OVA) in the presence of low levels of endotoxin (0.1μg per mouse) enhanced type 2 responses including IgE production and airway hyperreactivity. Identical treatment in the presence of high dose endotoxin (100μg per mouse) resulted in a type 1 response, with inhibition of IgE anti-

body expression. We have demonstrated previously that systemic (intraperitoneal; ip) administration of OVA to BALB/c strain mice stimulates a vigorous IgE antibody response. We have now investigated the impact of exogenous endotoxin on the induction of anti-OVA IgE antibody production. Animals (n=5) received 1% OVA supplemented with various concentrations of endotoxin (lipopolysaccharide from E coli serotype 055 B5) by ip injection on days 0 and 7. Seven days later, serum samples were analyzed for specific IgE antibody by homologous passive cutaneous anaphylaxis assay. Exposure to 1% OVA resulted in high titer IgE antibody (1 in 32) that was unaffected by co-administration of endotoxin (0.025 to 6.25μg per mouse), doses that encompass those reported to influence IgE antibody responses induced by in exposure to OVA. Endogenous endotoxin content of OVA preparations was measured by Limulus Ambeocyte Lysate (LAL) assay with a chromogenic end point. There was some inter-batch variation in endotoxin content of OVA, with samples varying from approximately 0.1 to 1μg per mg of protein. These concentrations equate to an endogenous endotoxin dose of 0.25 to 2.5μg per mouse. These data suggest that IgE responses to protein allergen administered by the ip route are less affected by endotoxin content than are those induced by in exposure to protein. Furthermore, the amounts of endogenous endotoxin contained within the protein preparation are unlikely to affect IgE responses.

664 FUNCTIONAL REGULATION OF HUMAN MICROSOMAL EPOXIDE HYDROLASE BY ALTERNATIVE GENE PROMOTERS AND SPLICING VARIANTS.

S. Liang, C. Hassett and C. Omiecinski. *Veterinary Science, The Pennsylvania State University, University Park, PA.*

The human microsomal epoxide hydrolase (EPHX1) is a key enzyme involved in bioactivation or detoxification of environmental and pharmaceutical compounds. Previously, we identified two alternative splicing variants in human tissues that differ in the noncoding exon 1 (Ex1 and Ex1b) region. The Ex1 variant is specifically expressed in the liver whereas the Ex1b variant is expressed in most human tissues. In this study, the alternative promoters for Ex1 and E1b were cloned and characterized. Transient transfection of the reporter constructs demonstrated that Ex1 promoter was active in the human hepatoma cell lines HepG2 and Huh7 but not in transformed kidney cells 293A, whereas the Ex1b promoter was active in all cells. These results are in accordance with our finding that the Ex1 variant is specifically expressed in the liver tissue, suggesting that HepG2 and 293A can be used as model systems to study the differential regulation of Ex1 and Ex1b variants. Mutagenesis analyses indicated that 200 bp and 300 bp regions upstream of Ex1 and Ex1b, respectively, contributed to the basal transcription of these two variants. Multiple cis-elements in the -200~Ex1 region were further identified as associated with the control of Ex1 variant expression. The cis-elements include a potential GATA binding site (-118 to -98), a CCAAT-binding factor site (-88 to -68), and a GC-rich site (-48 to -28). Several other EPHX1 splicing variants are reported in NCBI AceView data base. In the process of further characterizing these variants, we identified two other EPHX1 splicing variants by RT-PCR. One variant possesses an alternative exon 1 (Ex1b'), representing an extended sequence of Ex1b, and was detected in all cell lines tested. Another variant detected only in Huh7 cells contains Ex1b as exon 1 and possesses a deletion of exon 4. Together with our previous studies, these results indicate that functional regulation of EPHX1 is subjected to a complex program involving genetic polymorphism and altered regulation of gene expression. Supported by HIEHS grant ES-04978

665 MOLECULAR MECHANISMS OF SUBCELLULAR LOCALIZATION OF HUMAN GLUTATHIONE REDUCTASE.

L. K. Rogers, T. Tamura, B. J. Rogers, T. N. Hansen, S. E. Welty and C. V. Smith. *Columbus Children's Research Institute, Columbus, OH.*

Human Glutathione reductase (hGR) is encoded by a single nuclear gene, but GR protein and activities are found in cytoplasmic, mitochondrial, and nuclear compartments. Mitochondrial localization is attributed to an N-terminal, 43 amino acid mitochondrial targeting signal (MTS) that is coded by a 129 bp sequence immediately 5′ to the GR coding sequence, but the mechanisms involved in nuclear localization are unknown. Transfections of 293T cells with hGR cDNA without the MTS increased GR activities in cytoplasm only, whereas constructs including the MTS increased GR activities in the mitochondria and nuclei as well as the cytoplasm. A fusion protein consisting of MTS-GFP directed GFP immunofluorescence to the mitochondria. Examination of the GR amino acid sequence revealed a putative NLS in the C-terminal region of GR (416KRKTK420), but mutation of this sequence did not abolish nuclear GR localization. Deletion mutations of the MTS-hGR were prepared and transfected into 293T cells, GR activities were measured, and cells were analyzed by confocal microscopy. The (aa 2 to 15) MTS deletion gave lower mitochondrial GR activities than did MTS-hGR, but nuclear activities were not affected. The (aa 2 to 30) deletion gave higher cytoplasmic GR activities than did MTS-hGR, but the nuclear and mitochondrial activities were not increased above the activities in native cells. The deletion of (aa 42 to 50) which omits the last aa of the MTS and first 8 aa of hGR, including the second ATG, gave lower cytoplasmic, higher mitochondrial, and equivalent nuclear activities than did MTS-hGR. In contrast, a construct containing a point mutation of the second ATG gave lower cytoplasmic, unchanged mitochondrial and higher nuclear activities than did MTS-hGR. In conclusion, mitochondrial expression of GR is directed by the MTS, but nuclear localization of GR is determined in part by elements of the MTS between aa 15 and 30. The mechanisms through which cells may modulate subcellular expressions of GR, as in response to metabolic or toxicant-induced stresses remain to be studied.

666 CHARACTERIZATION OF FOUR MERCAPTURIC ACID URINARY METABOLITES OF 3-BUTENE-1, 2-DIOL.

S. L. Christopher and A. A. Elfarra. *Center for Molecular and Environmental Toxicology and Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI.*

3-Butene-1, 2-diol (BDD) is a major metabolite of 1, 3-butadiene (BD), a known rodent and human carcinogen. *In vitro* studies showed BDD can be readily oxidized to yield hydroxymethylvinylketone (HMVK), a Michael acceptor. In this study, male B6C3F1 mice and Sprague-Dawley rats were given BDD (25 - 200 mg/kg, i.p.) and the amounts of four HMVK derived mercapturic acids (4-(N-acetyl-L-cystein-S-yl-)-1-hydroxy-2-butanone [HB], 4-(N-acetyl-L-cystein-S-yl-)-1, 2-dihydroxybutane [DHB], 3-(N-acetyl-L-cystein-S-yl-)propan-1-ol [POH], and 3-(N-acetyl-L-cystein-S-yl-)propanoic acid [PA]) were measured. Mercapturic acids were extracted from urine, derivatized, and then quantified by GC/MS. In previous studies, urine DHB levels were measured to assess human BD exposure. While DHB, POH and PA have been reported as mouse or rat urinary BD metabolites, HB has not been previously identified as a metabolite of BD or its metabolites. The total amounts of the mercapturic acids excreted after BDD exposure were dose-dependent and similar between mice and rats given equivalent doses of BDD. The percentage of the dose present as the total mercapturic acids significantly increased with decreasing the dose in both species. The major mercapturic acid excreted by mice was DHB whereas rats excreted equivalent amounts of DHB and HB. The urine POH and PA amounts increased after BDD treatment but the levels of these two mercapturic acids were significantly lower in both species relative to DHB and HB and mice excreted more POH than PA. The results provide evidence that HMVK formation represents a prominent route for BDD metabolism in both mice and rats. This finding is significant because HMVK has potential to bind to macromolecules and disrupt cellular function thus contributing to BD toxicity. The observed species difference in BDD metabolic profile is consistent with the reported variability of the human DHB biomarker data. Collectively, the results may improve human biomonitoring studies. (supported by ES06841)

667 MECHANISTIC INVESTIGATION ON CYTOTOXICITY OF STYRENE.

J. Zheng, J. Chung and W. Yuan. *Pharmaceutical Sciences, Northeastern University, Boston, MA.*

Styrene (ST) is one of the most important industrial chemicals widely used in the production of many commercial polymers. Human exposures occur from such diverse sources as automobile exhaust, cigarette smoke, carpets, heating systems, painting and even food packaging. ST has been found to be toxic to respiratory system. Formation of styrene epoxide has been suggested to be initial step for cytotoxicity of ST, and CYP2E1 has been speculated as one of possible cytochrome P450 enzymes responsible for bioactivation of ST. To probe the role of CYP2E1, we exposed transgenic cell line expressing CYP2E1 and cHo1 cell line (wild-type <2E1-/->) to ST and found CYP2E1 cells were more susceptible to ST than wild-type cells, indicating participation of CYP2E1 in ST cytotoxicity. To investigate the structure-activity relationships of ST, CYP2E1 cells were exposed to ST analogs ethyl benzene (EB), vinylcyclohexane (VH), and ethylcyclohexane (EH). EB and VH were found as toxic as ST, but EH showed no toxicity to the cells. This indicates that unsaturation (i.e. olefins) of ST analogs is required for their toxicity. Epoxides are known to be derived from olefins. We propose that EB and VH are metabolized to corresponding epoxides, and the resulting epoxide metabolites cause cell injury. To probe the role of epoxide metabolites in their cytotoxicity, we used glutathione derivatives and epoxide hydrolaes inhibitors as modulators. Ethyl glutathione ester was found to decrease cytotoxicity of ST, EB and VH, while soluble epoxide hydrolase inhibitors as well as microsomal epoxide hydrolase inhibitors dramatically increased cytotoxic effect of ST and the derivatives. In conclusion, CYP2E1 is an enzyme responsible for bioactivation of ST. Unsaturation is a critical structure required for ST cytotoxicity, and epoxide metabolites may play an important role in ST-induced cytotoxicity.

668 METHOTREXATE (MTX) INDUCTION OF SULFOTRANSFERASES.

G. Chen. *Physiological Sciences, Oklahoma State University, Stillwater, OK.*

Phase I and phase II drug metabolizing enzymes are responsible for the detoxification of toxic compounds in food and drugs. Sulfotransferases (STs) belong to the phase II drug metabolizing enzymes. ST catalyzed sulfation is an important pathway in the metabolism of hydroxyl containing chemicals. Studies on drug metabolizing enzymes have focused mainly on the liver. Relatively less is known regarding detoxification processes in non-hepatic tissues. There have been reports on hormone regulation of STs since the 1980's. Xenobiotic induction of STs, especially intestinal STs, has not been well studied compared to other drug metabolizing enzymes. We have recently studied hepatic and intestinal STs induction by anti-cancer drugs tamoxifen (TAM), retinoic acid (RA), and methotrexate (MTX). Enzymatic assay, Western blot, and RT-PCR methods have been used to study changes in protein and mRNA levels after drug treatment in male and female Sprague-Dawley rats (10-12 weeks old) and in human Hep G2 and Caco-2 cell lines. The anti-folate, apoptosis inducing drug MTX was shown for the first time to be a novel xenobiotic inducer of rat liver and intestinal STs and human STs in Hep G2 and Caco-2 cells. The induction was found to be in transcription level. MTX has not been shown to induce other drug metabolizing enzymes. Like TAM and RA, intestinal inductions were found to be greater than the inductions found in liver. Further more, folic acid effects on MTX induction of STs were studied. Folic acid does not induce STs, but it can inhibit MTX induction of STs in a concentration-dependent manner. Currently, possible roles of folate receptor (FR) in the MTX induction of STs are being investigated.

669 BIOCHEMICAL COMPARISON OF ZEBRAFISH AND HUMAN ALDH2: USE OF ZEBRAFISH AS A MODEL FOR HUMAN ACETALDEHYDE METABOLISM AND TOXICITY.

N. Lassen[1], T. Estey[1, 2], V. Vasiliou[1, 2], R. Tanguay[3] and A. Pappa[1]. *[1]Pharmaceutical Sciences, UCHSC, Denver, CO, [2]Pharmaceutical Biotechnology, UCHSC, Denver, CO and [3]Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Ethanol is metabolized to acetaldehyde mainly by the alcohol dehydrogenase pathway (ADHs) and to a lesser extent by microsomal oxidation (CYP2E1) and the catalase-H_2O_2 system. Acetaldehyde, which is responsible for some of the deleterious effects of ethanol, is further oxidized to acetate by aldehyde dehydrogenases (ALDHs). The mitochondrial ALDH2 is the most effective ALDH that metabolizes acetaldehyde. The aim of this study was to clone, express and characterize the biochemical properties of the zebrafish (*Danio rerio*) ALDH2, in order to validate the use of this freshwater teleost as a model for human ethanol toxicity. We have cloned and sequenced the zebrafish ALDH2 cDNA, which is 1, 892 bp excluding the poly (A^+) tail, and has 5′ and 3′ untranslated regions of 5 bp and 335 bp, respectively. Zebrafish ALDH2 encodes a protein of 516 amino acids, including the first methionine (MW = 56, 562), which is approximately 75% identical to mammalian ALDH2 proteins. Western blot analysis showed that zebrafish ALDH2 is expressed at high levels in muscle, heart and brain with moderate expression in liver, eye and swim bladder. Recombinant zebrafish and human ALDH2 proteins were expressed using the baculovirus system and purified using single-step affinity chromatography with Sepharose-5′AMP 4B. Purification of human and zebrafish ALDH2 revealed single protein bands at the expected molecular weight of 56 kDa on SDS-PAGE. The zebrafish ALDH2 was found to be catalytically active with acetaldehyde (K_m = 11.5 μM) and propionaldehyde (K_m = 6.1 μM), and similar kinetic constants were found with the human recombinant ALDH2 protein (K_m = 3.2 μM and 2.4 μM, respectively). Based on the results of this study, the zebrafish is a reasonable model for studying acetaldehyde metabolism and toxicity. This work was supported by NIH R01 AA11885 and NIH R21 AA12783 Grant.

670 REGIO-SPECIFIC (ω TO ω-6) LAURIC ACID HYDROXYLATION IN HUMAN RECOMBINANT AND PEROXISOME PROLIFERATOR-TREATED JUVENILE CATFISH MICROSOMES.

M. L. Haasch[1, 2], A. W. Ford[1] and J. C. Allgood[1]. *[1]RIPS, ECHR, Environmental Toxicology Research Program, The University of Mississippi, University, MS and [2]Pharmacology, The University of Mississippi, University, MS.*

Most studies of lauric acid hydroxylation have focused on only two hydroxylation products, ω and ω-1. Modifications of an HPLC method (Lemaire et al., 1992) to include GC-MS (Buhler et al., 1997) have allowed more sub-terminal hydroxylation products including ω-2 to ω-6 to be quantified. Previously we have shown that in male rat liver microsomes, ω to ω-2 hydroxylation products of lauric acid, significantly increase in response to peroxisome proliferator chemicals (PPCs). Interestingly, adult catfish exposed by i.p. injection, to the PPCs, clofibrate (CLO) or the more potent ciprofibrate (CPR), did not exhibit any changes in hepatic microsomal lauric acid hydroxylase (LA-OHase) activities, although kidney microsomal ω and ω-6 products were increased significantly. In rainbow trout, ω-1 lauric acid hydroxylation is catalyzed by CYP2K1 and ω-6 by CYP2M1 (Buhler and Wang-Buhler, 1998). Induction of CYP2K1/2M1-like immunoreactive proteins in catfish by 48 hr i.p. injection of CLO and CPR has been shown (Haasch, 1996). Waterborne, 48 hr exposure of juvenile channel catfish (*Ictalurus punctatus*; size range 7.24 g to 34.32 g) to 1 ppm CLO (4.12 nM) or 2, 4-dichlorophenoxyacetic acid (2, 4-D; 4.52 nM) produced significant increases in both ω-1 and ω-5 hydroxylation products. Unexpectedly, ω-1 activity was absent in juvenile control and vehicle (DMSO) treated fish although we have previously shown that adults have relatively high ω-1 activity (Haasch, 1998). Regio-specific lauric acid hydroxylation was also investigated in human recombinant microsomes for isozyme control, CYP4A11 and CYP2E1. There is potential for sub-terminal hydroxylation products to act as second messengers in cellular signal transduction or to interfere with steroid biotransformation. These hypotheses will be investigated in future research. (Work supported by ES07929)

671 METABOLISM OF TATTOO PIGMENT YELLOW 74 USING RAT LIVER MICROSOMAL PROTEIN OR XANTHINE OXIDASE.

Y. Cui[1, 3], N. V. Gopee[1, 3], F. E. Evans[2], L. H. Couch[1, 3], M. I. Churchwell[1], D. R. Doerge[1] and P. C. Howard[1, 3]. *[1]Division of Biochemical Toxicology, NCTR, USFDA, Jefferson, AR, [2]Division of Chemistry, NCTR, USFDA, Jefferson, AR and [3]NTP Center for Phototoxicology, NCTR, USFDA, Jefferson, AR.*

Tattooing has increased in popularity in the past decade and is now considered a common form of personal artistic expression. Recent publications point out the increase in tattoo popularity has been accompanied by a change in the colorant in tattoo inks from inorganic salts to organic-based pigments. Very little is known concerning the pigments or combination of pigments in tattoo inks, or the biological availability, metabolism or toxicity of these pigments. In these initial studies, we examined the metabolism of Pigment Yellow 74 (PY74; CI 11741), a common monoazo pigment in yellow tattoo inks. Oxidative metabolism of PY74 was determined following aerobic incubation of PY74 with liver microsomes isolated from 3-methylcholanthrene treated male F344 rats. PY74 solubility in the incubations was increased by inclusion of bovine serum albumin to 10 mg/mL. Consumption of PY74 and formation of metabolites was linear for up to 30 min at 0.1 mg/mL microsomal protein. The predominant metabolite was isolated by HPLC and identified as a ring hydroxylation product (4-hydroxylation on the 2-methoxyaniline ring) using NMR and mass spectral (MS) methods. The identification of the second metabolite and determination of the CYP450 specificity of PY74 metabolism are in progress. PY74 contains a nitro group on the 2-methoxy-4-nitroaniline group that should be available for nitroreduction. PY74 was incubated with 0.2 University/mL xanthine oxidase under hypoxic conditions and the products were extracted. The nitroreduction of PY74 to the amino-derivative (NH_2-PY74) of PY74 was detected by comigration on HPLC (UV-VIS and fluorescence detection) with authentic NH_2-PY74, and additionally confirmed by MS analysis. These results demonstrate that PY74 can be metabolized by both aerobic oxidative and anaerobic nitroreductive pathways, and that these monoazo tattoo pigments should not be considered as inert chemicals.

672 REGULATION OF GLUCOCORTICOID-INDUCIBLE RAT HYDROXYSTEROID SULFOTRANSFERASE GENE EXPRESSION BY LIVER-ENRICHED TRANSCRIPTION FACTORS.

M. Alipour, J. R. Smigelski, A. Weckle and M. Runge-Morris. *Inst. Environment Health Sciences., Wayne State University, Detroit, MI.*

Rat hepatic hydroxysteroid sulfotransferase (SULT2A-40/41) is involved in the detoxication of bile acids and xenobiotics. Therefore, understanding its molecular regulation is essential. We previously showed that physiological doses of glucocorticoid (10^{-7}M) induce SULT2A-40/41 gene transcription *via* a glucocorticoid receptor (GR)-mediated mechanism. Since the proximal promoter region of the SULT2A-40/41 gene contains consensus binding sites for the CCAAT enhancer binding protein (C/EBP) liver-enriched transcription factors, the hypothesis is that ligand activation of the GR induces the nuclear expression of C/EBP transcription factors that in turn, transactivate SULT2A-40/41 gene expression. We determined that incubation of primary cultured rat hepatocytes with dexamethasone (DEX, 10^{-7}M) produced early increases in C/EBPα and C/EBPβ at 3h to 6h following DEX treatment. Subsequent inductions of SULT2A-40/41 mRNA and protein levels occurred at 24h and 48h, respectively. Transient transfections in primary cultured rat hepatocytes using SULT2A-40/41 reporter constructs demonstrated that cotreat-

ment with triamcinolone acetonide (TA, 10^{-7}M) and an expression plasmid containing dominant negative C/EBP effectively blocked TA-inducible SULT2A-40/41 transcription. These data implicate a role for GR-inducible C/EBPα and C/EBPβ in the transactivation of SULT2A-40/41 expression by physiological concentrations of glucocorticoid. Supported by NIH grant ES05823 and NIEHS Center Grant ES06639. Dominant negative C/EBP expression plasmid was a generous gift from Dr. Charles R. Vinson, National Cancer Institute, Bethesda, MD.

673 ONCOGENICITY EVALUATION OF NELFINAVIR MESYLATE IN RATS.

M. Zorbas[1], M. McClain[2], B. Jessen[1], W. Evering[1], G. Stevens[1], R. Gasser[3] and L. Burns Naas[1]. *[1]Pfizer Global Research & Development, San Diego, CA, [2]McClain Associates, Randolph, NJ and [3]Hoffmann-LaRoche, Basel, Switzerland.*

The oncogenic and immunotoxic potential of nelfinavir mesylate (NFV) were evaluated in a 2 year oral (gavage) carcinogenicity study in SD rats at dose levels of 100, 300, and 1000 mg/kg/d. At the end of treatment, increased incidences of thyroid follicular cell hyperplasia and neoplasms were observed at 300 (♂) and 1000 (♂/♀) mg/kg/d. No other adverse effects were observed, including immunotoxicity evaluated at 1 & 6 mo. Results from previous studies indicated a number of effects in the liver and thyroid as well as metabolic profiles that suggested NFV may cause thyroid hyperplasia/neoplasia secondary to hormone imbalance by altering thyroid hormone disposition. To confirm this hypothesis, the effects of NFV on gene expression in rat hepatocytes and liver slices (*in vitro*), thyroxin plasma clearance, and thyroid gland function were evaluated. Compared to controls, gene expression analyses demonstrated an increased expression of glucuronyltransferase (UGT) and CYP450 3A1 in NFV-treated rat hepatocytes and liver slices. In rats treated with NFV (1000 mg/kg/d) for 1 month, liver weights and centrilobular hepatocellular hypertrophy were increased and minimal to mild diffuse thyroid follicular cell hypertrophy and early follicular cell hyperplasia were evident in the thyroid gland. TSH levels were significantly increased (3 fold), while T3/T4 and reverse T3 levels were unchanged indicating that a compensated state to maintain homeostasis of T3/T4 had been achieved. Plasma ^{125}I-thyroxin clearance was increased and the plasma thyroxin $AUC_{0\text{-}48}$ was reduced by 24% compared to control. In conclusion, these data indicate that thyroid neoplasms observed in the NFV treated rats are secondary to thyroid hormone imbalance. Increased thyroxine disposition contributes to the effects of NFV on thyroid gland function and is likely a result of UGT induction. These results are consistent with a well-recognized rat-specific mechanism for thyroid neoplasms.

674 SAFETY EVALUATION OF STEALTH® LIPOSOMAL CKD–602.

B. E. Stewart[1], C. M. Engbers[1], N. B. Modi[1] and A. P. Mould[2]. *[1]ALZA Corporation, Mt View, CA and [2]Covance Laboratories, Madison, WI.*

The safety of STEALTH® liposomal CKD–602 (S–CKD602) was evaluated in IV single and repeat dose GLP toxicity studies in CD® (SD) IGS rats and beagle dogs. CKD–602 is a topoisomerase I inhibitor that is being developed by Chong Kun Dang Pharmaceutical Corporation in South Korea as an antitumor treatment. ALZA Corporation is developing a liposome formulation of CKD–602 in order to prolong the circulation time and target drug exposure to tumor tissue. In repeat dose studies S–CKD602 was administered for six cycles of single IV doses two and three weeks apart in rats (0.05 to 0.6 mg/kg·dose; n=12/sex·grp) and dogs (0.025 to 0.070 mg/kg·dose; n=6/sex·grp), respectively. Control groups included saline and placebo liposomes, and in some cases, nonliposomal (free) CKD–602. One treatment–related death occurred in the rat after five repeat doses of 0.6 mg/kg·dose of S–CKD602. In the dog, one death occurred after a single dose of 0.050 mg/kg S–CKD602, however, no deaths occurred with six administrations at the highest dose of 0.070 mg/kg·dose. Clinical signs of toxicity included fecal abnormalities, tooth abnormalities (rat only), and in the dog, rapidly reversible signs of a liposomal infusion reaction. Weight loss, decreased weight gain and decreased food consumption were seen after each dose administration in both species. Toxicokinetics were similar in males and females of both species, with increases in Cmax and AUC with increasing S–CKD602 dose. In the dog, the elimination half–life was approximately 1.3 h with free CKD–602 and approximately 9 to 18 h with S–CKD602. Dose–related, reversible myelotoxicity was seen in rats and dogs with administration of S–CKD602, with reticulocytes, white blood cells, and platelets most affected. Reversible microscopic changes in the rat and dog identified bone marrow (femur and sternum), thymus, gastrointestinal tissues and spleen as the target organs for toxicity by S–CKD602. The starting dose for Phase I Clinical studies, 0.1 mg/m^2, is one fifth the non–severely toxic dose in the dog of 0.025 mg/kg·dose (0.50 mg/m^2·dose).

675 2-[4-AMINO-3-METHYLPHENYL] 5-FLUORO BENZOTHIAZOLE, 5F-203, A DRUG USED IN BREAST CANCER TREATMENT UP-REGULATES NAG-1 EXPRESSION: A POSSIBLE MECHANISM OF ACTION.

J. M. Martinez[1], T. Sali[2], R. Okazaki[2], M. Hollingshead[3], C. Hose[4], N. Walker[1], A. Monks[4], S. Baek[2] and T. Eling[2]. *[1]LCBRA, NIEHS, Research Triangle Park, NC, [2]LMC, NIEHS, Research Triangle Park, NC, [3]Developmental Therapeutics Program, NCI, Frederick, MD and [4]SAIC Frederick Inc., NCI, Frederick, MD.*

A candidate anti-tumor drug 5F-203 to be evaluated in the treatment of breast cancer inhibits cell proliferation and has potent anti-cancer activity. 5F-203 activates the AhR that results in the induction of CYP1A1 and CYP1B1 in the human breast cell line, MCF-7. However, microarray analysis revealed the most highly induced gene was NAG-1/MIC/PLAB that has pro-apoptotic activity and anti-cancer activity in several models suggesting that NAG-1 may mediate the anti-cancer activity of 5F-203. In an orthotopic mouse model for breast cancer, treatment with the L-lysylamide prodrug, Phortress, reduced the size of the breast tumors in a dose-dependent manner. Furthermore, expression levels of NAG-1 as measured with real-time RT-PCR and the size of the tumors appeared to be inversely correlated. MCF-7 cells, constructed to express NAG-1, yielded smaller tumors as compared to control cells in the mouse orthotopic model. In cultured MCF-7 cells, 5F-203 at nanomolar concentrations greatly enhanced NAG-1 protein expression in a time dependent manner and was the most potent inducer of NAG-1 expression when compared to other anti-cancer chemicals. NAG-1 expression in HCT-116 human colorectal tumor cells, and in LNCaP human prostate tumor cells, was increased by 5F-203 indicating the response was not restricted to breast tumors. In contrast to other anti-cancer drugs, 5F-203 does not increase the transcriptional activity of the NAG-1 gene as measured in luciferase promoter constructs but appears to increase the half-life of NAG-1 mRNA. These results provide a rationale for the increase in protein expression by this drug. This study supports the hypothesis that the anti-cancer activity of 5F-203 is mediated by NAG-1 that in part, may be dependent on the stimulation of apoptosis. NAG-1 may be a suitable marker for estimating the efficacy of 5F-203.

676 PIFITHRIN-α IS A POTENT ARYL HYDROCARBON RECEPTOR AGONIST.

M. S. Hoagland, E. M. Hoagland, G. M. Ziegler and H. I. Swanson. *Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, KY.*

Pifithrin-α (PFTa), chemical inhibitor of p53-dependent apoptosis, is currently being developed to be used in chemotherapies to limit drug-induced toxicity in normal, p53+/+ cells. The mechanism by which PFTa inhibits p53, however, is unknown. Upon observing the structural similarities of PFTa to b-naphthoflavone as well as indole-containing compounds, we hypothesized that PFTa may act as an aryl hydrocarbon receptor (AhR) ligand. AhR activation induces gene expression of xenobiotic-metabolizing enzymes including CYP1A1 through dioxin response elements (DREs). We have found that PFTa acts as an AHR agonist that induces CYP1A1 expression in a DRE-dependent manner. Using gel shift and ligand binding assays, we have also shown that PFTa directly interacts with the AhR and induces formation of the AhR/ARNT DNA binding complex. Ongoing studies will determine whether inhibition of apoptosis by PFTa requires activation of the AhR signaling pathway.

677 PULMONARY FUNCTION EFFECTS OF A THREE-HOUR TOXICITY STUDY OF AMINOFLAVONE PRODRUG (NSC-710464) IN DOGS.

I. M. Grossi[1], M. Lynch[1], J. Merrill[1], J. Tomaszewski[2] and J. O. Peggins[2]. *[1]Toxicology, Battelle, Columbus, OH and [2]NCI, NIH, Bethesda, MD.*

Aminoflavone prodrug NSC-710464 exhibits excellent antineoplastic activity against the Caki-1 and A498 renal, MCF-7 breast, and OVCAR-5 ovarian cancer cell lines *in vitro* and *in vivo* against Caki-1 and A-498 xenografts. The objective of this study was to determine the effect of aminoflavone prodrug on pulmonary function in dogs treated once a week for three weeks. Dogs were administered a 3-hour continuous IV infusion of NSC-710464 at 0, 5.7 or 11.5 mg/kg (114 and 230 mg/m2) once per week for 3 weeks. Drug-related emesis occurred primarily after administration of the second and third doses. Increased respiratory rates with associated respiratory sounds were noted in the 11.5 mg/kg group. Swollen infusion sites were also noted in all NSC-710464 dosed animals. Dogs receiving aminoflavone had a 24% to 43% decline in diffusion lung capacity of CO (DLCO) values following the third dose. There was a similar decline in these animals diffusion constant of CO (KCO). At recovery, the DLCO and KCO values returned towards baseline values. There was no apparent change in total lung capacity (TLC-STPD), indicating no impairment of overall mechanical ventilation capacity. WBC

counts of treated dogs were consistently elevated in response to each infusion of the drug, and increased fibrinogen values were also noted in treated dogs. This was consistent with the thrombosis of the veins used for the infusions noted histopathologically. The increased fibrinogen response was also noted after recovery, but WBC values trended toward normal. Treatment related thrombosis was noted microscopically in all treated dogs and one vehicle dog. Drug-related histopathological lesions in the lung included multifocal chronic-active inflammation and thromboembolism. Thus, administration of the aminoflavone prodrug NSC-710464 by a three-hour continuous intravenous infusion at 5.7 or 11.5 mg/kg once a week for 3 weeks produced pulmonary toxicity in dogs.

678 EFFECT OF HUMAN APOB-100 ANTISENSE OLIGONUCLEOTIDE (ISIS 301012) ON THE EXPRESSION OF APOB-100 MNRA IN MONKEY.

T. Kim[1], T. Zanardi[2], M. Graham[1], R. Crooke[1], A. Levin[1] and S. Henry[1]. *[1]Toxicology/PKM, ISIS Pharmaceuticals, Carlsbad, CA and [2]Charles River Laboratories (SBi Division), Sparks, NV.*

ISIS 301012 is an antisense inhibitor of human ApoB-100. In humans, ApoB-100 plays a critical role in controlling cholesterol biosynthesis and is the principal apolipoprotein of triglyceride rich, VLDL and LDL-cholesterol. This apolipoprotein is synthesized exclusively in the liver of non-human primates and man. ISIS 301012 is also complementary with the cynomolgus monkey mRNA with the exception of two base pair mismatches. It has been shown to inhibit ApoB-100 expression both human and monkey hepatocytes *in vitro*. We characterized toxicity and the pharmacological activity of ISIS 301012 on liver ApoB-100 mRNA expression in monkeys after 4 weeks of treatment. ISIS 301012 was administered by 1-hour i.v. infusion at doses of 2, 4, and 12 mg/kg or s.c. injection at 2 and 20 mg/kg to monkeys. Doses were given on alternate days for the first 4 doses (loading) and every fourth day thereafter (maintenance). Results from this study reveal that acute and transient 1.2 to 1.3-fold increase in APTT was noted with high doses only during the first 4 hours after dosing. Basophilic granules containing oligonucleotide were observed in numerous tissues and are evidence of drug uptake by cells. After 1 week of treatment with 4 mg/kg, ApoB-100 mRNA expression in liver was reduced and the reduction was sustained up to 16 days after the cessation of treatment, consistent with the long half-life of the drug. There was a dose-dependent reduction in expression in liver after 4 weeks of treatment with up to 90% reduction of liver ApoB-100 mRNA in the highest dose group. When serum ApoB-100 protein was measured by serum ELISA, a dose-dependent decrease in ApoB-100 was observed. At the 12 mg/kg dose, ∽40% reduction in monkey ApoB-100 was noted. Collectively, these data suggest that ISIS 301012 is a potent inhibitor of ApoB-100 that produced minimal alterations in clinical pathology or histopathology at 20 mg/kg. The prolonged target reduction by ISIS 301012 suggests that weekly or bi-weekly dosing in humans will be possible.

679 CJC-1295, A LONG-ACTING GROWTH HORMONE RELEASE FACTOR ANALOGUE, IS WELL TOLERATED IN RATS UP TO 14 DAYS.

V. Iordanova[1], B. Lawrence[1], S. Morseth[2] and J. Castaigne[1]. *[1]ConjuChem, Montreal, QC, Canada and [2]Milestone Biomedical Associates, Frederick, MD.*

CJC-1295 is a synthetic modification of growth hormone releasing factor (GRF), the active core of growth hormone releasing hormone (GHRH) which predominately stimulates the pulsatile release of growth hormone (GH). By applying the Drug Affinity Complex technology to GRF, the peptide selectively and covalently bonds to circulating albumin after parenteral administration, thus prolonging its $t_{1/2}$, as seen in rats administered CJC-1295 ($t_{1/2}$=~20 h). The acute toxicity of CJC-1295 was evaluated in CD rats following single intravenous (IV) doses of 2, 4 and 8 mg/kg. CJC-1295 was generally well tolerated with transient decreases in food consumption at ≥2 mg/kg (↓62-90%), soft/mucoid stools (≥4 mg/kg), and decreased activity (8 mg/kg). Similar clinical signs were noted in a sub-chronic study in CD rats with every other day IV injections for 14 days at 0.25, 1 and 4 mg/kg, including transient decreases in food intake at ≥1 mg/kg (↓10-72%) and water consumption at 4 mg/kg (↓13-60%), seen on dosing days only; feces soft/mucoid/watery (≥0.25 mg/kg), absent stool (≥1 mg/kg), decreased activity (≥1 mg/kg), lacrimation (4 mg/kg), and decreases in weight gain in males at ≥1 mg/kg (↓8-26%). There were no effects on survival, ophthalmology, urinalysis, bone marrow, and gross pathology. Hematologically, red blood cell counts, hemoglobin and hematocrit were slightly reduced at ≥0.25 mg/kg (↓5-15%). Clinical chemistry changes included mild increases in calcium, cholesterol, albumin, globulins and total proteins at all doses. Although liver weight increases were seen at ≥0.25 mg/kg (↑8-25%), there were no corresponding microscopic changes. Microscopic finding consisted of minimal injection site hemorrhage/inflammation/necrosis at all doses. There was partial or complete recovery of the findings after a 14-day treatment-free period. In conclusion, CJC-1295 was well tolerated in rats following IV doses up to 4 mg/kg for up to 14 days.

680 CJC-1131, A LONG-ACTING GLP-1 ANALOGUE, EXHIBITS SAFETY AND TOLERABILITY IN RATS AND DOGS UP TO 91 DAYS.

S. Wen[1], B. Lawrence[1], S. Wilson[2] and J. Castaigne[1]. *[1]ConjuChem, Montreal, QC, Canada and [2]Milestone Biomedical Associates, Frederick, MD.*

CJC-1131 is an anti-diabetic compound being developed for the control of type 2 diabetes and is currently in Phase II clinical trials. By applying the Drug Affinity Complex (DAC) technology to glucagon-like peptide-1 (GLP-1), the hormone selectively and covalently bonds to circulating albumin after parenteral administration. The safety of CJC-1131 was evaluated in CD rats by subcutaneous (SC) every other day (q2d) administration at doses ranging from 0.05-1.8 mg/kg and in Beagle dogs by SC every three days (q3d) injections at doses of 0.04-4 mg/kg for 91 days. The toxicity profile was similar in both species. The primary test article-related effects included transient altered fecal output (e.g. few or absent feces, watery stools), decreased food and water consumption, and subsequent decreased body weight and/or body weight gains. Decreases in body weight gain in rats and dogs ranged from ~10-40% and ~25->100%, respectively, over 91 days with 4 mg/kg/q3d female dogs exhibiting a slight mean weight loss. Decreased activity was also observed in rats with secondary effects of salivation and material around nose. Occasional emesis was noted in dogs. Most changes in hematology and clinical chemistry parameters were not of biological or toxicological significance, and were consistent with those secondary to decreased food and water intake and decreased body weight. There was no consistent pattern to these changes that indicated any target organ toxicity and no histopathological findings that were attributed to treatment. The majority of the treatment-related findings exhibited reversibility during the four-week recovery periods in both species. In conclusion, with the exception of body weight effects, sub-chronic administration of CJC-1131 for 91 days was generally well tolerated up to 1.8 mg/kg/q2d in rats and up to 4 mg/kg/q3d in dogs with primarily exaggerated pharmacology-related effects.

681 FOUR-WEEK COMBINATION TOXICITY STUDY OF ANTI-VLA4 ANTIBODY ADMINISTERED IV AND IFN β-1A ADMINISTERED IM IN THE RHESUS MONKEY.

M. Cooper[1], V. Palmer[2], G. Beattie[4], J. Green[2], P. Martin[2] and N. G. Wehner[3]. *[1]Biomarker Development, Biogen, Cambridge, MA, [2]Preclinical and Clinical Development Sciences, Biogen, Cambridge, MA, [3]Safety Evaluation, Elan, South San Francisco, CA and [4]Sierra Biomedical, Sparks, NV.*

The objective of this study was to evaluate the potential toxicity of an anti-VLA4 antibody and IFN β-1a when administered alone and in combination to rhesus monkeys. Anti-VLA4 Ab is an anti-α4-integrin monoclonal antibody currently being developed for the treatment of multiple sclerosis. IFN β-1a is currently marketed for the treatment of this disease. Weekly administration of 30 or 60 mg/kg anti-VLA4 Ab I.V. for 4 weeks alone, or in combination with 30 μg IFN β-1a IM, followed by an 8-week recovery period was well tolerated. Test-article-related effects were limited to anti-VLA4 Ab-related alterations in hematology parameters. These effects likely reflect the pharmacologic activity of anti-VLA4 Ab and did not occur in animals administered only IFN β-1a. There was no evidence of an additive effect on hematology parameters when the drugs were co-administered. Hematology effects were generally reversible; some alterations persisted in high dose anti-VLA4 Ab animals, probably due to the persistence of the antibody. The individual PK profiles for Anti-VLA4 Ab and IFN β-1a were not affected by co-administration. Histologic findings consisted of lymphoid hyperplasia in the spleen and peripheral lymph nodes, and leukocytosis and focal leukocyte aggregates in the liver. These findings often appeared to be more prominent in animals administered anti-VLA4 Ab. Changes in the intramuscular injection sites were possibly related to IFN β-1a administration. The findings are consistent with hyperplasia of both T-lymphocyte and B-lymphocyte populations in the spleen. After an 8-week recovery period, spleen weights were considered fully or partially recovered and histological changes were resolved. Because the test article related histologic changes were of minimal or mild severity and reversible, they were considered to be of minor toxicologic significance under the conditions of this study.

682 RISING-DOSE TOLERABILITY STUDY OF A LYMPHOTOXIN BETA RECEPTOR AGONIST IN CHIMPANZEES.

M. Cooper[1], V. Palmer[2], T. J. Rowell[3] and J. Green[2]. *[1]Biomarker Development, Biogen, Cambridge, MA, [2]Preclinical and Clinical Development Sciences, Biogen, Cambridge, MA and [3]New Iberia Research Center, New Iberia, LA.*

LTBR mAb is a humanized monoclonal antibody that acts as an agonist to the lymphotoxin beta-receptor (LTBR). The LTBR is expressed on both neoplastic and normal tissues. Activation of the LTBR is expected to induce cell death or differentiation of neoplasms. LTBR mAb, which is being developed for treatment of

human solid tumors, is biologically active in humans and chimpanzees, but not macaques or other common laboratory species. The objective of this study was to determine the effects of LTBR mAb when administered intravenously in the chimpanzee and to determine basic pharmacokinetic parameters of LTBR mAb. Three male and three female chimpanzees were separated into two groups. Group 1 consisted of two males and two females. Group 2 contained one male and one female that had not been previously exposed to exogenous monoclonal antibodies. Group 1 was administered a single bolus intravenous infusion of LTBR mAb Placebo on Day 0 and were monitored for seven days post dose. Group 1 animals were then administered escalating doses of LTBR mAb at 1.0, 3.0, and 10.0 mg/kg at weekly intervals. Group 2 animals were administered a single bolus intravenous infusion of LTBR mAb Placebo on Day 21 then administered a single dose of LTBR mAb at 30 mg/kg on Day 28 to assess the effects of a single dose of LTBR mAb. This dose was selected as it represents an approximate 30-fold increase over the projected clinical dose of 1 mg/kg and would thus provide a reasonable assessment of potential toxicity. Samples were collected at various time points for pharmacokinetics, antibody concentrations, complete hematology panel, serum chemistries, coagulation profiles, cardiac parameters, and serum electrophoretic profiles. All animals survived the study. No clinical signs of test article-related toxicity were noted in any of the study parameters. LTBR mAb is thus concluded to be safe and well-tolerated when administered intravenously at doses up to 30 mg/kg in chimpanzees.

683 INTRAVENOUS PERI-POSTNATAL REPRODUCTION STUDY OF RHAT IN RATS.

M. S. Christian[1], E. M. Lewis[2], A. M. Hoberman[2] and R. A. Scotland[3]. *[1]Argus International, Inc., Horsham, PA, [2]Charles River Discovery and Development Services, Argus Division, Horsham, PA and [3]GTC Biotherapeutics, Inc., Framingham, MA.*

AT III deficiency, a rare hereditary disease, causes thromboembolic complications during pregnancy and childbirth. This study was conducted to support safe use of a transgenically-produced antithrombin human blood protein, rhAT, in pregnant women administered IV rhAT 3 days before and 3 days after delivery of an infant and to determine reproducibility of previous findings. In a previous study, in which female rats were treated IV with rhAT on DG 6 through DL 20 (~37 daily dosages), maternal body weights and feed consumption were increased and postnatal pup mortality to DL 5 was significantly increased at 210 mg/kg/day. Although these findings potentially were associated with prolonged treatment, it was more probable that they were associated with the techniques (purchased pregnant rats) and number and extent of maternal and pup observations. In the current study, pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats (30/group) were given rhAT or sodium citrate buffer (diluent) IV on DG 20 through DL 5, a more relevant treatment period (~8 daily dosages), at 0, 52.5, 105 or 210 mg/kg/day. Additional procedural differences from the previous study included onsite mating, multiple detailed daily clinical observations, a 24-hour litter watch (dams and pups), individual identification of pups, with daily clinical observations and body weights, and hematology and clinical chemistry parameters (dams and pups). Previous findings were not replicated. rhAT 105 and 210 mg/kg/day dosages produced transient increases in limb and/or facial swelling and pharmacologic increases in PT and APTT during gestation and/or lactation; maternal body weight and feed consumption were unaffected, findings consistent with studies in virgin female rats. rhAT dosages as high as 210 mg/kg/day did not adversely affect maternal reproductive parameters or pup *via*bility or growth. Thus, the previous findings were not replicated when a more appropriate, detailed study design was used.

684 SINGLE AND 28-DAY REPEATED INTRAMUSCULAR DOSE TOXICITY STUDIES OF BOTULINUM TOXIN TYPE A IN RATS.

W. S. Koh[1], M. K. Chung[1], Y. B. Kim[1], C. S. Ha[1], G. H. Yang[2], H. H. Chung[2] and T. C. Jeong[3]. *[1]Korea Institute of Toxicology, Daejeon, South Korea, [2]Medy-Toxicology, Asan, South Korea and [3]College of Pharmacy, Yeungnam University, Kyungsan, South Korea.*

Botulinum toxin type A was intramuscularly administered to Sprague-Dawley rats in both single and 28-day repeated dose toxicity studies. In the single dose toxicity study performed at 25, 50, 100, and 200 ng/kg, LD50 was estimated to be 70.71 ng/kg for males and 97.63 ng/kg for females. The major clinical signs observed were paralytic gait, decrease of locomotor activity, ananastasia, and death. The 28-day repeated dose toxicity study performed at dose levels of 1, 3, and 9 ng/kg/day showed an attenuation of body weight gain in a dose-dependent manner. This attenuation of body weight gain was considered due to the decrease of food and water consumption observed in the animals treated at 9 ng/kg/day and continued throughout a 4-week withdrawal period on completion of dosing. Paralytic gait was a common clinical sign observed in the animals treated at 3 ng/kg/day and muscle atrophy as well as paralytic gait were monitored at 9 ng/kg/day. Serum aspartate aminotransferase in males treated at 9 ng/kg/day was significantly higher than that of the vehicle-control males. No changes attributable to the administration of the botulinum toxin were observed in organ weight, ophthalmologic and hematological examinations. Histopathological examination of femoral muscle, which was the test substance-application site, revealed an atrophy of skeletal muscle. It showed a decrease in myofibre diameter and an increase of myofibre nuclei and intermyofibre connective tissue. The NOAEL of botulinum toxin type A is 1 ng/kg for 28-day repeated intramuscular dose toxicity in rats.

685 A HUMANIZED ANTI-TISSUE FACTOR MONOCLONAL ANTIBODY DOES NOT INCREASE THE RISK OF SURGICAL BLEEDING IN A CYNOMOLGUS MONKEY MODEL.

P. J. Bugelski[1], E. C. Martin[2], V. Mendenhall[2], A. R. Soderman[1], S. H. Tam[1], K. M. Picha[1], V. S. Khandekar[1] and G. Treacy[1]. *[1]Centocor, Inc., Malvern, PA and [2]Charles River Labs, Worcester, MA.*

A humanized IgG4 monoclonal antibody (mAb) that blocks human and cynomolgus monkey tissue factor (TF)-mediated coagulation was evaluated in a surgical bleeding model in cynomolgus monkeys. The purpose of the study was to determine if the anti-tissue factor mAb is associated with a significant risk of uncontrollable bleeding. Doses of 0.006, 0.06, 0.6, 6 and 60 mg/kg of the anti-tissue factor mAb caused a dose-dependent prolongation of PT and inhibited the LPS-induced up-regulation of prothrombin fragment F1+2 and fibrin D-dimer. Monkeys were anesthetized and a laparotomy performed. Four 6 mm diameter x 2 mm deep biopsies were cut from the liver, kidney and spleen and the resultant wounds covered with Surgicel®. Blood loss over a 1 hr observation period was quantified by weight and was the data expressed as % total blood volume. Anti-tissue factor mAb at a dose of 60 mg/kg did not inhibit the ability of Surgicel® to control bleeding. In contrast, heparin (300 University/kg) and activated protein C (25 mg/kg) significantly increased blood loss. Anti-tissue factor mAb may be a safe and effective means to control the undesirable activation of the coagulation system mediated by tissue factor.

686 A 4-WEEK SUBCUTANEOUS INJECTION TOXICITY STUDY OF PTH-FC WITH A 4-WEEK RECOVERY PERIOD IN THE ALBINO RAT.

J. E. Atkinson[1], C. Alcalde[1], M. E. Cosenza[1], S. Y. Smith[2], N. Doyle[2], C. R. Dunstan[3], C. Zimmermann[1] and P. Kostenuik[4]. *[1]Toxicology, Amgen Inc., Thousand Oaks, CA, [2]CTBR, Senneville, QC, Canada, [3]ANZAC Research Institute, Concord, NSW, Australia and [4]Metabolic Disorders, Amgen Inc., Thousand Oaks, CA.*

To determine the toxicity of PTH-Fc, Sprague-Dawley rats received subcutaneous injections twice weekly for 4-weeks. Upon completion of the dosing period a 4-week treatment-free recovery period was employed to determine the reversibility of any findings. Two hundred eighty-eight rats were assigned to 4 groups receiving 0, 0.2, 2 or 8 mg/kg/dose. Clinical observations, hematology, clinical biochemistry, toxicokinetic and antibody sampling were routinely performed throughout the experiment. Gross pathology, histopathology and organ weights were evaluated. Clinical observations seen in the high dose group only included, but were not limited to, lethargy (decreased activity, lateral recumbency), inappetence and abnormal breathing. Treatment-related mortality on Days 13 and 14 likely associated with hypercalcemia and/or uremia led to early euthanasia of the high dose group. Tissue mineralization was noted in, but not limited to, the heart, kidney and pancreas and is considered secondary to the hypercalcemia seen in the mid- and high-dose groups. Treatment-related changes were seen in the hematological parameters for both the mid- and high-dose groups. Biochemical parameters, including BUN and electrolyte values, notably changed for all groups receiving test article. Generally, changes seen in hematology and biochemistry parameters were reversible at the end of the recovery period and correlated to the histopathological findings upon the completion of the study. The high dose group exhibited a relative increase of heart, kidney and spleen weights in males and females when compared to controls. These differences are considered to be treatment-related. Based on findings of hypercalcemia, pathology changes in tissues, and changes in hematology and biochemistry parameters the NOAEL was 0.2 mg/kg.

687 DOSE RANGE-FINDING STUDY OF HALOFUGINONE (NSC-713205) IN RODENTS.

D. Kobs[1], P. J. Tosca[1], L. Bollinger[1], I. M. Grossi[1], J. Tomaszewski[2] and J. O. Peggins[2]. *[1]Toxicology, Battelle, Columbus, OH and [2]NCI, NIH, Bethesda, MD.*

Angiogenesis inhibitors such as halofuginone have shown great potential in the treatment of various cancers. They possess anti-metastatic and anti-proliferative properties due to their ability to suppress extracellular matrix deposition, cell proliferation and angiogenesis. Rats were utilized to determine the maximally tolerated

dose and relative drug toxicity of halofuginone when administered twice weekly for 4 consecutive weeks. Thirty male Fischer 344 rats received intravenous doses of 0, 0.75, 1.5, 3.0, 3.5, or 4.0 mg/kg halofuginone. A high incidence of drug-related mortality occurred in rats at the two highest dose levels. Dosing was suspended following the third dose administration for the 3.5 and 4.0 mg/kg groups due to the moribund condition of the animals. A drug related effect at 3.5 and 4.0 mg/kg was noted by marked clinical observations (lethargy, hunched posture, red eye/nasal discharge, unresponsive, alopecia, thin appearance, soiled anal region and urine stain) and group mean body weight decreases. Changes in hematology and serum chemistry parameters were also noted. Histological review of the bone marrow and spleens of all 3.5 and 4.0 mg/kg rats indicated toxicologically significant hematopoietic depression. Several serum chemistry parameters were also decreased in the 3.5 and 4.0 mg/kg groups. Decreases in TP and ALB were attributed to gastrointestinal toxicity (diarrhea), which corresponded to intestinal lesions seen microscopically (necrosis, edema, dilation, hyperplasia, and/or ulcers). Elevated BUN and CREA were noted in many unscheduled-death rats, related to dehydration secondary to the reported diarrhea. Rats in the 3.0 mg/kg group and lower had few or no clinical signs (non-drug related). Based on these findings, doses of halofuginone at 3.5 mg/kg and higher produced high mortality, bone marrow depletion and gastrointestinal toxicity. Findings at doses of 1.5 and 3.0 mg/kg were not as severe and reversible. The maximally tolerated dose in rats appears to be between 3.0mg/kg and 3.5mg/kg.

688 28-DAY ORAL (GAVAGE)TOXICITY STUDY OF *Se*-METHYLSELENOCYSTEINE IN DOGS.

W. Johnson[1], J. Lopez[1], R. Morrissey[2], C. Ip[3], I. Kapetanovic[4] and D. McCormick[1]. [1]*IIT Research Institute, Chicago, IL,* [2]*Pathology Associates, Chicago, IL,* [3]*Roswell Park Memorial Institute, Buffalo, NY and* [4]*National Cancer Institute, Bethesda, MD.*

Se-Methylselenocysteine (MSC) is an organoselenium compound being developed for breast cancer prevention. This study was conducted to characterize the toxicity of subchronic oral administration of MSC in dogs. Beagle dogs (4/sex/group) received daily oral (gavage) exposure to MSC (in purified water) at doses of 0, 0.15, 0.3, or 0.6 mg/kg/day for 28 days. Toxicology endpoints included body weight, food consumption, clinical observations, clinical chemistry, hematology, coagulation, urinalysis, ophthalmology, electrocardiography, organ weights, gross pathology, and microscopic pathology. The high dose of MSC induced weight loss in two male dogs; one of these dogs also exhibited hypoactivity, hypothermia, salivation, and decreased food consumption. No other effects on body weight, food consumption, or other clinical signs of toxicity were observed in any dog exposed to MSC. Modest anemia was observed in both sexes exposed to the high dose of MSC; slight anemia was seen in males exposed to the middle dose. Fibrinogen levels were increased in high dose males. No treatment-related alterations in clinical chemistry or urinalysis parameters were observed, and the results of ophthalmic and electrocardiographic evaluations were within normal limits. Absolute and relative thymus weights were decreased in males receiving the high dose of MSC; no other organ weight changes were seen. Treatment-related microscopic lesions were observed in the liver, duodenum, ileum, thymus, and epididymides of MSC-treated dogs. Although changes in one or more organs were seen in all dose groups, only hepatic changes (peliosis hepatic and midzonal vacuolar degeneration) present in high dose males were considered to be toxicologically significant. On the basis of body weight loss, clinical signs, anemia, and microscopic liver toxicity seen in high dose males, the No Observed Adverse Effect Level (NOAEL) for MSC in beagle dogs in this study was defined as 0.3 mg/kg. (NCI N01-CN-15140)

689 ACUTE AND SUB ACUTE ORAL TOXICITY OF VEGPANZYME IN MICE AND RATS.

S. N. Shah[1], A. University. Burhan[1], S. L. Bodhankar[1], S. P. Risbud[2] and S. Kurundkar[3]. [1]*Pharmacology, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, India,* [2]*Advanced Biochemicals Ltd.,, Thane, Maharashtra, India and* [3]*Raj Biotech (India) Pvt. Ltd.,, Satara, Maharashtra, India.* Sponsor: H. Mehendale.

Vegpanzyme, a mixture of proteolytic enzymes is used to treat GIT disorders. The objective of the study was to evaluate the acute and subacute oral toxicity of Vegpanzyme in mice and rats. Wistar rats of either sex weighing 100 to 150g and Swiss Albino mice weighing 18 to 22g were used. Mice received 50, 300, 2000 and 5000mg Vegpanzyme/kg(AOT 425, OECD guidelines).In subacute toxicity study 4 groups of each gender (4M&4F) received distilled water, 100, 200 or 400mg Vegpanzyme/kg/day for 28 days.Body weight and food consumption was recorded. On day29, the animals were sacrificed. Haematological and clinical chemistry parameters were measured. Histology of rat organs was performed. No clinical signs of toxicity or mortality was seen during the 14 days after the end of treatment period. The LD50 was >5000 mg Vegpanzyme/kg and the animals were free from any toxic effect on haemoglobin, RBC, WBC, DLC and ESR at the doses of 100, 200 and 400mg/kg. At 400mg/kg a weak (non significant) hypoglycemic effect was observed. Vegpanzyme did not alter Blood cholesterol, triglycerides, HDL and VLDL compared to vehicle treatment. Vegpanzyme 400 and 100mg/kg decreased serum urea, potassium concentration and creatinine respectively. Vegpanzyme(100, 200&400 mg/kg) produced increase in plasma alkaline phosphatase (ALP) and decrease in ALT and AST. The histopathology of liver and kidneys indicated minimal changes. Histopathology indicated consistent but insignificant changes of haemorrhage and congestion in spleen. At 100 and 400mg/kg Vegpanzyme caused minimal, focal MNC in myocardial tissue. No abnormality was detected in stomach. At the lower doses of 100 and 200mg Vegpanzyme/kg did not alter the body weight of rats. However, at higher doses of 400mg/kg a nonsignificant reduction in body weight was observed. Vegpanzyme did not alter organ weight of rats. It was thus concluded that Vegpanzyme at the doses of 100 and 200mg/kg is non-toxic. However, at higher dose, the drug is likely to alter function of spleen and heart.

690 A NON-HUMAN PRIMATE MODEL OF COLLAGEN-INDUCED ARTHRITIS: ITS ONSET AND THE EFFECTS OF METHOTREXATE.

H. Kasai, H. Sameshima, H. Tsusaki, N. Horai, S. Kojima, H. Tokado and R. Nagata. *DSR Laboratories, SNBL, Kagoshima, Japan.*

Collagen-induced arthritis (CIA) has been used in several species, primarily rodents, in order to evaluate rheumatoid arthritis. However, little data exists of CIA in non-human primates, and most data is from rhesus monkeys. In this study, we selected cynomolgus monkeys, which are commonly used in safety and efficacy studies. The objectives were to identify whether they can be employed in a CIA model by investigating the onset to arthritis and any effects of methotrexate (MTX, Wako Pure Chemical Industries, Ltd., Japan). Forty-five females, weighing 2.0 to 3.5 kg, were sensitized with an emulsion of type II bovine collagen and Freund's complete adjuvant. The emulsion was administered 3 times at 3-week intervals. The area of the interphalangeal (IP) joints of all limbs were measured sequentially using calipers. The mean IP area was measured in order to evaluate the onset of CIA. Animals in which the mean IP area increased 105% or greater from the 1st sensitization were defined as CIA animals. Five animals were selected from this group and were treated with MTX at 0 (control), 3, or 10 mg/animal twice weekly for 8 weeks. CIA was induced in 32/45 animals (72%) at 2 weeks after the final sensitization. The 3rd sensitization produced a marked increase in the IP area, whereas the 1st and 2nd sensitization did not. When the CIA animals were treated with MTX, increased IP area in control group did not change or slightly decreased during the treatment period. In the MTX groups, percentile changes in IP area were decreased 55% and 71% at 3 mg/animal (n=2) and 10 mg/animal (n=2), respectively, compared with the pre-treatment values. The histopathological results of the MTX groups showed repair of the destroyed joint. It is concluded that the CIA model in cynomolgus monkeys can be used for the evaluation of efficacy for rheumatoid arthritis when changes in IP area are used as primary end point as an established drug demonstrates a therapeutic effect in this model.

691 REAL-TIME PCR ASSAY FOR THE QUANTITATION OF SEROTYPE 5 ADENOVIRUS VECTOR DNA IN GENOMIC DNA EXTRACTED FROM TISSUES AND FLUIDS.

M. Wu[1], M. Subramanyam[2] and D. Enke[1]. [1]*Biomarker Development, Biogen, Cambridge, MA and* [2]*Biogen, Cambridge, MA.* Sponsor: J. Green.

The FDA has mandated that quantitative PCR-based assays be developed to assess the safety of gene therapy viral vectors. Specifically, these assays are to be used to determine the biodistribution of the vector distal to the site of injection and in germline tissues. A TaqMan® real-time PCR assay was developed for the quantitation of adenovirus type 5 gene therapy vector viral genome copy number. The assay is specific for a target sequence in the d1309 region of the adenoviral vector genome. A standard curve is generated by plotting the threshold cycle (CT) number versus the log of copy number, and the standard vector copy number in each test sample is determined from the sample CT number by interpolation from the standard curve. The standard curve ranges from 10 to 1x106 copies of standard vector in a background of genomic DNA. Intermediate assay precision averaged 0.8% CV by CT value (14.4% CV by interpolated copy number) across the standard curve range. Based on the tightness of the assay precision, a decision was made to test samples in duplicate on a single plate. Plate acceptance criteria are based on the acceptance criteria of the standard curve. According to acceptance criteria set by the manufacturer Applied Biosystems, TaqMan® standard curves should have a slope of -3.32 ± 0.5 and a correlation coefficient (R2) of > 0.99. In addition, No Template Control (NTC) wells should return a value of zero copies as a control for contamination. Sample acceptance criteria are based on the CV of sample copy number returned. Samples which return copy numbers with >20% CV between duplicates should be retested. This assay satisfies the current Food and Drug Administration (FDA) guidelines for PCR-based biodistribution assays as defined by Biological Response Modifiers Advisory Committee (BRMAC).

692 APOPTOSIS INDUCED BY INTERACTIONS BETWEEN MOLDY HOUSE MICROBES.

P. Penttinen[1, 2], J. Pelkonen[2, 3], K. Huttunen[1] and M. Hirvonen[1]. *[1]Department of Environmental Health, National Public Health institute, Kuopio, Finland, [2]University of Kuopio, Kuopio, Finland and [3]Kuopio University Hospital, Kuopio, Finland.* Sponsor: M. Viluksela.

Exposure to complex mixture of bacteria and fungi in moldy buildings is a potential cause of inflammatory related symptoms among the occupants. In this study, capability of these microbes to cause apoptotic cell death was investigated in more details. In addition, microbial interactions on apoptosis was identified in mouse macrophages, when the microbes were cultured separately and together on the same culture plate. The fungal strain *Stachybotrys chartarum* and the gram-positive bacterial strain *Streptomyces californicus* were selected in this study based on their characteristic occurrence in water damaged buildings. These microbes were co-cultivated on the same agar plate. The both strains were also cultured separately and the strains were combined at the same proportion as the co-cultivated combination of *S. chartarum* and *Str. californicus* (1:5). Mouse macrophages (RAW264.7) were exposed to combination of spores and spores individually. In the dose response study the macrophages were exposed to six doses (1×10^4-3×10^6 spores/ml) for 24 hours. For the time course study the macrophages were exposed to the dose of 3×10^5 spores/ml for 4, 8, 16 and 24 hours. Apoptosis was measured by flow cytometry using propidium iodide (PI) staining of permeabilized cells. Changes of caspase-3 activity in mouse macrophages were detected by fluorometric substrate cleavage assay. Flow cytometric analysis of DNA fragmentation showed, that there was 2.6±0.1 % apoptotic cells when exposed (3×10^5 spores/ml at 24 hr) to separately cultured combination, but the amount of apoptotic cells increased to 12.4±1.3 % when exposed to co-cultivated combination. Furthermore co-cultivation increased the caspase-3 activity by 2.4-fold compared to separately cultured combination. Altogether, this data suggests that co-culture of *S. chartarum* and *Str. californicus* leads to microbial interactions, which significantly affect on the ability of microbes' spores to cause apoptosis in mammalian cells.

693 DIFFERENCES IN THE ONSET OF APOPTOSIS IN OLFACTORY SENSORY CELLS OF MICE, RATS AND MONKEYS GIVEN AN INTRAVENOUS INJECTION OF MAXIMUM TOLERATED DOSE (MTD) OF VINCRISTINE, A VINCA-ALKALOID ANTITUMOR DRUG.

K. Kai, H. Satoh, T. Suzuki, M. Yoshida, Y. Shikanai, T. Kajimura, M. Kato and K. Furuhama. *Drug Safety Research Laboratory, Daiichi Pharmaceutical Co., Ltd.,, Tokyo, Japan.*

We have previously reported that vincristine sulfate (VCR) causes apoptosis in the olfactory sensory cells of male mice following an intravenous injection. In the present study, we investigated differences in the onset of apoptosis in the olfactory epithelium of BALB/c mice and Crj:CD(SD)IGS rats given an intravenous injection of VCR at a MTD and estimated 10% lethal dose (LD10), and of common marmoset monkeys receiving MTD. Namely, VCR was intravenously administered once to mice (1.17 and 1.95 mg/kg), rats (0.21 and 0.35 mg/kg) and monkeys (0.35 mg/kg) of both sexes. The dosing day was designated as Day 1 in this study. The animals were serially necropsied on Days 2, 5 and 10, and the nasal tissue and sciatic nerve were examined light-microscopically. The olfactory sections of mice on Day 2 were subjected to the terminal deoxyribonucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling (TUNEL) assay and electron-microscopic observation. In mice, VCR elicited cell deaths with condensation and fragmentation of nuclei in the olfactory epithelia and vomeronasal organs in males given 1.95 mg/kg and in females receiving 1.17 mg/kg or more from Day 2. On Day 10, demyelination in the sciatic nerve was noted in both sexes at 1.17 mg/kg or more. TUNEL-positive cells were also recognized in the basolateral olfactory epithelium. Ultrastructurally, condensation and margination of chromatin were observed in sensory cells of the basolateral olfactory epithelium. However, no morphological changes related to VCR treatment were observed in rats and monkeys of either sex. In conclusion, these results demonstrate that MTD of VCR evokes severe olfactory apoptosis only in mice, especially in females.

694 EFFECTS OF STAINLESS STEEL MANUAL METAL ARC WELDING FUMES ON DNA DAMAGE AND APOPTOSIS INDUCTION *IN VITRO* AND *IN VIVO*.

M. D. Taylor, J. R. Roberts, C. E. Solano-Lopez, S. S. Leonard, X. Shi and J. M. Antonini. *HELD/PPRB, NIOSH, Morgantown, WV.*

Epidemiological studies examining the potential association of occupational inhalation of welding fumes and increased incidence of lung cancer have not reached definite conclusions. Stainless steel (SS) welding fumes are of particular interest because they contain potential human carcinogens, such as chromium and nickel. Animal studies addressing toxicological responses related to carcinogenicity are currently lacking, although SS welding fumes have been shown to be mutagenic *in vitro*. The goals of this study were to examine the potential for SS welding fumes to damage DNA and induce apoptosis, events that are associated with possible carcinogenic activity. In a previous study using electron spin resonance, the generation of hydroxyl radicals from Cr(VI) in the SS fume (1.0 mg/ml) was observed. In the current study, SS fume at the same concentration caused plasmid DNA strand breakage *in vitro* under similar conditions. To examine the effects of SS fumes on apoptosis *in vivo*, male Sprague-Dawley rats were intratracheally instilled with SS fumes from manual metal arc welding (1.0 mg/100 g bw), Cr(VI) (0.2 mg/100 g bw) as a positive control, or the saline vehicle. On days 1, 3, 6, 8, and 10, the rats were euthanized and their left lungs were excised and cryo-sectioned. Apoptosis was examined in the tissue sections by TUNEL assay. Increased numbers of apoptotic cells were found in lung tissue treated with either the SS fume or the positive control, Cr(VI), as compared to lungs from saline-treated animals. These preliminary studies indicate that SS welding fumes can damage DNA *in vitro* and induce apoptosis *in vivo*. Studies are ongoing to determine if the apoptotic event is associated with an increase in oxidative DNA damage in lung tissue.

695 INHALED OZONE INDUCES DNA-DNA CROSS-LINKING IN EXPOSED RAT LUNG.

D. H. Bowser[1], M. Sisco[1], K. Baker[1], K. Salnikow[2], R. B. Schlesinger[3], M. D. Cohen[1] and J. T. Zelikoff[1]. *[1]Environmental Medicine, New York University School of Medicine, Tuxedo, NY, [2]NCI, Frederick, MD and [3]Biology, Pace University, Pleasantville, NY.*

Ozone (03) exposure has been shown to cause a variety of debilitating respiratory disturbances including possibly cancer. The prevalence of 03 as the dominant oxidant of world-wide photochemical smog, its occupational utilization and its application as a direct or adjunct therapy for treatment of various diseases make it a global human health concern. As the major target of 03 inhalation, lung tissue has been reported to sustain both cytotoxic and genotoxic damage as well as cause neoplastic transformation *in vivo* and *in vitro*. However, whether exposure to O3 can cause DNA-DNA cross-linking is as of yet unknown. Thus, this study examined whether long-term /chronic 03 exposure could induce pulmonary genomic DNA-DNA cross-linking. Adult male rats were exposed nose-only, 5h/d, 5d/wk for 4, 8, 12, 24 and 48 wks to filtered air or one of three different O3 concentrations (i.e., 0.1, 0.3 and 0.6 ppm). Rats were sacrificed 3d after the cessation of exposure lungs were removed and flash frozen and DNA-DNA cross-links determined by gel electrophoresis. Effects of 03 appeared to be time- and dose- dependent. Animals exposed to 0.3 ppm 03 for 8 wks demonstrated significantly elevated levels of cross-linking compared to controls and 0.1 and 0.6 ppm 03 exposed rats; exposure for twelve wks to 0.3 and 0.6 ppm 03 resulted in greater DNA cross-linking than controls or the 0.1 ppm 03 group; inhalation exposure of rats for twenty four wks to all three concentrations caused a significant dose-related increase in cross-linking; at 48 wks rats exposed to 0.3 ppm O3 demonstrated significantly elevated levels of cross-linking (compared to air controls). These findings suggest that long-term inhalation of 03 can produce genotoxic events that could be associated with neoplastic changes. NIOSH # OH03607.

696 ROLE OF INDUCIBLE NITRIC OXIDE-DERIVED NITRIC OXIDE IN SILICA-INDUCED PULMONARY INFLAMMATION AND INJURY.

P. C. Zeidler[1, 2], A. F. Hubbs[1, 2] and V. Castranova[1, 2]. *[1]PPRB, NIOSH, Morgantown, WV and [2]West Virginia University, Morgantown, WV.*

Our lab has demonstrated previously that exposure of rats to silica resulted in induction of inducible nitric oxide synthase (iNOS) in alveolar macrophages (AM) and alveolar type II epithelial cells (TII). The production of nitric oxide (NO) by these pneumocytes and resultant NO-dependent damage (nitrotyrosine residues) have been related temporally and anatomically with silica-induced inflammation and granuloma formation (Am J Physiol Lung Cell Mol Physiol 283:L485, 2002). The objective of the present study was to determine if these associations represent a causal role for NO in silica-induced lung disease. To address this question, the sub-chronic response of C57BL/6J wild type (WT) mice and iNOS knockout (KO) mice to aspiration of silica (40 mg/kg) was compared 42 days post-exposure. Exposure of WT mice to silica was marked by the following sub-chronic responses: pulmonary damage (increased LDH and albumin levels in lung lavage fluid, and increased lung weight), inflammation (increased lung lavage polymorphonuclear leukocytes, TNF-alpha, MIP-2 and TGF-beta, and histological evidence of alveolitis and lipoproteinosis), oxidant stress (increased zymosan-stimulated chemiluminescence from AM, and decreased total antioxidant levels in lung lavage fluid), and

fibrosis (increased lung hydroxyproline levels). Absence of the iNOS gene in KO mice resulted in a significant decrease in markers of pulmonary damage, inflammation, oxidant stress and fibrosis 42 days after silica exposure. These data indicate that NO production plays a causal role in the progression of silica-induced lung disease.

697 ROLE OF NITRIC OXIDE IN MEDIATING ALVEOLAR MACROPHAGE RESPONSES TO DIESEL EXHAUST PARTICLES.

J. Y. Ma[1], H. Zhao[1], M. W. Barger[1], J. K. Ma[2] and V. Castranova[1]. *[1]HELD, NIOSH, Morgantown, WV and [2]School of Pharmacy, WVU, Morgantown, WV.*

Diesel exhaust particles (DEP) are known to suppress the innate immune responses of alveolar macrophages (AM) against bacterial infection. In particular, although DEP induce AM production of nitric oxide (NO) through particle stimulation, the organic component of DEP strongly inhibits pathogen-induced NO production by AM. The present study examined whether NO may affect the secretion of pro- and anti-inflammatory cytokines by AM resulting in suppression of the host defense in response to DEP exposure. Male Sprague-Dawley rats were intratracheally (IT) instilled with saline or DEP (35 mg/kg body weight). To inhibit DEP-induced inducible nitric oxide synthase (iNOS), another group of rats were treated with an iNOS inhibitor, aminoguanidine (AG), by i.p. injection of 100 mg/kg AG, 30 min prior to and 3, 6 and 9 h after IT instillation of DEP or saline. Rats were sacrificed at 1 day post-DEP exposure, and AM were harvested. The results show that DEP induced iNOS expression in AM, which was not affected by AG. AG did not affect DEP-induced inflammatory markers in the lung (neutrophil infiltration and protein content), but significantly lowered the DEP-induced iNOS activity in AM. The production of nitrite by DEP-exposed AM was reduced from 52 ± 16 to 18 ± 4 μM by AG treatment. AG treatment inhibited the secretion of TNF-α, pro-inflammatory cytokine, by AM in response to DEP exposure (from 3424 ± 676 to 1511 ± 428 pg/ml). DEP exposure induced AM production of IL-10, an anti-inflammatory cytokine that is often induced by intracellular pathogens to dampen the host defense mechanism. Inhibition of iNOS activity by AG further enhanced this DEP-induced IL-10 production (from 38 ± 20 to 199 ± 86 pg/ml), suggesting that NO, by inhibiting IL-10, plays an important anti-bacterial role. In summary, this study shows that inhibition of NO by AG significantly decreases TNF-α secretion, while further enhancing IL-10 production by DEP-exposed AM. This change in pro-/anti-inflammatory cytokine balance may make the lung more susceptible to bacterial infection.

698 *IN-VITRO* INFLAMMATORY AND CYTOTOXIC RESPONSES TO AMBIENT AIR PARTICULATE SAMPLES COLLECTED DURING LONG-RANGE TRANSPORT (LRT) OF FOREST FIRE SMOKE TO HELSINKI, FINLAND.

P. Jalava[1, 3], R. O. Salonen[1], A. I. Halinen[1], M. Sillanpaa[2], S. Saarikoski[2], R. Hillamo[2] and M. Hirvonen[1]. *[1]Department of Environmental Health, National Public Health Institute (KTL), Kuopio, Finland, [2]Air Quality Research, Finnish Meteorological Institute, Helsinki, Finland and [3]University of Kuopio, Kuopio, Finland.* Sponsor: M. Viluksela.

Ambient air particulate (PM) samples from three different air pollution situations in autumn 2002 were studied: normal seasonal sample and two episodes of LRT forest fire smoke. The samples were collected with a high volume cascade impactor in three size fractions: Fine2 (F2, PM1-0.2), Fine1 (F1, PM2.5-1) and Coarse (CO, PM10-2.5). Episodes affected mostly the F2 fraction: the total mass concentration increased 5-10 times and the $PM_{2.5}$ mass concentrations for K+, oxalate and malonate increased also strongly. Cultures of mouse macrophage cell line (RAW 264.7) were exposed in a dose-related manner (15, 50, 150 and 300 μg/ml) to the PM samples for 8 or 24h. We measured nitric oxide (NO) production by the Griess method, production of cytokines (TNFα, IL-1, IL-6, IL-10 and MIP-2) immunochemically (ELISA) and cytotoxicity with the MTT test. Both the size fraction and the air pollution situation affected the PM induced cytotoxic and proinflammatory responses. The CO and F1 fractions were roughly equipotent but at the same time more potent inducers of cytotoxicity and cytokine production than the F2 fractions. The potency difference in TNFa production was 100-fold. The first forest fire episode increased TNFa production most within F2 fractions, whereas within F1 fractions, the second forest fire episode and the normal seasonal sample gave the larger responses. In NO and MIP-2 production, and cytotoxicity, the normal seasonal F2 fraction was more potent than the forest fire episodes. Thus, the PM chemistry changes caused by the LRT-episodes of forest fire smoke were differently reflected in the toxicity of the F2 and F1 fractions.

699 NRF2 PLAYS A CRITICAL ROLE IN CONFERRING PROTECTION AGAINST INFLAMMATION IN A MOUSE MODEL OF ASTHMA.

T. Rangasamy[1], J. Guo[2], S. Srisuma[1], S. N. Georas[2], T. W. Kensler[1], W. A. Mitzner[1] and S. Biswal[1]. *[1]Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD and [2]Department of Medicine, Johns Hopkins University, Baltimore, MD.*

The critical role of Nrf2, a b-zip transcription factor that positively regulates several antioxidant enzymes and cytoprotective proteins, against inflammatory response in lung was investigated in a murine model of asthma. Both Nrf2 +/+ and -/- mice were sensitized (on day 0 and 14) and challenged (on day 24th, 26th and 31 st) with ovalbumin (OVA). In response to OVA challenge, there was a progressive increase in the infiltration of inflammatory cells, particulary eosinophils (80 %) into the lungs of Nrf2 -/- than Nrf2 +/+ mice from 1st to 3rd OVA challenge. Staining of the lung sections with PAS and H&E also showed increased mucus cell metaplasia and infiltration of eosinophils into the lungs of the OVA treated Nrf2 -/- mice. There was an elevated level of of TH-2 cytokines (IL-4 and IL-13) in the BAL fluid of OVA treated Nrf2 -/- mice. In response to acetylcholine, OVA treated Nrf2 -/- mice showed increased airway hyperresponsiveness than Nrf2 +/+ mice. Activation of Nrf2 in the lungs of Nrf2 +/+ mice in response to OVA was confirmed by EMSA. Real Time PCR analysis showed the increased transcriptional induction of an array of antioxidant genes in the lungs of OVA treated Nrf2 +/+ mice. Increased eosinophilia, mucus cell metaplasia, airway hyperresponsiveness and elevated level of TH-2 cytokines in Nrf2-deficient mice in response to allergen suggests the pivotal role of Nrf2 in the protection of lungs against ovalbumin induced asthmatic response. (This work was supported by grants: P50 CA058184-09 and NIEHS center grant P30 ES 03819)

700 LACK OF NRF2 AUGMENTS LIPOPOLYSACCHARIDE INDUCED INFLAMMATION IN MICE LUNGS.

S. Srisuma, R. Thimmulappa, T. Rangasamy, K. Mai, T. W. Kensler and S. Biswal. *Johns Hopkins University, Baltimore, MD.*

Lung inflammatory diseases such as acute lung injury and chronic obstructive pulmonary disease are predominantly associated with oxidative stress. Recent findings on the transcriptional targets of Nrf2 revealed its regulatory role on several detoxifying enzymes, antioxidants and NADPH-regenerating enzymes suggest its protective function against a wide spectrum of toxicants. To evaluate the protective role of Nrf2 against inflammation in lungs, we employed the murine model of lipopolysaccharide (LPS) induced acute lung injury. LPS caused greater increase in wet/dry lung weight, inflammatory cells, TNF-a level in bronchoalveolar lavage fluid of Nrf2 -/- mice than those of Nrf2 +/+ mice. Electrophoretic mobility shift assay showed greater increase of NF-κB activity in the Nrf2 -/- lungs than Nrf2 +/+ lungs in response to LPS, which may be responsible for differences in inflammation. LPS treatment also resulted in higher oxidative stress in the lungs of Nrf2 -/- than Nrf2 +/+ mice as determined by the ratio of GSH/GSSG. mRNA levels of antioxidant genes such as g-glutamylcysteine synthetase (regulatory subunit), glutathione reductase, heme oxygenase and the specific activity of glutathione peroxidase, gluathione reductase, and glucose-6-phosphate dehydrogenase were significantly higher in the lungs from Nrf2 +/+ mice compared to Nrf2 -/- mice after LPS challenge. Results demonstrated that Nrf2 plays a significant role in protection against endotoxin-induced lung inflammation and resulting lung injury by elevating antioxidant defense systems (supported by P50 CA058184-09 and NIEHS Center grant-P30-ES03819).

701 ROLE OF TOLL LIKE RECEPTOR-4 IN OZONE-INDUCED PRODUCTION OF INFLAMMATORY MEDIATORS AND TOXICITY.

A. J. Connor, J. D. Laskin and D. L. Laskin. *Joint Graduate Program In Toxicology, Rutgers University and UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ.*

Studies from our laboratory have demonstrated that the toxicity of inhaled ozone is due, in part, to cytotoxic inflammatory mediators including nitric oxide, generated from inducible nitric oxide synthase (NOSII), tumor necrosis factor alpha (TNFα), and PGE2 produced *via* cyclooxygenase-2 (COX-2) released from activated alveolar macrophages. Toll like receptor-4 (TLR-4) is a transmembrane protein important in macrophage-mediated inflammatory responses initiated by lipopolysaccharide. Engagement of TLR-4 leads to activation of the transcription factor NF-κB which regulates NOSII, COX-2 and TNFα. In the present studies we analyzed the role of TLR-4 in ozone-induced macrophage activation, inflammatory mediator production, and toxicity. Treatment of control C3H/OuJ mice with ozone (0.8 ppm, 3 hr) resulted in alveolar epithelial injury as measured by protein accumulation in bronchoalveolar lavage fluid (BAL). In contrast, BAL fluid protein

was at control levels in C3H/HeJ which possess a mutated TLR-4, demonstrating that they are protected from ozone-induced injury. In OuJ mice, ozone inhalation caused a time-dependent induction of NOSII and COX-2 protein expression in the lung which was detected in histologic sections. Whereas NOSII was localized mainly in alveolar macrophages, COX-2 staining was noted throughout the alveolar epithelial regions. Ozone-induced expression of NOSII and COX-2 was markedly attenuated in lungs from HeJ mice. Taken together these data demonstrated that TLR-4 plays an important role in the pathogenesis of inflammation and tissue injury induced by ozone. Supported by NIH grants ES04738, GM34310, ES007148 and ES05022.

702 GENOMIC ANALYSIS OF PHOSGENE-INDUCED LUNG INJURY.

A. M. Sciuto[2], J. F. Dillman[1], C. S. Phillips[1], L. M. Dorsch[1], A. I. Hege[1] and T. S. Moran[2]. *[1]Applied Pharmacology, USAMRICD, Aberdeen Proving Ground, MD and [2]Neurotoxicology, USAMRICD, Aberdeen Proving Ground, MD.*

Phosgene is a toxic gas used widely in industry for the production of many synthetic products, such as polyfoam rubber, plastics, and dyes. Exposure to phosgene results in a latent (3-24 hr) potentially life-threatening pulmonary edema and irreversible acute lung injury. A genomic approach was utilized to investigate the molecular mechanism of phosgene-induced lung injury. CD-1 male mice were exposed whole-body to either air or a concentration x time (c x t) amount of 32 mg/m3 (8 ppm) phosgene for 20 min (640 mg x min/m3). Lung tissue was collected from air- or phosgene-exposed mice at 0.5, 1, 4, 8, 12, 24, 48, and 72 h post-exposure. RNA was extracted from the lung and used as starting material for the probing of oligonucleotide microarrays to determine changes in gene expression following phosgene exposure. The data were analyzed using principal component analysis (PCA) to determine the greatest sources of data variability. A two-way analysis of variance (ANOVA) based on agent exposure and time was performed to identify the genes most significantly changed as a result of phosgene exposure. These genes were rank ordered by p-values and categorized based on molecular function and biological process. Some of the most significant changes in gene expression reflect changes in glutathione synthesis and redox regulation of the cell, including upregulation of glutathione S-transferase alpha-2, glutathione peroxidase 2, and glutamate-cysteine ligase, catalytic subunit (also known as gamma-glutamyl cysteine synthetase). This is in agreement with previous observations describing changes in redox enzyme activity after phosgene exposure. We are also investigating other pathways that are responsive to phosgene exposure in order to identify mechanisms of toxicity and potential therapeutic targets.

703 TRANSCRIPTIONAL REGULATION IN RESPONSE TO CARBON NANOTUBES IN HUMAN BRONCHIAL EPITHELIAL CELLS AS DETECTED BY MICROARRAY ANALYSIS.

N. Keshava[1], A. R. Murray[2], O. Gorelik[4], S. Arepalli[4], V. Z. Gandelsman[5], V. Castranova[2, 3] and A. A. Shvedova[2, 3]. *[1]TMBB, NIOSH, CDC, Morgantown, WV, [2]PPRB, NIOSH, CDC, Morgantown, WV, [3]Physiology & Pharmacology, WVU, Morgantown, WV, [4]Materials & Processes Branch, Lockheed Martin Corporation, Engineering Directorate, Houston, TX and [5]Nanotube Team, GBTech, Inc., NASA-JSC, Houston, TX.*

Carbon nanotubes are new members of carbon allotropes with unique physical properties used for novel applications in the electronics, aerospace and computer industries. Previously, we have reported that exposure of BEAS-2B cells to single wall carbon nanotubes (SWCNT) caused ultra-structural and morphological changes, cytotoxicity, apoptosis, and induced oxidative stress as shown by the formation of free radicals, accumulation of peroxidative products, and depletion of antioxidants. In this study, we have investigated the effect of SWCNT on alterations in gene expression. Exponentially growing human bronchial epithelial cells were exposed to 0.06, 0.12 or 0.24 mg/ml of SWCNT for 18 h. Total RNA was used for the preparation of double stranded cDNA. A biotin labeled cRNA transcript was synthesized, fragmented and hybridized to Affymetrix U133A oligonucleotide microarray. The arrays were stained with streptavidin-phycoerythrin and biotinylated anti-streptavidin antibodies. The differential gene expression data analysis was performed using Affymetrix microarray suite 5.0 software. The 3'/5' cRNA transcript ratios for both GAPDH and ?-actin were found to be consistent between control and treatment groups over a period of 18 h exposure. Altered gene expression patterns were observed in 187 RNA transcripts with a signal log ratio of at least 0.8 (1.7 fold change). Altered genes include genes involved in metabolism (CYP1B1, aspargine synthetase, NNMT), cell cycle control (p21, CD24, GAS1, PA26) and others (matrix metalloproteinase, ferrodoxin reductase, cathepsin L2). In conformity with our earlier study, it appears that SWCNT alter a variety of genes that include those involved in oxidative stress.

704 *IN VIVO* EXPOSURE TO DRINKING WATER ARSENIC MODIFIES EXPRESSION OF GENES IN THE MOUSE LUNG.

A. S. Andrew[1], A. Barchowsky[2], J. C. Davey[1], N. V. Soucy[1], D. D. Mayka[1], C. R. Lantz[3], A. Hayes[3] and J. W. Hamilton[1]. *[1]Pharmacology & Toxicology, Dartmouth Medical School, Hanover, NH, [2]Environmental & Occupational Health, University of Pittsburgh, Pittsburgh, PA and [3]Cell Biology & Anatomy, University of Arizona, Tucson, AZ.*

Chronic ingestion of arsenic has been associated with increased incidence of vascular and cardiovascular disease, diabetes, hyperkeratosis, and cancer in multiple organs, including the lung. However, the disease risks and biological effects associated with arsenic ingestion at lower levels commonly found in the US remain unclear. To investigate these effects, C57BL/6 mice ingested drinking water with or without 50 ppb arsenic (the previous US maximum contaminant level) for five weeks. RNA from three control and three arsenic exposed animals was labeled and hybridized independently on six independent Affymetrix mouse 430(A) arrays, each containing over 14 k full length genes. Signals were normalized using Robust Multichip Analysis (RMA) and statistical analysis for differential gene expression between the control and arsenic exposed groups was assessed using a Bayesian approach. We ranked differentially expressed genes in ascending order by the p-values and our initial focus was placed on the 30 genes with p-values <0.005. As we have observed previously, the levels of expression for the majority of the genes in the arsenic exposed group were decreased in comparison to controls. Genes involved in transcriptional regulation, DNA repair, and T-cell mediated immune response showed significant decreases in expression, while increases were observed in insulin regulating and xenobiotic metabolism genes. These data indicate that significant biological effects occur at drinking water arsenic concentrations routinely found throughout the US The specific pattern of gene changes that were observed will also guide further mechanistic studies of arsenic-induced disease. Supported by NIEHS SBRP grants ES07373 and ES04940.

705 ESTROUS CYCLE ALTERS PULMONARY METABOLISM OF NAPHTHALENE (NA).

L. S. Van Winkle[1], G. L. Baker[1], R. L. Stelck[1], C. D. Brown[1], C. G. Plopper[1] and A. R. Buckpitt[2]. *[1]VM:APC, UC Davis, Davis, CA and [2]VM:Molecular Biosciences, UC Davis, Davis, CA.*

Tobacco smoke exposure is causally associated with pulmonary diseases in both men and women. However, risk for cancers of the peripheral lung is higher in women and suggests gender and lung region-specific differences in pulmonary epithelial responses to tobacco smoke. NA is the most abundant PAH in sidestream tobacco smoke. Random cycling female mice are more susceptible than males to NA-induced pulmonary cytotoxicity. NA lung toxicity has been shown to depend on bioactivation to a toxic intermediate by cytochrome P450s. This study addressed the issue of whether metabolism of NA in proximal and distal airways of female mice differed by estrous cycle stage and from male mice. Intact cycling female mice separated into three categories by estrous cycle stage: proestrus (estradiol low, progesterone low), estrus (estradiol high, progesterone low) and noncycling diestrus (estradiol moderate, progesterone high). Cycle stage was determined using vaginal cytology. NA metabolic products were measured in microdissected proximal (bronchi and trachea) and distal (bronchioles) airways using HPLC. In both proximal and distal airways, total NA metabolism as well as formation of NA glutathione conjugate 2 (derived from the 1R, 2S-epoxide) were significantly greater ($P < 0.05$) in mice at the proestrus stage of the estrous cycle compared to females in estrus, to noncycling females or to males. Formation of NA dihydrodiol increased significantly in the distal airways of the proestrus mice ($P < 0.05$) compared to all other groups. However, in the proximal airways, estrus and proestrus mice had similar rates of NA dihydrodiol formation which differed significantly from the rates in noncycling females and in males. We conclude that NA metabolism is influenced by the estrous cycle in the lungs of female mice and varies by airway level. Further, the pattern of changes suggests that NA conjugate formation may be influenced by estradiol, but dihydrodiol formation in proximal airways is influenced by progesterone. Supported by: University of California TRDRP grants 11RT-0258, 12IT-0191.

706 SITE-SPECIFIC INDUCTION OF PULMONARY CYTOCHROME P450 ISOZYME 3A1 BY 1-NITRONAPHTHALENE IN NEONATAL RATS.

K. C. Day, G. L. Baker, C. G. Plopper and M. V. Fanucchi. *Center for Comparative Respiratory Biology and Medicine, University of California, Davis, Davis, CA.*

Neonatal rats are more susceptible to severe pulmonary injury by bioactivated environmental cytotoxicants than adult animals. Cytochrome P450 (CYP) monooxygenases play an important role in the bioactivation of these environmental cytotoxicants such as nitropolyaromatic compounds. 1-Nitronaphthalene (1NN), a

by-product of diesel exhaust and cigarette smoke, produces pulmonary injury at lower doses in neonatal rats than adult rats. To determine whether 1NN alters the expression of pulmonary CYP monooxygenases, we examined RNA expression in laser capture microdissected and blunt microdissected airways, protein expression and CYP3A1 activity during the first 24 hours post 1NN treatment in 7-day old and adult male Sprague-Dawley rats. Rats were injected IP with 0 or 100 mg/kg of 1NN dissolved in corn oil and killed at 0, 1, 2, 3, 6, 24 hours post-treatment. CYP3A1 RNA expression was significantly induced at 6-hrs post treatment in proximal airway epithelium and pulmonary vein of 7-day old rats, but not in adult rats. CYP3A1 protein expression increased 2-hrs post treatment in 7-day old rats but not in adult rats. Proximal airways of 7-day old and adult rats were heavily labeled with immunoreactive CYP3A1. There was little to no labeling in the distal airways. The vascular smooth muscle surrounding the proximal airway was heavily labeled throughout the time course. Differences in CYP3A1 activity levels were also determined from both neonatal and adult lung microsomes using the substrate Midazolam. We conclude that elevation in gene and protein expression for pulmonary CYP isozyme 3A1 is induced by the bioactivated cytotoxicant 1NN in neonatal rats but not adult rats. This suggests that a potential mechanism for the increased susceptibility of neonatal rats to pulmonary injury by a bioactivated cytotoxicant (1NN) is by induction of a key isozyme CYP3A1. NIH ES06700, USEPA (R827442010) and ES 004311

707 THE EFFECT ON PUP VIABILITY AND GROWTH DURING NOSE-ONLY INHALATION EXPOSURE OF WISTAR-HAN RATS FOR PRE AND POST NATAL STUDIES.

M. Stoute[1], S. Maquire[2], K. Robinson[1], A. Viau[1] and C. Banks[1]. *[1]CTBR, Senneville, QC, Canada and [2]GlaxoSmithKline, Ware, United Kingdom.*

Due to the increased focus on the effects of inhaled pharmaceutical products on reproduction, a study was undertaken to determine the effects on the *via*bility and growth of pups when their dams were placed in restraint tubes (for nose only inhalation exposure) for 60 minutes starting from Day 15 of gestation to parturition and again from Day 1, 2, 3 or 4 to Day 10 post partum. Fifty mated female Wistar Han rats were randomized into 5 groups of 10. Group 1 animals were used as a room control. Groups 2 to 5 animals were acclimatized to restraint tubes over a period of 3 days (15, 30 and 60 minutes, respectively), from Day 13 of gestation, followed by a period of 60 minutes each day in the restraint tubes until Day 20 of gestation. Following parturition, the Group 2, 3, 4 and 5 dams were restrained for 60 minutes daily, from Day 1, 2, 3 and 4 post partum (pp), respectively. Tube restraint continued to Day 10 pp for each group. The dams were checked daily for mortality, signs of poor health, abnormal behavior and signs of parturition. Detailed clinical examinations were performed weekly on the dams and the pups were checked daily. Body weights and food intake were measured during gestation until Day 10 pp for the dams and pup body weights were measured on Days 0, 1, 4, 7 and 10 pp. The dams and pups were euthanized at the end of the study. There were no deaths or adverse clinical signs and no effect upon body weights, food intake or maternal performance including numbers of live pups for the restrained F0 dams. There were no adverse effects upon pup *via*bility, pup body weight or clinical condition due to the pups separation from their dams. Daily tube restraint for 60 minutes starting from Day 15 of gestation to parturition and again from Day 1, 2, 3 or 4 to Day 10 pp had no effects upon maternal performance or pup *via*bility and growth.

708 INHALATION TOXICITY OF GASOLINE & FUEL OXYGENATES: NEUROTOXICITY.

J. P. O'Callaghan[1], C. M. Felton[1], B. K. Mutnansky[1] and W. C. Daughtrey[2]. *[1]Molecular Neurotoxicology, Centers for Disease Control and Prevention-NIOSH, Morgantown, WV and [2]211b Research Group, American Petroleum Institute, Washington, DC.*

In compliance with the Clean Air Act Section 211b for fuel and fuel additive registration, petroleum and oxygenate manufacturers have conducted comparative toxicology testing of evaporative emissions of gasoline alone, and gasoline plus ether and alcohol oxygenates. Here we present results from the neurotoxicity component of this program. The functional observation battery (FOB) with the addition of motor activity (MA) testing, hemotoxylin and eosin staining of brain tissue sections, and brain regional analysis of glial fibrillary acidic protein (GFAP) were used to assess behavioral changes, neuropathology and gliosis, respectively, following inhalation exposure to the test agents. Seven vapor condensates of gasoline (GVC) or gasoline+ether [MTBE (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), diisopropyl ether (G/DIPE)] or alchohol [ethanol (G/EtOH), t-butyl alcohol (G/TBA)] oxygenates were evaluated. Sprague-Dawley rats of both sexes were exposed to the test agents (2000-20, 000 mg/m^3)by inhalation for 13 weeks (6 hrs/day, 5 days/week). FOB and MA were conducted on non-exposure days, at least 16 days post-initiation of exposures; neuropathology and GFAP analyses were conducted at the study termination (13 weeks). A recovery group was included to monitor resolution of behavioral changes. FOB and MA data for all agents, except G/TBA, were negative. G/TBA behavioral effects resolved during recovery. Neuropathology was negative for all groups. Analyses of GFAP revealed multi-brain region increases (as great as 150% of air controls) limited largely to males of the G/EtOH group. Small changes (mostly increases) in GFAP were observed for other test agents but these effects were not consistent across sex, brain region or exposure concentration. The results are consistent with the incidence of a mild gliosis in males of the G/EtOH group. Results of these studies will be used for comparative risk assessments of gasoline/oxygenate blends.

709 INHALATION TOXICITY OF GASOLINE & FUEL OXYGENATES - REPRODUCTIVE TOXICITY ASSESSMENT.

T. M. Gray[1], K. P. Hazelden[3], D. R. Steup[1], J. P. O'Callaghan[2], G. M. Hoffman[3] and L. G. Roberts[1]. *[1]API, 211b Research Group, Washington, DC, [2]Molecular Neurotoxicology, CDC-NIOSH, Morgantown, WV and [3]Reproductive Toxicology, Huntingdon Life Sciences, Inc., East Millstone, NJ.*

In compliance with the Clean Air Act Section 211b for fuel and fuel additive registration, the petroleum industry and oxygenate manufacturers conducted comparative toxicology testing on evaporative emissions of gasoline alone and gasoline containing ether or alcohol oxygenates. Seven vapor condensates of gasoline (GVC), or gasoline-ether [MTBE (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), diisopropyl ether (G/DIPE)], or alcohol [ethanol (G/EtOH), t-butyl alcohol (G/TBA)] were evaluated for reproductive toxicity in Sprague-Dawley rats. G/ETBE, G/TAME, G/DIPE, G/EtOH, and G/TBA were assessed for one-generation; GVC and G/MTBE were assessed over two generations. Additionally, GVC and G/MTBE offspring were evaluated for quantitative changes in regional brain glial fibrillary acidic protein (GFAP) content. All inhalation exposures were 6 h/d, 7 d/w, at levels of 2000 - 20, 000 mg/m3. All exposures increased male kidney weight consistent with light hydrocarbon nephropathy. In adult rats, frequent findings across exposure groups included decreased body weight gain and increased liver weight, most commonly in males. Spleen weight decreased with G/TBA. G/EtOH and G/TBA exposure resulted in higher prostate weight, but no pathological changes occurred to reproductive organs in any study. Except for decreased food consumption during lactation (G/TAME) and a minor increase in time to mating (G/TBA), there were no reproductive findings. Offspring effects included reduced weight gain during lactation (G/TAME) and decreased spleen weight (G/TBA). Results of these studies will be used for comparative risk assessments of gasoline and gasoline/oxygenate blends.

710 ANALYTICAL CHARACTERIZATION OF AEROSOLIZED JET PROPELLANT 8 (JP-8) IN AN EXPOSURE CHAMBER ATMOSPHERE.

K. Frank[1], J. Campbell[1], M. Witten[2], M. Bartlett[3] and J. Fisher[1]. *[1]Interdisciplinary Toxicology Program, University of Georgia, Athens, GA, [2]Pediatrics, University of Arizona, Tuscon, AZ and [3]Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA.*

Jet propellant 8 (JP-8) is a kerosene-based fuel that is composed of hundreds of hydrocarbons and is used by the military. The most common route of JP-8 exposure is through inhalation. Our laboratory is constructing a physiological model for JP-8 to understand its uptake, deposition at target organs, and clearance in animals exposed to JP-8 aerosol and vapor. Analytical methods were developed to identify and quantify JP-8 hydrocarbons in the vapor and aerosol phases of animal exposure chambers. Using a nose-only (mouse) exposure chamber at the University of Arizona, samples of JP-8 aerosol were collected on glass fiber filters and vapor on charcoal tubes. Aerosol samples were also collected on cascade impactor plates. Five samples were collected from 60 minute exposures and three samples were collected from 15 minute exposures. Each exposure was conducted with no animals in the chamber. The impactor plates were analyzed gravimetrically to determine the aerosol chamber concentration of JP-8 in mg/m3. In addition, charcoal tubes, glass fiber filters, and impactor plates were extracted with chloroform and analyzed by GC/MS. Preliminary results indicate that this aerosol exposure system produces a JP-8 chamber atmosphere that consists of 5-10% aerosol and the remainder is vapor. Analysis of the hydrocarbon composition of the aerosol and vapor phases is ongoing. The composition of JP-8 hydrocarbon aerosol fraction collected on glass wool fibers compares favorably with that of the cascade impactor plates. Funded by AFOSR [grant no F49620-03-1-0157].

711 INHALATION TOXICITY OF GASOLINE & FUEL OXYGENATES: TEST SAMPLE PREPARATION.

W. C. Daughtrey, M. Henley, D. M. Burnett, P. Podhasky and R. D. White. *API 211(b) Research Group, Washington, DC.*

In compliance with the Clean Air Act Section 211b requirements for fuel and fuel additive registration, the petroleum industry and oxygenate manufacturers conducted comparative toxicology testing on evaporative emissions of gasoline alone

and gasoline containing ether or alcohol oxygenates. To mimic real world exposures, a distillation method was employed that produced test material similar in composition to the headspace vapor from an automotive fuel tank at near maximum in-use temperatures. Gasoline vapor was generated by a single-step distillation from a 1000-gallon Pfaudler glass-lined kettle wherein approximately 15 to 20 % of the starting material was slowly vaporized, separated, condensed and recovered as test sample. This fraction was termed gasoline vapor condensate (GVC) and was prepared for each of the seven test samples, namely: gasoline alone (GVC), or gasoline + an ether (MTBE, ETBE, TAME, or DIPE), or gasoline + an alcohol (EtOH or TBA). Oxygenate concentrations were generally higher in the vapor phase than the liquid fuel starting material. Additional details about the methodology for generating the GVCs and data on their respective chemical compositions will be presented in the poster. The GVCs were analyzed under GLP conditions using capillary GC or GC MS methods. These materials were used as test samples for the subsequent inhalation toxicology studies described in the accompanying series of abstracts. These studies included evaluations for subchronic toxicity, neurotoxicity, immunotoxicity, genotoxicity, reproductive and developmental toxicity, and chronic toxicity / carcinogenicity. Results of these studies will be submitted to the USEPA and be used for comparative risk assessments of gasoline and gasoline/oxygenate blends.

712 A SIMPLE METHOD FOR COMPARING THE TOXICOLOGIC POTENTIAL OF EMISSIONS FROM VEHICLES USING DIFFERENT FUELS.

C. A. Lapin[1], W. B. Bunn[2] and T. W. Hesterberg[2]. *[1]Lapin & Associates, Glendale, CA and [2]International Truck and Engine Corporation, Chicago, IL.*

In June 2000, the South Coast Air Quality Management District (SCAQMD) of southern California sought to improve air quality through a series on rules promoting the purchase of alternative fueled vehicles over diesel fueled vehicles for local government fleets. With some exceptions, diesel fueled vehicles can only be purchased if their exhaust emissions meet 1) required nitrogen oxides and particulate matter levels and 2) equivalent "toxic risk" to alternative fueled vehicles. The SCAQMD has a proposed procedure for determining "toxic risk" equivalency that focuses solely on cancer potency weighted emissions. This paper describes simple method that builds upon the SQAQMD proposal by also looking at all emissions that have potential air health concerns at ambient levels, not just those with cancer unit risk factors (URF). For emissions with a URF, because of the controversy surrounding the California Office of Environmental Health Hazard Assessment diesel exhaust URF, this paper uses three different potency weighted comparisons: mixed, PM based, and chemical specific. For emissions with Reference Exposure Levels, mass emission rates for each compound are compared. Other measures, such as *in vitro* mutagenicity are also compared when available. The resulting comparisons provide a summary matrix for use by decision makers. The method in this paper was used to compare recent emissions tests of diesel and compressed natural gas (CNG) transit buses (Ayala et al., Diesel Engine Emissions Reduction Conference, 2003). Trapped-equipped diesel transit buses were found to have equivalent potential "toxic risk" when compared to CNG buses equipped with oxidation catalysts. In conclusion, a simple method is described that provides decision makers with a comprehensive and clear way to use available emission data to facilitate a straightforward comparison of the "toxic risk" potential of vehicles using different fuels.

713 INHALATION TOXICITY OF GASOLINE & FUEL OXYGENATES - CHRONIC TOXICITY.

J. Benson[1], C. R. Clark[2], E. B. Barr[1], A. P. Gigliotti[1], T. H. March[1], C. A. Elliott[1], A. P. Gomez[1], B. M. Tibbetts[1] and R. White[2]. *[1]Lovelace Respiratory Research Institute, Albuquerque, NM and [2]API 211(b) Research Group, Washington, DC.*

In compliance with the Clean Air Act Section 211(b) for fuel and fuel additive registration, the petroleum industry and oxygenate manufacturers have conducted comparative toxicology testing of evaporatve emissions from gasoline alone, and gasoline plus ether and alcohol oxygenates. This abstract presents results from chronic toxicity testing of inhaled baseline gasoline vapor condensate (gasoline) and gasoline containing MTBE (G/MTBE). Groups of 50M/50F F344 Crl BR rats were exposed in H2000 whole body inhalation chambers to gasoline or G/MTBE at vapor concentrations of 2000 mg/m3 (low level), 10, 000 mg/m3 (mid level) and 20, 000 mg/m3 (high level) 6 hr/day, 5 d/wk for 104 wk. There were no clinical signs of toxicity attributable to inhalation of either vapor condensate. Survival was not adversely affected by chronic inhalation of either vapor condensate. Mean group body weights of mid and high level male and female gasoline-exposed rats, and of high level male and mid and high level female G/MTBE-exposed rats were significantly below control values for most of the study. Group mean terminal body weights of high level and mid level gasoline exposed rats and high level G/MTBE exposed rats were significantly below control values. Kidney-to-brain weight ratios for high and mid level gasoline exposed females and for high level G/MTBE-exposed males were significantly greater than control. Gross observations at necropsy were chiefly related to lesions commonly present in aged F344 rats. Information on target organ effects and carcinogenicity is pending histopathological evaluations.

714 INHALATION TOXICITY OF GASOLINE & FUEL OXYGENATES: 13-WEEK SUBCHRONIC/NEUROTOXICITY STUDY.

G. Hoffman[1], C. A. Schreiner[2], C. Parker[2] and T. Gray[2]. *[1]Huntingdon Life Sciences Inc., East Millstone, NJ and [2]API 211(b) Research Group, Washington, DC.*

In compliance with the Clean Air Act Section 211(b) for fuel and fuel additive registration, the petroleum industry has conducted comparative toxicology testing of evaporative emissions of seven vapor condensates of gasoline (GVC), or gasoline+ether [methyl tertiary butyl ether (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), diisopropyl ether (G/DIPE)], or alcohol [ethanol (G/EtOH), t-butyl (G/TBA)] oxygenates. Here we present results of a 13-week inhalation study. Results of satellite studies evaluating genetic toxicity, GFAP and immunotoxicity are addressed in accompanying abstracts. Sprague-Dawley rats were exposed to target concentrations of 0, 2000, 10000, or 20000mg/m3 of each test material, 6 hr/day, 5d/wk for 13 wks. Air-control and 20000mg/m3 groups contained 20 rats/sex; 10/sex terminated at wk 13; 10/sex maintained for 4wk untreated recovery. Other groups contained 10 rats/sex. At wk 13 necropsy, 5 rats/sex/group were transcordally perfused and nervous system tissues prepared for neuropathology; all other rats were prepared for routine microscopy. Body wt gain and food consumption were comparable to concurrent controls for all test materials except at the high dose of G/EtOH (both sexes) and G/ETBE (males only) where decreased gain was seen during exposure but was resolved in recovery rats. Neurobehavioral test results (motor activity [MA], functional observational battery [FOB]) were comparable to controls for all test materials except for G/TBA where dose-related trends in MA and grip strength were observed during exposure but were not seen in recovery rats. Neuropathology was negative in all animals. Increased kidney wt and light hydrocarbon nephropathy (LHN) were observed in treated male rats in all studies. Effects were reversible or nearly reversible after 4wk recovery. LHN is unique to male rats and is not relevant to human toxicity. Results of these studies will be used for comparative risk assessment of gasoline and gasoline/oxygenate blends.

715 INHALATION TOXICITY OF GASOLINE & FUEL OXYGENATES: MICRONUCLEUS AND SISTER CHROMATID EXCHANGE TESTS.

C. Schreiner[1], G. Hoffman[2], C. Mason[2] and R. Gudi[3]. *[1]API 211(b) Research Group, Washington, DC, [2]Huntingdon Life Sciences Inc., East Millstone, NJ and [3]BioReliance, Rockville, MD.*

In compliance with the Clean Air Act Section 211(b) for fuel and fuel additive registration, the petroleum industry has conducted a series of comparative multi-endpoint studies of evaporative fractions of seven vapor condensates of gasoline (GVC), or gasoline+ether [methyl t- butyl ether (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), diisopropyl ether (G/DIPE)], or alcohol [ethanol (G/EtOH), t-butyl (G/TBA)] oxygenates. Here we present results from micronucleus (MN) and sister chromatid exchange (SCE) tests. Sprague-Dawley rats (5/sex/group used for both studies) were exposed to target concentrations of 0, 2000, 10000, or 20000mg/m3 of each test material, 6 hr/day, 5d/wk for 4 weeks. Positive control groups (5 rats/sex/group) were used for each study; rats were given cyclophosphamide IP, 24hr prior to sacrifice at doses of 5mg/kg for the SCE study and 40mg/kg for the MN study. At treatment end, blood was collected from the abdominal aorta for the SCE test [cultures established within 24 hr of collection], and femurs removed for the MN test. Femoral bone marrow was prepared and stained using a modified Feulgen method. Approx. 21hr after initiation of culture, cells were exposed to 5ug/ml bromodeoxyuridine (BrdU); colcemid was given at 68hr of culture. Cells were harvested after 72hr in culture and processed for SCE evaluation. No significant increased incidence of micronuclei in polychromatic erythrocytes was observed for any test material. Statistically significant increases in SCE over several doses were observed in rats given GVC alone or G/MTBE; females appeared more sensitive than males. G/TAME induced increased SCE in both sexes at the highest dose only. Although several instances of increased SCE were observed in cultured lymphocytes, gasoline and gasoline/oxygenate blends did not induce cytogenetic effects in bone marrow of exposed animals. Results of these studies will be used for comparative risk assessment of gasoline and gasoline/oxygenate blends.

716 INHALATION TOXICITY OF GASOLINE & FUEL OXYGENATE: IMMUNOTOXICITY.

K. L. White[1], V. L. Peachee[1], S. R. Armstrong[2] and L. E. Twerdok[2]. *[1]ImmunoTox, Inc., Richmond, VA and [2]API 211(b) Research Group, Washington, DC.*

In compliance with the Clean Air Act Section 211b for fuel and fuel additive registration, the petroleum industry and oxygenate manufacturers have conducted comparative toxicology testing of evaporative emissions of gasoline alone, and gasoline plus ether and alcohol oxygenates. Here we present results from the immunotoxicity component of this program. The antibody-forming cell (AFC) response to the T-dependent antigen, sheep erythrocyte (sRBC), was used to determine the affects of the gasoline vapor condensates on the humoral immune component of the immune system. Seven vapor condensates of gasoline (GVC) or gasoline + an ether [MTBE (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), diisopropyl ether (G/DIPE)] or an alcohol [ethanol (G/EtOH), t-butyl alcohol (G/TBA)] oxygenates were evaluated. Female Sprague-Dawley rats were exposed to the test agents (2, 000, 10, 000 and 20, 000 mg/m^3) by inhalation for 4 weeks (6 hrs/day, 5 days/week). Animals were immunized with sRBC four days prior to sacrifice. Spleens were removed the day after the last exposure and were shipped overnight express to ImmunoTox, Inc. where the plaque assays were conducted. Exposure to GVC, G/MTBE, G/TAME, and G/TBA did not result in significant changes in the IgM AFC response to sRBC, when evaluated as either specific activity (AFC/10^6 spleen cells) or as total spleen activity (AFC/spleen). However, exposure to G/EtOH and G/DIPE resulted in a dose-dependent decrease in the AFC response when evaluated as either AFC/10^6 spleen cells or AFC/spleen. The decrease, ranging from 63-86% reached the level of statistical significance at the high (20, 000 mg/m^3) exposure level. Exposure to G/ETBE resulted in a statistically significant decrease in the AFC response when evaluated as either AFC/10^6 spleen cells or AFC/spleen, which reached the level of statistical significance at the middle (10, 000 mg/m^3) and high (20, 000 mg/m^3) exposure levels, ranging from 70-76%. Results of these studies will be used for comparative risk assessments of gasoline and gasoline/oxygenate blends.

717 BIOCHEMICAL CHANGES IN RESPIRATORY TISSUES OF RATS EXPOSED TO ETHYL TERT-BUTYL ETHER.

K. M. Broadwell and R. Schatz. *Northeastern University, Boston, MA.*

Ethyl tert-butyl ether (ETBE), a fuel oxygenate, is added to gasoline to improve combustion and reduce automobile emissions. Studies assessing ETBE respiratory effects are limited. Analysis of bronchoalveolar lavage fluid (BALF) and nasal lavage fluid (NLF) are sensitive tools in assessing suspected respiratory toxicants. In this study, rats were exposed to ETBE (500 and 250 ppm for 6 hours) and sacrificed both immediately and 24 hours following exposure. Significant increases in sialic acid (SA) (93%) and lactate dehydrogenase (LDH) (162%) were seen in NLF of rats sacrificed 24 hours post-exposure to 500 ppm ETBE. Significant increases were observed in protein content (101% and 174%) and acid phosphatase (ACP) (31% and 117%) at 0 and 24 h post exposure in NLF, respectively. There were significant increases in LDH activity (98%), gamma-glutamyl transferase (GGT) (149%), and ACP (86%) at 24 h post-exposure in BALF following a 500 ppm exposure. Following exposure to 250 ppm, there were significant increases in SA (46%), LDH (78%), and ACP (89%) 24 h post-exposure in NLF. ETBE is metabolized to acetaldehyde, a known respiratory irritant and free radical inducer. *In vitro* incubation with acetaldehyde (0.1mM -1mM) with liver, lung, and nasal mucosa microsomes was investigated in a reactive oxygen species (ROS) assay. Acetaldehyde addition produced dose-dependent increases in ROS formation in hepatic, pulmonary, and nasal microsomes (maximal values of 153%, 154%, and 217% for each organ system, respectively). Following an *in vivo* exposure to ETBE, there was a significant increase in ROS formation in lung microsomes (172% at 500 ppm) and nasal mucosa microsomes (181% and 140 % at 500 and 250 ppm, respectively) immediately following exposure. Biochemical changes in lavage fluid parameters indicate that ETBE is a respiratory toxicant. A possible mechanism of toxicity may be bioactivation of ETBE in target organs and subsequent formation of ROS.

718 CATEGORIZING SIGNS AND SYMPTOMS FOR EMERGENCY RESPONSE PLANNING LEVELS.

G. V. Alexeeff and W. D. Wang. *OEHHA, Cal/EPA, Oakland, CA.*

The National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGLs) reviews the scientific literature and identifies adverse effects that may be discomforting (AEGL-1), disabling (AEGL-2), or increasing the likelihood of death (AEGL-3). These levels are to assist emergency response planning. In this analysis, 91 chemical documents were reviewed to evaluate their consistency in classifying signs and symptoms to discrete severity levels. The starting points and adverse effects associated with specific AEGLs were identified. AEGL-1 values based on human data include effects such as headaches, nausea, vomiting, and mild nervous system responses. AEGL-1 values based on animal signs primarily included eye and respiratory irritation. An emphasis on notable discomfort for the AEGL-1 resulted in a lack of reliance on irritation and other mild adverse effects in humans. The signs and symptoms encountered often did not easily conform with AEGL definitions. Some human effects found to occur below the AEGL-1 included mild eye and respiratory irritation, chest tightness, cough, and slight headache. AEGL-2 values were primarily identified based on animal data such as bronchiolar lesions, pulmonary edema, irreversible ocular lesions, incapacitation, ataxia and malformations. Some AEGL-2 values were identified based on human effects and included exposures producing unbearable irritation or considered intolerable by experimental subjects. AEGL-3 values were almost exclusively based on animal lethality studies. AEGLs are derived for once-in-a-lifetime exposures during chemical emergencies. In contrast, most air toxics standard setting is designed to address exposures of the susceptible public to routine emissions from facilities or motor vehicles where mild adverse effects become more important due to repeated or prolonged exposures. Consequently, the adverse effects categories and the AEGLs themselves should not be applied as protective standards in situations other than emergency response planning.

719 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR THREE ALIPHATIC AMINES: ALLYLAMINE (AA), CYCLOHEXYLAMINE (CYC), AND ETHYLENEDIAMINE (EDA).

S. Milanez[1], L. Koller[2], M. McClanahan[3], D. Krewski[4] and K. Bakshi[5]. *[1]Oak Ridge National Laboratory, Oak Ridge, TN, [2]Loren Koller & Associates, Corvallis, OR, [3]Centers for Disease Control and Prevention, Doraville, GA, [4]University of Ottawa, Ottawa, ON, Canada and [5]National Research Council, Washington, DC.*

AA, CYC, and EDA are basic compounds with ammonia-like or fishy odors. AEGLs are airborne concentrations above which toxic effects of specified severity could occur in humans from one-time exposure for 10 min to 8 hrs: AEGL-1 (discomfort), AEGL-2 (disability), and AEGL-3 (death). AEGLs were developed from animal and human inhalation studies by exponential scaling ($C^n \times t = k$) and use of interspecies and intraspecies uncertainty factors and modifying factors. AEGL-1 values for 10 min to 8 hrs were based on the endpoint of sensory irritation for AA and CYC (0.42 ppm; 1.8 ppm), but were not developed for EDA due to lack of data. AEGL-2 values were associated with respiratory irritation as well as cardiovascular lesions (AA), ocular irritation (CYC), and kidney swelling (EDA). The AEGL-2 values for CYC and EDA for 10 min to 8 hrs (11-2.7 ppm; 12-4.8 ppm) were similar, but values were ~3-fold lower for AA (3.3-1.2 ppm). This indicates that AA will elicit non-lethal toxic effects in humans at lower air concentrations than CYC or EDA. The AEGL-3 endpoint for all three amines was the lethality threshold. Animals that died had lung edema and hemorrhage (AA); ocular opacity and lung and bladder lesions (CYC); but no toxic effects were described for EDA (just death). AEGL-3 values for 10 min to 8 hrs were comparable for CYC (38-9.4 ppm) and EDA (25-10 ppm) but spanned a greater range for AA (150-2.3 ppm). The greater values for AA at <4 hrs may have been due to limiting AA metabolism (to acrolein and H_2O_2, known to occur in mammals). The developed AEGL values reflect the similarities and differences in the chemical structure and mode of action of the three amines. [This abstract presents Interim AEGL values that are subject to change pending further review.] ORNL is managed by UT-Battelle LLC for the US Department of Energy under contract DEAC0500OR22725.

720 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR URANIUM HEXAFLUORIDE.

C. Bast[1], G. Rusch[2], D. Krewski[3] and K. Bakshi[4]. *[1]Oak Ridge National Laboratory, Oak Ridge, TN, [2]Honeywell, Morristown, NJ, [3]University of Ottawa, Ottawa, ON, Canada and [4]National Research Council, Washington, DC.*

AEGL values represent one-time exposures for increasingly severe effects with AEGL-1 being least and AEGL-3 being most severe. Uranium hexafluoride (UF_6) is a volatile solid. When airborne, it hydrolyzes immediately on contact with moisture to form hydrofluoric acid (HF) and uranyl fluoride (UO_2F_2). Thus, an inhalation exposure to uranium hexafluoride is actually an inhalation exposure to a mixture of both fluorides. Pulmonary irritation, corrosion, or edema may occur from the hydrofluoric acid component. Renal injury may be observed from the uranium component. In the absence of chemical-specific data, a stoichiometric adjustment factor of 4 was applied to the hydrogen fluoride AEGL-1 values to obtain AEGL-1 values for uranium hexafluoride (3.6 mg/m^3). AEGL-1 values were derived only for the 10-minute, 30-minute, and 1-hour time points. AEGL-2 values (28 mg/m^3, 19 mg/m^3, 9.6 mg/m^3, 2.4 mg/m^3, and 1.2 mg/m^3, for 10-min, 30-min, 1-hr, 4-hr, and 8-hr, respectively) were based on renal pathology in dogs exposed to 192 mg/m^3 UF_6 for 30 minutes. AEGL-2 values were adjusted by interspecies and intraspecies UFs of 3 each. In the absence of an empirically derived chemical-specific scaling exponent for renal effects, temporal scaling was performed using n=3 when

extrapolating to shorter time points and n = 1 when extrapolating to longer time points using the equation, $c^n \times t = k$. The AEGL-3 values (216 mg/m^3, 72 mg/m^3, 36 mg/m^3, 9.0 mg/m^3, and 4.5 mg/m^3 for 10-min, 30-min, 1-hr, 4-hr, and 8-hr, respectively) were based on an estimated lethality threshold in rats. Values were divided by a total uncertainty factor of 10 and scaled across time using the exposure-concentration duration relationship $c^1 \times t = k$, shown to be valid for lethality in rats from pulmonary effects of UF6. [This abstract presents Interim AEGL values subject to change pending further review.] [1]Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the US Department of Energy under contract DEAC0500OR22725.

721 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR HYDROGEN CHLORIDE.

R. Young[1], C. Bast[1], J. Hinz[2], D. Krewski[3] and K. Bakshi[4]. *[1]Oak Ridge National Laboratory, Oak Ridge, TN, [2]USAF, Brooks AFB, TX, [3]University Ottawa, Ottawa, ON, Canada and [4]National Research Council, Washington DC, DC.*

AEGL values represent one-time exposures for increasingly severe effects with AEGL-1 being least and AEGL-3 being most severe. Hydrogen chloride is an upper respiratory irritant at relatively low concentrations and may cause damage to the lower respiratory tract at higher concentrations. Regression analyses of the exposure-concentration duration relationship for lethality in rats and mice determined that the relationship is $c^1 \times t = k$. The AEGL-1 values were based on a 45 minute no-adverse-effect-level of 1.8 ppm in exercising adult asthmatics. No uncertainty factors were applied because the study population consisted of sensitive humans. The 1.8 ppm value was held constant across all exposure durations because there is adaptation to the slight irritation that defines AEGL-1. The 10-minute AEGL-2 value (100 ppm) was derived by dividing the mouse RD50 of 309 ppm by a factor of 3 to estimate an irritation threshold. AEGL-2 values for 30-min, and 1-hr (43 ppm, 22 ppm, respectively) were based on severe nasal or pulmonary histopathology in rats exposed to 1300 ppm for 30 minutes. The values were adjusted by intraspecies and interspecies Uncertainty Factors (UFs) of 3 each and a modifying factor of 3, for a total of 30. Time scaling utilized the regression relationship above. The 4- and 8-hour values (11 ppm) were derived by applying a modifying factor of 2 to the 1-hr AEGL-2 value. The 10-min, 30-min, 1-hr, and 4-hour AEGL-3 values (620 ppm, 210 ppm, 100 ppm, 26 ppm, respectively) were based on an estimated lethality threshold in rats. Values were divided by a total uncertainty factor of 10 and time-scaled using the regression relationship above. The 8-hour value (26 ppm) was set equal to the 4-hour value because low concentrations of HCl are well scrubbed in the nasal passages. These Interim AEGL values are subject to change pending further review. [1]Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the US Department of Energy under contract DEAC0500OR22725.

722 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR HYDROGEN FLUORIDE.

S. Talmage[1], L. Gephart[2], D. Krewski[3] and K. Bakshi[4]. *[1]Oak Ridge National Laboratory, Oak Ridge, TN, [2]Exxonmobil Biomedical Sciences, Inc., Annandale, NJ, [3]University of Ottawa, Ottawa, ON, Canada and [4]National Research Council, Washington, DC.*

AEGL values represent one-time exposures for increasingly severe effects with AEGL-1 being least and AEGL-3 being most severe. AEGL values were developed for hydrogen fluoride (HF), a colorless, highly irritating gas. Data on irritant effects in humans and lethal and sublethal effects in six species of mammals were available for development of AEGL values. Regression analyses of the exposure duration-concentration relationship for lethality for the animal species determined a relationship of $C^2 \times t = k$. The AEGL-1 value was based on a clinical study in which 3 ppm was the threshold for irritation. The 3 ppm value was divided by an intraspecies uncertainty factor (UF) of 3 to protect sensitive individuals. The resulting 1 ppm value was used across all exposure durations because there is adaptation to the slight irritation that defines the AEGL-1. The 10-minute AEGL-2 and AEGL-3 values were based on 10-minute no-effect (950 ppm) and lethal threshold (1700 ppm) values, respectively, in cannulated rats. The values were adjusted by intraspecies and interspecies UFs of 3 each for a total of 10. The 30-minute through 4-hour AEGL-2 values were based on a 1-hour exposure to 243 ppm in which dogs showed signs of sensory irritation. The value was divided by a total UF of 10 and time scaled to the longer exposure durations using the regression relationship above. The 8-hour value was set equal to the 4-hour value because at this concentration (12 ppm), HF is well scrubbed in the nasal passages. The 10-minute through 4-hour AEGL-3 values were based on the threshold for lethality in mice, a 1-hour exposure to 263 ppm. Adjustment was by an uncertainty + modifying factor of 6; time-scaling used the regression relationship above. The 8-hour value was set equal to the 4-hour value. [This abstract presents interim AEGL values subject to change pending further review.] [1]ORNL, managed by UT-Battelle, LLC, for the US Department of Energy under contract DEAC0500OR22725.

723 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR TOLUENE DIISOCYANATE.

C. S. Wood[1], S. J. Barbee[2], D. Krewski[3] and K. Bakshi[4]. *[1]Oak Ridge Nat. Lab., Oak Ridge, TN, [2]Arch Chemicals Inc., Norwalk, CT, [3]University of Ottawa, Ottawa, ON, Canada and [4]National Research Council, Washington, DC.*

2, 4- and 2, 6-Toluene diisocyanate (TDI) cause irritation and sensitization of the respiratory tract. Only irritation effects were considered in establishing AEGL values. AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods of 10 min, 30 min, 1 hr, 4 hr, and 8 hr. AEGL-1 (discomfort) values (0.02, 0.02, 0.02, 0.01, 0.01 ppm, respectively) were based on exposure of fifteen asthmatics to 0.01 ppm TDI for 1 hr, then after a rest of 45 min, to 0.02 ppm TDI for 1 hr. No statistically significant differences in lung function parameters were observed, but nonpathological bronchial obstruction was measured in several individuals; eye and throat irritation, cough, chest tightness, rhinitis, dyspnea, and/or headache were reported. AEGL-2 (disability) values (0.24, 0.17, 0.083, 0.021, and 0.021 ppm, respectively) were based on human data. Exposure of volunteers to 0.5 ppm for 30 minutes resulted in severe eye and throat irritation and lacrimation. A higher exposure concentration was intolerable. Extrapolations were made with the equation $C^n \times t = k$ using default values of n = 3 for extrapolating to shorter durations and n = 1 for extrapolating to longer durations. An uncertainty factor of 3 was applied to account for sensitive individuals. Animal data were used to derive AEGL-3 (death) values (0.65, 0.65, 0.51, 0.32, and 0.16 ppm, respectivley). The 4-hr mouse LC_{50} of 9.7 ppm was divided by 3 to estimate a threshold of lethality and scaled as described for AEGL-2. A total uncertainty factor of 10 was applied which includes 3 to account for sensitive individuals and 3 for interspecies extrapolation. [This abstract presents Interim AEGL values that are subject to change pending further review.] Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the US Department of Energy under contract DEAC0500OR22725.

724 ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR ETHYLENIMINE (EI) AND PROPYLENIMINE (PI).

J. H. Moyer[1], K. A. Davidson[1], M. A. McClanahan[2], D. Krewski[2] and K. S. Bakshi[4]. *[1]Oak Ridge National Laboratory, Oak Ridge, TN, [2]CDC (retired), Atlanta, GA, [3]University of Ottawa, Ottawa, ON, Canada and [4]National Res. Council, Washington, DC.*

AEGL values represent threshold exposure limits for the general public and are applicable to emergency exposure periods of 10, 30, and 60 min, 4 hrs, and 8 hrs. The three AEGL levels (discomfort, disability, and death, respectively) are distinguished by varying degrees of severity of toxicity. EI PI are very reactive alkylating agents that cause blistering similar to that of nitrogen mustard. EI and PI vapors are irritating to the skin, eyes, and respiratory tract; PI, however, is less toxic than EI. The effects of both chemicals may be delayed depending upon the concentration. Data were not available for deriving AEGL-1 values for either EI or PI. Death and/or extreme respiratory difficulty occurred at EI concentrations ≥25 ppm for 1 to 8 hrs. Therefore, AEGL-2 values for EI (33, 9.8, 4.6, 1.0, and 0.47 ppm, respectively) were based a no-observed-effect-level (NOEL) for extreme respiratory difficulty (10 ppm for a 4-hr exposure) in guinea pigs. AEGL-3 values for EI (51, 19, 9.9, 2.8, and 1.5 ppm, respectively) were based on an estimation of the lethality threshold (LC_{01}) of 15 ppm for rats exposed to EI for 8 hrs. AEGL-2 values for PI (83, 25, 12, 2.5, and 1.2 ppm, respectively) were based on the relative potency factor of 5 for PI compared with EI. AEGL-3 values for PI (170, 50, 23, 5.1, and 2.4 ppm, respectively) were based on the NOEL for lethality (500 ppm for 30 min) in guinea pigs. Interspecies and intraspecies uncertainty factors of 3 and 3, respectively, (total UF = 10) were applied to the NOEL to derive the AEGL values. The equation $C^n \times t = k$, where n = 0.91 for the guinea pig studies and n = 1.1 for the rat study, was utilized for time scaling across the 10-min to 8-hr time frames. [This abstract presents interim AEGL values that are subject to change pending further review.] Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the US Department of Energy under contract DEAC0500OR22725.

725 INTERIM ACUTE EXPOSURE GUIDELINE LEVELS (AEGLS) FOR ETHYLENE OXIDE.

K. A. Davidson[1] and K. Blackman[2]. *[1]Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN and [2]FEMA, Washington, DC.*

AEGL values represent threshold exposure limits for the general public and are applicable to emergency exposure periods of 10 min, 30 min, 60 min, 4 hrs, and 8 hrs, and the three levels are distinguished by varying degrees of severity of toxicity. Ethylene oxide (EO) is a direct alkylating agent that is readily absorbed from the respiratory tract of both humans and animals. Inhalation of ethylene oxide vapor has been shown to affect the eyes, respiratory tract, central and peripheral nervous systems, hematopoietic system, and the developing fetus of humans and/or animals. AEGL-1 values were not developed for EO because the odor detection

threshold (≥260 ppm) and concentrations causing mild sensory irritation would be above the AEGL-2 levels. AEGL-2 values (80, 80, 45, 14, and 7.9 ppm for 10 min, 30 min, and 60 min, 4 hrs, and 8 hrs, respectively) were based on a developmental toxicity study in rats. Uncertainty factors (UFs) of 3 for interspecies sensitivity and 3 for intraspecies variability (total UF = 10) were applied to the exposure concentration of 100 ppm for 6 hrs that caused growth retardation in rat fetuses. The equation, C^n x t = k, where n = 1.2, was utilized for time scaling across the relevant exposure durations. The 10-min value is set the same as the 30-min value because of the uncertainty of scaling from a 6-hr study to 10 min. AEGL-3 values (360, 360, 200, 63, and 35 ppm, respectively) were based on an estimation of the lethality threshold (LC_{01} = 628 ppm) utilizing a 4-hr acute inhalation study in rats. Application of UFs and the time scaling method were the same as described for AEGL-2 values. [This abstract presents interim AEGL values that are subject to change pending further review.] Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the US Department of Energy under contract DEAC0500OR22725.

726 INTERIM INDOOR HEALTH GUIDANCE LEVEL FOR FORMALDEHYDE.

R. Lam, R. J. Blaisdell and M. A. Marty. *CalEPA/OEHHA, State of California, Oakland, CA.*

Since 2000 the California Department of Health Services (CDHS) has been using OEHHA's chronic Exposure Reference Levels (RELs) as guidance levels in controlling the release of pollutants from indoor products. The chronic REL for formaldehyde is 2 ppb (3 *mu*g/m3) and is often found above that level in indoor air. OEHHA's chronic RELs are intended to protect the general public who could be exposed to a pollutant continuously for a 24-hour time period. CDHS requested OEHHA to provide an 8-hour indoor health standard for formaldehyde with consideration that at present time it is impossible to completely eliminate formaldehyde emission from wood products. The acute 1-hour REL of 76 ppb (94 *mu*g/m3) for formaldehyde was derived from a human study with eye irritation as the adverse health effect. An 8-hour interim indoor REL (IREL) of 27 ppb (33 *mu*g/m3) was derived directly from this human study using a modification of Haber's Law, Cn * T =K, where n=2. An uncertainty factor of 10 was used to account for intraindividual variability in the human population. This interim number has been used for assessing human exposure to formaldehyde on a typical 8-hour workday or school day. The interim IREL for formaldehyde represents the current best scientific judgment, but there may be need for periodic revision of the number when new scientific data become available. It is believed that inhalation of formaldehyde at 27 ppb or below, will not have adverse noncancer health effects, primarily irritation, for most people. However, highly sensitive groups especially those that respond immunologically to the chemical may be effected at levels below this concentration. Health effects at or below this level can also result from combined exposures to various chemicals or from exposure to the same chemical by multiple routes.

727 UPDATING USEPA'S AMBIENT WATER QUALITY CRITERIA FOR ARSENIC (AS): TOXICITY AND BIOACCUMULATION.

T. R. Henry[1], T. Linton[2], W. Clement[2], D. McIntyre[2] and C. Abernathy[1]. *[1]Office of Water, USEPA, Washington, DC and [2]Great Lakes Environmental Center, Columbus, OH.*

The Clean Water Act requires the USEPA (EPA) to develop, publish and periodically revise AWQC. Human health AWQC are numeric values for pollutant concentrations in ambient waters considered to be protective of human health. In 2000, USEPA updated the methodology for deriving AWQC to reflect advances in key areas such as risk assessment, exposure assessment and bioaccumulation. EPA published partial updates to AWQC, incorporating new information on toxicity and fish consumption, for 83 chemicals in 2000 and proposed updates to 15 chemicals in 2002. As, for which a major reassessment is ongoing, was not included in the 2000 or 2002 updates. Key components of the As criterion reassessment are the toxicology and bioaccumulation inputs. The toxicological basis for the existing As criteria is skin cancer. EPA has considered recent information on target tissues and dose-response relationships for As toxicity and concluded that the cancer potency factor should be based on bladder and lung cancers and therefore, is currently revising the slope factor. To assess the bioaccumulation potential of As, a literature review was conducted to identify data on the relative amounts of inorganic and organic As in aquatic organisms and surface waters. The literature search results have been reviewed for applicability in deriving trophic-level specific bioaccumulation factors, as per the 2000 human health methodology. The present data set is insufficient to derive bioaccumulation factors for any As species other than total As. The proportion of total As in freshwater fish that is inorganic appears to be considerably higher than that previously reported for marine fish and shellfish, and indicates that As speciation data from marine species should not be used for freshwater species. The statements expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of EPA.

728 ARSENIC SPECIATION AND BIOACCUMULATION STUDIES SUPPORT HIGHER AMBIENT WATER QUALITY CRITERION (AWQC).

R. A. Schoof[1], L. Williams[1] and J. W. Yager[2]. *[1]Integral Consulting, Inc., Bellevue, WA and [2]Electric Power Research Institute, Palo Alto, CA.*

The AWQC for protection of human health for arsenic (As) is currently 0.14 µg/L for fish ingestion. Two principal assumptions used to derive the current arsenic AWQC need to be reevaluated when these studies are revised, including the assumption that all arsenic in fish has toxic potential comparable to inorganic As (As-i), and the use of a bioaccumulation factor (BAF) of 44. The majority of the arsenic in fish is composed of complex organic arsenicals that are relatively nontoxic. Available studies of As speciation in seafood support the following assumptions regarding the fraction of total As (As-tot) that is As-i in various kinds of seafood: freshwater fish, 0.1, marine and estuarine fish, 0.01, crustaceans, 0.01, and molluscs, 0.02. The fractional contribution of these seafood categories to the total fish consumption value of 17.5 g/day is as follows: freshwater fish, 0.19, marine and estuarine fish, 0.37, crustaceans, 0.41 and molluscs, 0.03. Multiplying these intake fractions by the corresponding As-i fraction indicates that an average of 2.7% of As-tot in seafood consumed is As-i. The BCF of 44 was intended for broad application to freshwater and estuarine environments, and is based on consumption-weighted values for oysters (350) and fish (1). However, available studies of As bioaccumulation in seafood suggest that no increases in tissue As concentrations occur in freshwater fish exposed to water As concentrations less than 100 µg/L. Additional studies are needed to verify this finding and to assess bioaccumulation in estuarine species and shellfish. On an interim bases, a BAF of 1 should be used to derive a revised As AWQC for fish consumption. When this BAF and an adjustment of 0.027 to reflect the average fraction of As-tot that is As-i and the current oral cancer slope factor are incorporated into the AWQC equation, the resulting value is 99 µg/L. This analysis demonstrates that the current As AWQC for fish consumption may be more than 700-fold lower than needed to protect human health.

729 ESTIMATING A RELATIVE SOURCE CONTRIBUTION FOR DRINKING WATER IN ARSENIC (AS) RISK ASSESSMENTS.

I. S. Dooley[1], C. O. Abernathy[1], M. Devitt[2] and A. Kotros[2]. *[1]Environmental Protection Agency, Washington, DC and [2]The Cadmus Group, Watertown, MA.*

In 1996, the EPA charged the National Academy of Sciences/National Research Council (NRC) to review the Agency's draft risk assessment of As in food and drinking water, considering the available studies. NRC issued a report in 1999 and EPA used many of the NRC's recommendations and data to quantify bladder and lung cancer risks for its 2001 arsenic drinking water regulation. After publishing the final arsenic rule on January 22, 2001, the Agency asked the NRC to re-evaluate the Agency's risk assessment, available studies, and the effect of data uncertainties and differences in populations related to As. One uncertainty examined is water consumption. The 2001 NRC report commented on the Taiwanese water consumption rates applied in the 1999 NRC report and EPA's 2001 drinking water risk assessment and characterization. In this paper, we use US Army data to examine some parameters that can affect water consumption to estimate drinking water consumption in Taiwan. We used both temperature and relative humidity to develop heat categories along with 3 different levels of work (light, medium or heavy activity) to estimate the water intake levels for Taiwanese (adjusted to 50 kg). During the warm season (or growing season), water intake estimates ranged from 4.3 L/day (light activity) to 6.4 L/day (medium to heavy activity). During the cooler months, the lower bound estimates were taken from the Army's minimum recommendations of 1.7 to 2.1 L/day. These calculations were based on only "working" hours and average temperatures to calculate water needs. For most of the year, water intake is near 2 L/day, but during growing season, they may reach 2 to 3 times the 2 L/day rate generally used by EPA to estimate water consumption. This methodology needs to be further validated prior to its use in risk assessments. [These views are those of the authors and do not necessarily represent the views of EPA or the Cadmus Group.]

730 RISK ASSESSMENT FOR CHEMICALS IN DRINKING WATER: ESTIMATION OF RELATIVE SOURCE CONTRIBUTION.

R. A. Howd, J. P. Brown and A. M. Fan. *Office of Environmental Health Hazard Assessment, Cal/EPA, Oakland, CA.*

In estimating health-protective levels of chemicals in drinking water, one of the factors that should be considered is the proportion of the total permissible dose that is derived from water, versus other exposure routes. This is applied only for chemicals

or toxic effects presumed to involve a toxicity threshold (primarily non-cancer effects), in which the combined daily dose from all exposure routes is used for calculation of allowable exposure levels. However, the calculation of the relative source contribution (RSC) is usually rather crude due to a lack of appropriate data, and the methods for estimation of an RSC in the absence of good data involve a large component of professional judgment. USEPA (USEPA) policy provides limited guidance on calculating RSC. Values in the range of 0.2 to 0.8 (20 to 80%) are allowed, with a default value of 0.2 (20%) of total permissible exposure in the absence of data. OEHHA has generally followed this guidance, although it has also used a value of 1.0 (100%) in its derivation of Public Health Goals (PHGs) for drinking water when human data were used, which was uncontrolled for environmental exposures. Application of the principles for development of RSCs can be illustrated by examining the values and rationale used in the development of PHGs. Examples of the range of values include aluminum (1.0, acute human study), beryllium (0.2, animal study), cadmium (0.2, chronic human study), copper (0.8, acute human study), ethylbenzene (0.2, chronic animal study), fluoride (1.0, chronic human studies), Freon-11 (0.4, subchronic animal study), nickel (0.3, animal reproductive study), simazine (0.2, chronic animal study), thallium (0.2, subchronic animal study), 1, 2, 4-trichlorobenzene (0.2, subchronic animal study), and xylene (0.4, chronic human study). The entire set of 69 chemicals for which PHGs have so far been finalized provides a larger database for illustration of the application of RSC development principles.

731 INTERACTION PROFILE FOR CHEMICALS IN RURAL WELL WATER.

J. Colman[1] and H. Pohl[2]. *[1]Syracuse Research Corp., Syracuse, NY and [2]ATSDR, Atlanta, GA.* Sponsor: P. McGinnis.

Atrazine, deethylatrazine, simazine, diazinon and nitrate frequently occur together in rural well water. The potential impact of this mixture on public health was evaluated using an approach developed by ATSDR. Because pertinent data were not available for the whole mixture, evaluation of health effects, mechanistic and joint toxic action data for individual components and various combinations of components was performed in the ATSDR Interaction Profile. The profile recommends that reproductive health hazard of atrazine, deethylatrazine and simazine be assessed with the hazard index (HI). Neurological effects of diazinon and hematological effects of nitrate are to be assessed with separate hazard quotients (HQs). This approach is used when HQs of components are ≥0.1. The Weight of Evidence (WOE) for interactions indicates high confidence in the dose additivity assumption (HI) for triazine components and uncertainty regarding potential effects of diazinon or nitrate on reproductive toxicity of these components. WOE analysis suggests that the triazine components may potentiate neurological toxicity of diazinon such that the HQ may underestimate the hazard. No information regarding impact of interactions on hematological toxicity of nitrate was available so uncertainty is high for this endpoint. Although components of this mixture have not been classified as carcinogens, triazines may interact with nitrate (as the metabolite nitrite) to form N-nitrosoatrazine and N-nitrososimazine, which are more genotoxic than the parent triazines. Confidence in the WOE prediction that this chemical interaction may result in carcinogenicity is medium because although adequate cancer data are lacking for these compounds, data for other N-nitrosamines and their precursors support the prediction. When screening criteria are exceeded (HI>1 for reproductive effects of triazine components, HQ close to or >1 for neurological effects of diazinon, and/or HQ>1 for nitrate), further evaluation is needed using biomedical judgment and community-specific health outcome data and taking into account potential carcinogenicity of nitrosamines that may be formed.

732 REGULATORY DETERMINATION FOR HEXACHLOROBUTADIENE IN DRINKING WATER.

D. Wong and J. Du. *USEPA, Washington, DC.*

The 1996 Safe Drinking Water Act Amendment (SDWA) requires EPA to establish a list of contaminants to aid the Agency in regulatory priority setting for its drinking water program. The first Contaminant Candidate List (CCL) was published on March 2, 1998; and hexachlorobutadiene (HCBD) was one of the 19 chemicals in the regulatory determination priority category. The SDWA requires EPA to make regulatory determinations for no fewer than five contaminants in the regulatory determination priority category. Regulations are only recommended when all the following three criteria are satisfied: 1) The chemical must have adverse health effects, 2) It must occur or is likely to occur in drinking water at concentrations of health concern, and 3) Regulation of this chemical provides a meaningful opportunity to reduce human risk. The available toxicological data indicate that HCBD can cause adverse health effects in animals, and probably also in humans at high dose. In rodents, there is clear evidence of renal damage resulting from acute, subchronic, and chronic exposures to HCBD by the oral route. Under EPA's 1999 draft Guidelines for Carcinogen Risk Assessment, HCBD is classified as likely to be carcinogenic to humans by the oral route of exposure. However, the available data on occurrence, exposure, and other risk considerations suggest that, because HCBD does not occur in public water systems at frequencies and levels of public health concern, regulating HCBD will not present a meaningful opportunity for health risk reduction for persons served by public water systems. (The opinions expressed in this abstract are those of the author and not necessarily those of EPA.)

733 DERIVATION OF A DRINKING WATER ACTION LEVEL FOR 2-MERCAPTOBENZOTHIAZOLE.

A. Gebhart[1], M. H. Whittaker[2] and F. Hammer[1]. *[1]Water Program, Underwriters Laboratories, Northbrook, IL and [2]ToxServices, Washington, DC.*

2-Mercaptobenzothiazole (MBT) is used as a vulcanization accelerator in rubber products that come into contact with potable drinking water. When such products are submitted for ANSI/NSF Standard 61 certification, any chemical extracting from these products must be below an appropriate action level. As defined by Standard 61, a Total Allowable Concentration (TAC) is the maximum concentration of a nonregulated contaminant allowed in a public drinking water supply, and the Single Product Allowable Concentration (SPAC) is 10% of the TAC. In order to determine an action level for MBT, a comprehensive health effects evaluation was performed. Epidemiological investigations indicate that occupational exposure to MBT results in an increased risk of death from bladder cancer. Genotoxicity investigations provide evidence indicating that MBT has the potential to induce mutations and chromosome aberrations. Studies in rats and mice chronically exposed to MBT identified increases in tumors, such as adrenal, pituitary, liver, and renal pelvis tumors. The biological significance of most of these tumors is questionable due to a lack of dose-response, and the effect of test article vehicle (corn oil) upon tumor rates. A review of the epidemiological and toxicological datasets for MBT indicates that the induction of renal pelvis transitional cell tumors is the most sensitive health effects endpoint upon which to base a TAC and SPAC. A linearized multistage model was used to extrapolate to low-dose MBT exposure due to the genotoxicity of MBT. A TAC of 100 μg/L was derived for MBT, and is based upon an LED10 of 164.18 mg/kg/day and a 20% relative source contribution factor. A SPAC of 10 μg/L was derived by multiplying the revised TAC by 0.10. These action levels are based upon the most sensitive health effects endpoint, as well as current risk assessment methodologies.

734 HUMAN HEALTH RISK ASSESSMENT OF FURFURAL TO DETERMINE DRINKING WATER ACTION LEVELS.

A. Ewing[1], A. Phelka[2], G. Ball[3] and C. McLellan[4]. *[1]NSF International, Ann Arbor, MI, [2]NSF International, Ann Arbor, MI, [3]NSF International, Ann Arbor, MI and [4]NSF International, Ann Arbor, MI.* Sponsor: M. Dourson.

A human health risk assessment was conducted by NSF International to determine acceptable levels of furfural in drinking water according to NSF/ANSI Standard 60/61, Annex A. The scientific literature relating to human and laboratory animal exposure to furfural was reviewed. Significant treatment-related responses were limited to liver lesions in high-dose male rats. A chronic rat bioassay completed by the NTP (1990) was selected as the key study from which oral risk values were derived. Groups of 50 F344/N rats of each sex were administered furfural by gavage at adjusted doses of 0, 21 or 43 mg/kg-day. Significant liver lesions were observed at each of the two administered doses, preventing the identification of a NOAEL. However, the available data suggested that a dose level should exist below which no significant treatment-related responses were observed. Benchmark dose modeling after conversion to human equivalent doses, was used to derive a BMDL10 of 2.9 mg/kg-day using centrilobular necrosis of the liver as the critical, treatment-related and precursor response. The logistic model provided the best fit, although five other dichotomous models gave similar results. Uncertainties of 3x for interspecies variability, 10x for intraspecies variability, 3x for database deficiencies and 1x each for less-than-lifetime extrapolation and LOAEL to NOAEL extrapolation were incorporated into the oral RfD derivation. An oral RfD of 0.03 mg/kg-day, with a 20% relative source contribution for drinking water was used to derive NSF-specific risk management values, including a Total Allowable Concentration of 0.2 mg/L and a Single Product Allowable Concentration of 0.02 mg/L.

735 HUMAN HEALTH RISK ASSESSMENT FOR *p*-CHLORO-*m*-CRESOL TO DETERMINE DRINKING WATER ACTION LEVELS.

J. Durham, V. S. Bhat, G. L. Ball and C. J. Mclellan. *Toxicology Services, NSF International, Ann Arbor, MI.* Sponsor: M. Dourson.

A risk assessment to determine acceptable levels of *p*-chloro-*m*-cresol in drinking water was conducted according to Annex A of NSF/ANSI Standard 60/61. Scientific literature in humans and animals was reviewed. *p*-Chloro-*m*-cresol tested

positive in one *Salmonella* reverse mutation assay in Strain TA97 with metabolic activation but tested negative in all tested strains in four other *Salmonella* studies. *p*-Chloro-*m*-cresol tested negative in chromosomal aberration and unscheduled DNA synthesis assays. The weight of evidence suggested *p*-chloro-*m*-cresol is not genotoxic. In a developmental study, a NOAEL of 100 mg/kg-day was chosen based on clinical signs of toxicity and decreased mean body weight gain in dams and also based on a statistically significant decrease in mean fetal weight per litter. The critical study chosen for this risk assessment was a two-year feeding study in which rats were administered *p*-chloro-*m*-cresol at 0, 21, 103.1, or 558.9 mg/kg-day in males and 0, 27.7, 134.3, or 743.5 mg/kg-day in females, as indicated by the authors of the study. In mid-dose females and low-dose males, a statistically significant increase in the number of pituitary adenomas was observed. In mid- and high-dose males, a statistically significant increasing trend of testicular interstitial cell adenomas was observed. Since all neoplastic effects were within historical control ranges, they were not considered relevant. In high-dose males, an increased incidence of unilateral papillary necroses, truncated papillae, and cortical dilations and fibrosis of the kidneys were observed. These renal effects were considered to be the critical effects. Using a NOAEL of 103.1 mg/kg-day and an uncertainty factor of 1000x to account for interspecies extrapolation, intraspecies variability, and database deficiencies, an oral RfD of 0.1 mg/kg-day was calculated. The resulting Short-Term Exposure Level was calculated to be 1 mg/L, and the chronic exposure levels, Total Allowable Concentration and Single Product Allowable Concentration, were calculated to be 0.7 mg/L and 0.07 mg/L, respectively.

736 RISK ASSESSMENT FOR DRINKING WATER CONTAMINANTS: ASBESTOS, BARIUM, BERYLLIUM, CHLOROBENZENE, 1, 1- DICHLOROETHANE, DIETHYLHEXYLADIPATE, ETHYLENE DIBROMIDE, HEXACHLOROBENZENE, SILVEX, 1, 1, 2, 2-TETRACHLOROETHANE AND TOXAPHENE.

A. M. Fan, R. A. Howd, G. Alexeeff, M. Sullivan, T. Parker, J. Avalos, P. Painter, D. Ting, N. Butler, C. Vidair and L. Jowa. *Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland/Sacramento, CA.*

Risk assessments were performed for 11 chemicals for development of public health goals (PHGs) in drinking water. PHGs are non-regulatory concentrations in water that are not anticipated to produce adverse health effects following a lifetime exposure, based on health considerations. Toxicological evaluations included both animal and human studies, and carcinogenic and non-carcinogenic endpoints. The PHGs established based on carcinogenic endpoints are: Asbestos, 7E6 fibers/L (>10 um), mice; 1, 1-dichloroethane, 3 ppb, rats; ethylene dibromide, 10 ppb, rats and mice; hexachlorobenzene, 0.03 ppb, rats; 1, 1, 2, 2-tetrachloroethane, 0.1 ppb, mice; and toxaphene, 0.03 ppb, mice. PHGs based on non-cancer endpoints are: Barium, 2000 ppb, absence of cardiovascular effects (hypertension), human; beryllium, 1 ppb, gastrointestinal tract lesions, dog; diethylhexyladipate, 200 ppb, fetotoxicity, rat; chlorobenzene, 200 ppb, liver pathology, dog; and silvex, 25 ppb, liver pathology, dogs. The assessments included the consideration of sensitive populations, cancer potency values, relative source contribution, uncertainty factors, and water consumption intake. These PHGs were finalized September 2003 after extensive periods for scientific peer review and public input.

737 HEALTH RISKS TO FETUSES, INFANTS AND CHILDREN (PROPOSED STAGE 2 DISINFECTANT/ DISINFECTION BYPRODUCTS): A REVIEW.

Q. Zhao[1], N. Chiu[2], J. Du[2], A. Bathija[2], J. Donohue[2] and D. Wong[2]. *[1]TERA, Cincinnati, OH and [2]USEPA, Washington, DC.*

The 1996 Safe Drinking Water Act requires that any new regulations for a drinking water contaminant must address the risk to sensitive populations, including children. In the development of Stage 1 regulations for the disinfectants and disinfectant by-products (DBPs), a children's risk report was developed in 1998 entitled "Health Risks to Fetuses, Infants, and Children (Final Stage 1 D/DBP Rule)." A corresponding Stage 2 D/DBP document updates the Stage 1 document on health risks to fetuses, infants, and and includes a number of new developmental, reproductive and cancer studies in animals, in addition to new epidemiology data on developmental and reproductive effects. The new document includes proposals for new Maximum Contaminant Level Goals (MCLGs) for three DBPs: monochloroacetic acid (MCA), trichloroacetic acid (TCA), and chloroform. MCLGs and Maximum Residential Disinfectant Level Goals (MRDLGs) promulgated in 1998 for six DBPs and three disinfectants are unchanged, but the health effects data base as it relates to the fetuses, infants and children has been updated. At the present time, the data necessary for quantifying risks are not available for four DBPs, these are: monobromoacetic acid (MBA), bromochloroacetic acid (BCA), dibromoacetic acid (DBA) and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX).

738 DERIVATION OF A LITRE-EQUIVALENT MODIFYING FACTOR TO ACCOUNT FOR MULTIROUTE EXPOSURES IN SETTING DRINKING WATER GOALS FOR TRIHALOMETHANES (THMS).

K. Krishnan[1] and R. Carrier[2]. *[1]Occupational and Environmental Health, Universite de Montreal, Montreal, QC, Canada and [2]Water Quality and Health Bureau, Health Canada, Ottawa, ON, Canada.*

In establishing the drinking water goals for THMs, the importance of inhalation and dermal routes associated with showering and bathing should be evaluated. The objective of the present study was to derive a modifying factor, in terms of L-equivalent, for each of the THMs by evaluating the relative contribution of inhalation and dermal exposures associated with household use of tap water. The approach involved the derivation of an overall L-equivalent for dermal and inhalation exposures to THMs during showering/bathing as follows: L-equivalent = (Kp x A x t x F x 0.001) + (Pw/a x Qp x t x F x 0.001), where Kp = skin permeability coefficient (cm/hr), A = area of skin exposed (sq. cm), t = duration of exposure (hr), F = fraction of the dose that is absorbed (unitless), Pw/a = water-to-air factor (L/cub. metre), Qp = alveolar ventilation rate (L/hr), and 0.001 = conversion factor (L to cubic metre, or cubic cm to L). To solve the above equation for chloroform (CLFM), bromoform (BROM), bromodichloromethane (BDCM), and dibromochloromethane (DBCM), the numerical values of Kp, A, Pw/a and Qp were obtained from the published literature, whereas t was set to 30 min (duration of bathing) and F was estimated using a physiologically-based pharmacokinetic model. The L-equivalents, reflective of the contribution of dermal and inhalation exposures during 30-min bathing, were 2.6, 2.1, 2.1 and 2.2 for CLFM, BDCM, DBCM and BROM, respectively. These numbers for a 6-yr old child were 1.5, 1, 1, and 1.1 for CLFM, BDCM, DBCM and BROM, respectively. Whereas contribution of the inhalation route was more important than dermal route for CLFM, the opposite was true for the three other THMs. The litre-equivalent modifying factors derived in this study, based on the consideration of the relative importance of dermal and inhalation routes during bathing, should be useful for setting drinking water goals for THMs.

739 HUMAN HEALTH RISK ASSESSMENT FOR ENVIRONMENTAL RESIDUES OF ATOMOXETINE.

J. P. Bercu, J. M. Fiori and R. D. Meyerhoff. *Toxicology and Drug Disposition, Eli Lilly and Company, Greenfield, IN.*

Atomoxetine (Strattera®) is a selective norepinephrine reuptake inhibitor recently approved by the FDA for the oral treatment of attention-deficit/hyperactivity disorder. A risk assessment was performed for the impact on human health from possible trace atomoxetine residues in surface water. This included calculations of representative atomoxetine levels in the environment and safe levels for children and adults. Assessment of a possible exposure level was derived from FDA guidance for estimating the amount of atomoxetine that could be eliminated and enter publicly owned treatment works. Based on the calculations, the upper level of residual atomoxetine that might be in the surface water is about 46 ng/L. In order to determine safe levels for atomoxetine, nonclinical and clinical safety endpoints were reviewed. The recommended starting doses for atomoxetine are 0.5 mg/kg/day for children and 40 mg/day for adults. Doses are increased after a minimum of 3 days to a target daily dose of 1.2 mg/kg/day for children and 80 mg/day for adults. Based on levels of water and fish consumption, exposure to atomoxetine is at least 100, 000 times lower than the recommended starting dose for therapy. Thus, there is no concern for human health resulting from exposure to residues of atomoxetine in water.

740 EVALUATION OF NONCANCER HEALTH RISK ASSOCIATED WITH EXPOSURE TO CHEMICAL MIXTURES IN FISH.

S. J. Baird[1] and T. S. Bridges[2]. *[1]Menzie-Cura & Assoc., Inc., Winchester, MA and [2]US Army Engineer Research and Development Center, Vicksburg, MS.* Sponsor: L. Hicks.

Bioaccumulative chemicals found in contaminated sediments move through the aquatic food chain and result in PCBs, TCDD, pesticides, metals and PAHs in fish tissue. Evaluation of the human health noncancer hazard from exposure to these mixtures of chemicals typically considers one chemical at a time, with the total noncancer hazard index (HI) evaluated by summing the individual chemical hazard quotients (HQ) based on the critical effect. When an estimated HI is >1, the mixture is reevaluated based on the target organs (TO) of the critical effect (Risk Assessment Guidance for Superfund, USEPA, 1989). However, effects of toxicological significance can occur in additional TOs at doses similar to that eliciting the critical effect. Ignoring these additional effects may lead to an underestimate of noncancer hazard associated with an exposure. We evaluated the potential health impact of not considering these effects. We calculated TO-HIs by dividing an esti-

mated average daily dose for each chemical by the toxicologic target organ doses (TTD) (i.e., TO specific RfDs) for all significant effects for all chemicals in a mixture typically found in fish tissue. We used TTDs developed by ATSDR, presented in Interaction Profiles (ATSDR, 2001), as well as TTDs developed following USEPA guidance for the derivation of RfDs. The influence of dose rate and timing of the exposures, and the potential for interactions within the exposure-dose-response pathway (i.e., toxicokinetics, toxicodynamics) were considered. The TO-HIs were 3 to 5 times higher than HIs based on critical effects only for neurological, hematological, reproductive, and hepatic TO systems for a mixture of PCBs, DDE, methylmercury, zinc, arsenic, cadmium and chromium(VI). Our results suggest that human health risk from exposure to a mixture of chemicals typically found in fish could be underestimated when effects other than the critical effects are omitted from the hazard evaluation. These results support the need for continued efforts to understand the risk from chemical mixtures.

741 FACTORS AFFECTING EXPOSURE AND RISK TO DOMOIC AID VIA SHELLFISH CONSUMPTION FOR HIGH RISK POPULATIONS.

N. L. Judd[1, 2], W. C. Griffith[1, 2] and E. M. Faustman[1, 2]. [1]*Environmental Health, University Washington, Seattle, WA and* [2]*Institute for Risk Analysis and Risk Communication, Seattle, WA.*

The Pacific Northwest is a unique location for study of health risks associated with shellfish contaminated with domoic acid because there are groups with diverse diets who consume high levels of seafood, and domoic acid contamination is a common phenomenon in this region. Our studies have evaluated which groups may be most at risk and the importance of different dietary and behavioral factors for determining domoic acid health risks for these groups. Recent reports of total seafood consumption by some tribal groups and recent Asian and Pacific Islander (API) immigrants are 5X the national average, and average shellfish consumption by tribal groups may be 25X the national average. Tribal members also collect shellfish where domoic acid contamination is common. In both tribal and API communities, cultural practices result in consumption behaviors, like drinking shellfish cooking water and consumption of the crab hepatopancreas, which may make them more vulnerable to both acute high level and chronic low-level domoic acid exposure. We have previously shown that these dietary behaviors may increase risk from some contaminants (e.g. PCBs) by more than 10X. Shellfish monitoring practices may not be sufficient to evaluate risk from chronic exposures. The limit of detection is high (1 mg/kg) relative to the tolerable limit for domoic acid acute toxicity (20 mg/kg). Thus, there may be potential for chronic health risks from low level (non-detected) domoic acid exposure for these high consumption groups. This contributes to uncertainties about exposure and toxicity which limit ability to assess risk. We have evaluated four additional dietary and behavioral factors which influence domoic acid exposure from shellfish. To prioritize data needs identified for these dietary and behavioral factors, we have developed a model for evaluating domioc acid health risks from shellfish consumption. Supported by the Center for the Study and Improvement of Regulation at Carnegie Mellon University.

742 BIOACCESSIBILITY TESTING OF PAH CONTAMINATED SOIL AND ITS APPLICATION IN HUMAN HEALTH RISK ASSESSMENT.

M. J. Chappel[1], B. Birmingham[1], B. Gizyn[1], B. Leece[3], University. Klee[3], J. Bestari[2] and K. Solomon[2]. [1]*Standards Development Branch, Ontario Ministry of the Environment, Toronto, ON, Canada,* [2]*CNTC, University of Guelph, Guelph, ON, Canada and* [3]*Risk Assessment, Dillon, Cambridge, ON, Canada.*

In human health risk assessment, ingestion of soil is considered to be a major route of exposure for soil-borne contaminants. However, only a fraction of a chemical measured in soil may be available to impact human health. Bioaccessibility (*in vitro*) studies are designed to estimate the fraction that may be accessible for absorption following ingestion. A bioaccessibility study was conducted on polycyclic aromatic hydrocarbon (PAH) contaminated soils collected from a community in S. Ontario where elevated levels of carcinogenic and non-carcinogenic PAH have been detected. The objectives were to estimate a location-specific bioaccessibility factor value for a PAH mixture in soil to determine more accurate exposure point concentrations and to refine the exposure assessment portion of the risk assessment based on site-specific considerations. Soil samples were tested using a modified *in vitro* digestion process. Soil characteristics and properties were also evaluated. Results indicated that PAHs were consistently recovered from the digestive fluid. Average recovery ranged from 53 to 67% (+/-3%). The average bioaccessibility factor (BAF) values for unfiltered non-carcinogenic PAH samples was 0.09 (+/- 0.04), while BAF for unfiltered carcinogenic PAH samples was 0.07 (+/- 0.04). The BAF values were, on average, 20% higher for non-carcinogenic PAHs when compared to carcinogenic PAHs, and 20% higher between unfiltered and filtered samples. Although individual sample PAH BAFs ranged from 0.01 to 0.20 for the soil type tested, the combined average BAF for all PAHs was 0.08. The effect of other variables, including soil concentration were also assessed. Although the results of the *in vitro* bioaccessibility study were used for adjusting intakes of soil component, the results should be interpreted with caution, as *in vivo* validation has not taken place.

743 MODELING RISKS FROM INTERMITTENT EXPOSURES TO LEAD: EFFECT OF EXPOSURE MODEL AVERAGING TIME.

M. H. Follansbee[1], R. M. Lorenzana[2], R. Troast[3], J. M. Klotzbach[4] and G. L. Diamond[1]. [1]*Environmental Science Center, Syracuse Research Corp., Syracuse, NY,* [2]*R10, USEPA, Seattle, WA,* [3]*OSRTI, USEPA, Washington, DC and* [4]*Syracuse Environmental Research Associates, Syracuse, NY.*

Lead exposures often involve both long-term exposures to relatively constant exposure levels (e.g., yard soil) and intermittent exposures elsewhere (e.g., seasonal visits to a park), resulting in blood lead concentrations (PbB) that vary on a temporal scale with the intermittent exposure pattern. Prediction of short-term PbB arising from intermittent exposures requires a model that can reliably simulate lead exposures and biokinetics on a temporal scale equivalent to the exposure events. If exposure model averaging times (EMATs) exceed the shortest exposure duration that characterizes the intermittent exposure, uncertainties will be introduced into risk estimates because the exposure concentration must be time-averaged. The International Commission of Radiological Protection (ICRP) model simulates lead intakes that can vary over time spans as small as one day, allowing for simulation of intermittent exposures to lead, and resulting PbB, as a series of discrete daily exposure events. The ICRP model was used to explore the potential magnitude of uncertainty introduced into risk estimates (expressed as the probability of a specified PbB) from time averaging of exposures. Results of these analyses suggest that standard approaches to time averaging that estimate the long-term daily exposure concentration can, in some cases, produce substantial under-prediction of short-term variations in PbB predicted for intermittent exposures. In general, risk estimates will be improved by simulation of exposure and biokinetics of lead on shorter time scales that more closely approximate the actual temporal dynamics of the exposure. In applications of existing regulatory models that implement EMATs of 1 year or more, however, alternative approaches to time averaging approaches that more reliably predict short-term elevations in PbB are recommended. [Views expressed in this report are those of the authors and do not represent the views of the USEPA.]

744 LEAD AND COPPER AT MILITARY SMALL ARMS RANGES; EVALUATION, RISK ASSESSMENT, AND BIOAVAILABILITY ISSUES.

D. Bannon[1] and B. R. DeShields[2]. [1]*Center for Health Promotion and Preventive Medicine, US Army, APG, Aberdeen, MD and* [2]*Blasland, Bouck, and Lee, Inc., Engineers and Scientists, San Rafael, CA.* Sponsor: G. Reddy.

An estimated 2000-3000 military small arms ranges exist in the continental US, ranging in size from a few to hundreds of firing lanes. These sites are all commonly contaminated with lead (Pb) and copper (Cu) from copper-jacketed lead bullets. Bullets can be entire, fragmented, or oxidized, and risk assessment methods attempt to address the health and environmental effects of these varied forms. Over time the ultimate fate of the metals is oxidation to compounds of Pb and Cu that interact with the soil particles, forming a vehicle for metal ingestion. Human health and ecological concerns primarily stem from exposure to Pb, although copper can also pose a hazard. How risk is assessed depends on whether the targeted use is residential, industrial, or continued military. For Pb, well established human models of soil ingestion and absorption are used into which default parameters are placed, while ecological risk assessment relies on less precise exposure estimates and toxicity values, including the hazard quotient (a ratio of the estimated ingested dose of a metal to the dose of a metal supplied in a controlled laboratory toxicity study) for indicator species such as small mammals or birds. For Cu, no such models exist, and the hazard quotient is used. Relative bioavailabilty (RBA) measurements adjust the absorption of metals to account for chemical forms of the metal and soil properties of the site, using soluble salts as a reference. For Pb, this adjustment factor is based on *in vivo* swine surrogates of human digestion and absorption, allowing the setting of realistic and cost-effective cleanup goals. Current research focuses on the establishment of reliable *in vitro* methods for bioavailability assessment. A modified method for ecological receptors is also examined. For small arms ranges, rapid *in vitro* methods for bioavailability measurement would expedite decision making and remediation.

745 EFFECTS OF LEAD BIOAVAILABILITY ON OUTCOME OF ADULT AND CHILD BIOKINETIC MODELS.

I. S. Chaudhuri and M. Garcia. *Risk Assessment, ENSR International, Westford, MA.*

A risk evaluation was conducted to develop lead remedial goals for lead present in thermally treated foundry sand. The foundry used a method that involved roasting waste foundry sand at a temperature of 800C. for about 45 minutes. Roasting waste

foundry sand results in the formation of insoluble lead silicate. This roasting method results in reducing leachable lead concentrations to less than 5 mg/L as measured by USEPA's Toxicity Characteristic Leaching Procedure (TCLP). The lead leachate from foundry sand before thermal treatment is approximately 30 mg/L, therefore this technique significantly reduces the amount of leachable lead. Information in the literature and the TCLP data indicate that lead silicate has very low bioavailability in the body. USEPA's Adult Lead Exposure Model (ALEM) for adults and Integrated Exposure Uptake Biokinetic (IEUBK) model for children assumes the form of lead to be soluble lead acetate, which is a much more bioavailable form of lead than lead silicate. The ALEM assumes that soluble lead acetate in water and food has 20% absolute bioavailability. The relative bioavailability of lead bound in a soil matrix is 60%. Therefore, the absolute bioavailability of lead in soil is 12% (i.e., 60% x 20%) in the ALEM. The IEUBK model assumes that soluble lead acetate in water and food has 50% absolute bioavailability. The relative bioavailability of lead bound in soil is 60%, similar to the ALEM. Therefore, the absolute bioavailability of lead in soil is 30% (i.e., 60% x 50%) in the IEUBK model. Information from the literature indicates that the relative bioavailability of 60% is too high for lead silicate. Based on various studies, we determined a more appropriate relative bioavailability of 20%. Using this factor resulted in an absolute bioavailability of 4% in the ALEM and 10% in the IEUBK model. These factors resulted in remedial goals of 2, 800 mg/kg lead in sand using the ALEM, and 1040 mg/kg lead in sand using the IEUBK model, which are higher than remedial goals calculated using the default bioavailability factors (929 and 400 mg/kg, respectively).

746 PEDIATRIC BIOAVAILABILITY OF LEAD IN SOIL AND DUST: ESTIMATES FROM SOIL, HOUSE DUST, AND BLOOD AT THE BUNKER HILL SUPERFUND SITE.

M. L. Stifelman[1], S. Spalinger[2] and I. H. von Lindern[2]. [1]*Region X, Office of Environmental Assessment, USEPA, Seattle, WA and* [2]*TerraGraphics, Inc., Moscow, ID.*

More than a century of mining and smelting at Bunker Hill contaminated soil and interior dust with lead and other metals. Following closure of the smelter in 1981, lead in soil and dust were identified as primary exposure pathways for children. Soil and dust have been sampled annually as contaminated soil was replaced with clean soil at schools, parks, businesses, and approximately 2, 200 residences. Annual voluntary blood lead screening has recruited over 50% of exposed children every year since 1988. Blood lead levels have decreased as soil and dust lead levels have declined as the soil cleanup progressed. Bioavailability was estimated by comparing lead uptake from over 5, 000 blood lead measurements paired with estimates of lead intake from thousands of soil and dust measurements. Lead intakes were estimated using a range of assumptions of the relative importance of lead levels in 1) house dust, 2) residential soil from a child's own yard, 3) soil from neighboring yards, and 4) the mean concentration from all yards in a town. The impact of various exposure assumptions on the estimated intake was minimal because of intercorrelations among the four sources of exposure. From 1988 to 2002, the estimated aggregate bioavailability of soil and dust averaged 18% over a range of 12-23%. Higher bioavailability coincided with increased intakes of dust relative to soil, suggesting that lead in dust is more bioavailable than soil. Attempts to separate soil and dust bioavailability were sensitive to soil and dust ingestion rate assumptions. However, assuming 12% soil and 24% dust bioavailability explains the annual variation in aggregate soil/dust bioavailability observed from 1988 to 2002. Greater bioavailability of dust may be caused by smaller particles in dust relative to soil which may account for an increase in bioavailability (due to greater surface area) and ingestion rate (smaller particles are more likely to cling to hands and fingers).

747 ASSESSMENT OF METALS EXPOSURE ASSOCIATED WITH SUBSISTENCE USE OF CARIBOU COLLECTED NEAR A MINING TRANSPORT ROAD IN NORTHWEST ALASKA.

M. R. Garry[1, 2], L. J. Yost[2], S. S. Shock[2] and W. J. Shields[2]. [1]*University of Washington, Seattle, WA and* [2]*Exponent, Bellevue, WA.*

Caribou have historically been, and remain, an integral part of life for native northern Alaskans, for both subsistence and cultural reasons. Historical transport of ore concentrate over a 50-mile haul road from the Red Dog lead/zinc mine in northwest Alaska to the seaport has been associated with elevated concentrations of lead on moss growing near the road, and with concerns regarding subsistence foods. Although caribou herds range widely, they sometimes overwinter in areas near the road. As part of a comprehensive assessment of potential impacts to human health and the environment associated with metals released during overland transport of ore concentrate, caribou were evaluated for their potential contribution to dietary metals exposure during subsistence use. In spring 2002, 10 caribou were harvested from near the mine and the haul road. Muscle, liver, and kidney tissues were dissected at the time of harvest, frozen immediately, and shipped for analysis of lead, zinc, cadmium, and arsenic. Caribou metals concentrations were compared to concentrations in caribou collected from the Red Dog mine area in 1996, and concentrations in caribou harvested from other areas of northern Alaska without known anthropogenic sources. There were no consistent elevations of metals in the 2002 Red Dog caribou relative to the comparison groups. Lead was slightly elevated (two- to three-fold) in liver (2.70±1.72 mg/kg) relative to the other northern Alaska herds, but was two- to eight-fold lower in muscle tissue (0.104±0.088 mg/kg). Muscle lead was similar to the typical lead concentrations in meat, fish, and poultry in the United States, which range from 0.002 to 0.159 mg/kg. Zinc was lower in all tissues sampled. Cadmium and arsenic were similar in all tissues sampled. Based on the lack of consistent differences and the results of preliminary health risk estimates, there appear to be little or no potential human health impacts from subsistence use of caribou in the area of the Red Dog mine.

748 RELATIVE BIOAVAILABILITY OF ARSENIC FROM SOIL AFFECTED BY CCA-TREATED WOOD AND DISLODGEABLE ARSENIC FROM CCA-TREATED WOOD COLLECTED FROM RESIDENTIAL STRUCTURES.

S. W. Casteel, T. J. Evans, M. A. Miller and S. E. Turnquists. *Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO.*

Assessment of health risks from oral exposure to arsenic in chromated copper arsenate (CCA)-treated wood requires knowledge of the amount of arsenic absorbed from the gastrointestinal tract into the body. The enteric absorption of arsenic from soil adjacent to CCA-treated wood utility poles, placed in service in 1988, was measured in a juvenile swine model. In a related study the swine model was also used to measure the absorption of arsenic from dislodgeable material obtained from the surface of weathered (1-3 years) residential structures composed of CCA-treated wood. The arsenic concentrations in the test materials were 320 ?g/g in the utility pole soil and 3500 ug/g in the dislodgeable material. In each dosing trial, groups of five semi-fasted swine were orally dosed with sodium arsenate (0, 30, or 60 ug/kg/day), soil (60, or 120 ug/kg/day) or dislodgeable material (30, 60, or 120 ug/kg/day) twice a day for either 12 (dislodgeable material) or 15 (soil) days. The amount of arsenic absorbed by each pig was estimated by measuring the amount excreted in the urine during 48 hour collections on days 6 to 7, 8 to 9 and 10 to 11. The amount of arsenic excreted by each pig was plotted as a function of the amount administered and the slope of the best-fit straight line through the data approximated the urinary excretion fraction (UEF). The relative bioavailability (RBA) of arsenic in a test material was calculated as a ratio (UEFtest/UEFref) of the UEFs. Using sodium arsenate as a soluble reference form of arsenic in each independent study, the RBA estimates for both test materials were 49% for soil and 29% for dislodgeable material. Both estimates are significantly lower than the default value of 80%-100% employed when reliable site-specific data are lacking. Use of these data will improve the accuracy of risk estimates for humans who may incidentally ingest soil adjacent to CCA-treated wood or dislodgeable material from CCA-treated wood structures.

749 DEVELOPMENT OF A RISK ASSESSMENT TO EVALUATE HUMAN HEALTH RISKS FROM EXPOSURE TO TEBUCONAZOLE USED AS A WOOD PRESERVATIVE.

B. K. Shipp[1], E. M. Dube[1], B. D. Beck[1], M. R. Seeley[1], K. A. Radloff[1], S. Schettler[1] and C. Petito Boyce[2]. [1]*Gradient Corporation, Cambridge, MA and* [2]*Gradient Corporation, Seattle, WA.*

Background: Copper azole Type B (CA-B) is a new pressure-applied, waterborne wood preservative, consisting of copper and tebuconazole (TEB). TEB is an effective fungicide used to protect the integrity of wood used primarily for outdoor structures. **Objective:** Risk assessments have rarely been conducted for preservatives used in treated wood applications. Our objective was to apply toxicity criteria for TEB used as a treated wood preservative to a human health risk assessment (HHRA). **Approach:** We reviewed animal studies to identify no observed effect levels (NOELs) for acute and chronic exposures to TEB *via* ingestion, dermal contact, and inhalation. **Results:** Based on animal studies, developmental effects are the main toxicological concern regarding high-dose exposure to TEB. We identified an acute oral NOEL of 10 mg/kg-day, based on a developmental toxicity study in mice; an acute/chronic dermal NOEL of 1, 000 mg/kg-day, based on no effects observed at the highest dose tested in developmental studies in mice, rats, and rabbits; a chronic oral NOEL of 3 mg/kg-day based on adrenal gland effects in dogs; and an acute/chronic inhalation NOEL of 1.9 mg/m^3-day (adjusted for continuous exposure) based on the induction of liver microsomal enzymes in rats. Doses up to and including the maximum tolerated dose do not appear to be carcinogenic in animals. We assessed potential human health risks from occupational and non-occupational

exposures to TEB used as a wood preservative based on these NOELs and a margin of exposure (MOE) approach. Using reasonable maximum exposure (RME) assumptions, MOEs for residential exposures to TEB ranged from 1.1 x 10^4 for a child (from dermal contact with soil) to 2.4 x 10^7 for an adult handler (from incidental ingestion of dislodgeable residue). **Conclusion:** No appreciable risk of adverse health effects from exposures to TEB in CA-B-treated wood are expected based on the sufficiently large MOEs calculated for the exposure scenarios in our HHRA.

750 PROBABILISTIC CANCER RISK ASSESSMENT OF WORKERS EXPOSED TO CREOSOTE DURING PRESSURE TREATMENT OF WOOD.

T. Long[1], C. R. Kirman[1], J. Butala[3] and R. Tardiff[2]. *[1]The Sapphire Group, Beachwood, OH, [2]The Sapphire Group, Bethesda, MD and [3]Toxicology Consultants, Gibsonia, PA.*

Creosote is a complex coal tar-derived mixture consisting of polynuclear aromatic hydrocarbons (PAHs) used as a preservative in pressure-treated commercial wood products. A cancer risk assessment was conducted for occupational exposure associated with pressure-treatment of wood. For exposure assessment, a recent exposure study in creosote workers served as the primary source of information and dose estimation. In the exposure study, measurements with personal air samplers and passive, whole-body dosimeters were made for 108 dermal and 88 inhalation work shifts job activities involving creosote exposure. Total dermal doses ranged from 0.0141 to 49.6 mg/kg, and were lognormal in distribution. No carcinogenic PAHs were identified in air samples and CTPV's were detected in only one of 88 samples (<0.1mg/m3). For the toxicity assessment, cancer potency was estimated based on a study of coal tar mixtures in mice exposed orally for two years. In this study, dose-related increases in hepatocellular adenomas and carcinomas, alveolar and bronchiolar adenomas and carcinomas, forestomach papillomas and carcinomas, small intestine adenocarcinomas, and other tissue tumors were reported. For dose-response relationships, the tissue sites were grouped into two categories: (1) point-of-contact tumors, consisting of forestomach and small intestines; and (2) systemic tumors, consisting of all other tissue sites. Monte Carlo methods were used to characterize the variability and uncertainty associated with exposure and dose-response components. The estimates of cancer risk generally fall within the range of USEPA acceptable risk levels (5x10-6 to 1x10-4). It is concluded that over the course of a working lifetime, wood treating workers exposed to creosote will experience no more than one additional cancer per 10, 000 workers. In a population of about 600 to 3700 workers, the number actually involved in pressure treatment of wood, less than one new cancer case could be expected to occur as a result of occupational creosote exposure.

751 GEOSPATIAL ANALYSIS OF THE EFFECTIVENESS OF THE RESIDENTIAL DUST CLEANUP PROGRAM IN LOWER MANHATTAN FOLLOWING THE ATTACK ON THE WORLD TRADE CENTER.

M. Maddaloni[1], C. Nace[1], W. Thayer[2], E. Gabriel[2], M. Ramsey[2], B. Allen[2], P. Goodrum[2] and G. Diamond[2]. *[1]Region 2, USEPA, New York, NY and [2]Environmental Science Center, Syracuse Research Corporation, Syracuse, NY.*

In response to the concerns of the residents of Lower Manhattan regarding the potential presence of residual contamination from the collapse of the World Trade Center (WTC) buildings, the USEPA (EPA) and its federal, state and city partners developed a comprehensive plan to ensure that residences potentially affected by the collapse of the WTC had been properly cleaned. Upon the request of residents located in Lower Manhattan (south of Canal Street), USEPA and its partners arranged for the cleanup of residential units, using certified contractors, with follow-up testing for air-borne asbestos and total fibers. In a subset of residences, data on metals loading in settled dust (ug / sq. ft. of sampled surface area) were collected prior to and following cleanup. As part of the assessment of the effectiveness of the cleanup effort, we analyzed the data, using methods from point pattern analysis, to determine if the post-cleanup data exhibited any spatial pattern that might support the hypothesis that exceedance of health-based benchmarks were attributable to residual contamination from the collapse of the WTC buildings. We tested the hypothesis using Monte Carlo-type statistical tests that are appropriate for data that were not collected using random sampling methods (cleanups and sampling were performed at the request of residents of Lower Manhattan); the tests consider the geographic location of the sampled buildings and the number of samples collected from each building. The point pattern analysis was performed using GeoSEM: GIS software that was developed by SRC for the application of spatial statistics in human and ecological risk assessment. The results of our analysis do not support the hypothesis that post-cleanup exceedance of the health-based benchmarks are related to the collapse of the WTC buildings.

752 REENTRY CRITERIA FOR DIOXIN AND DIOXIN-LIKE COMPOUNDS FOR BUILDING SURFACES.

J. Greene[1], G. Brorby[1] and D. Paustenbach[2]. *[1]Exponent, Oakland, CA and [2]ChemRisk, San Francisco, CA.*

Building reentry criteria for dioxin TEQ, as measured by surface wipes, have varied greatly over the past 15 years, from as low as 1 ng/m^2 to as high as 125 ng/m^2. While these values have all been derived from either the cancer slope factor (CSF) or relatively high no observable adverse effect levels, they are highly variable, inconsistent in their use of exposure parameters, and lacking complete transparency in the calculations. Recently, the World Trade Center Indoor Air Taskforce calculated a reentry criterion of 2 ng TEQ/m^2 for a residential exposure. This number was based on the EPA's draft CSF of 1 x 10^6 $(mg/kg\text{-}day)^{-1}$, various exposure parameters, dermal absorption values, and a cancer risk criterion of 1 x 10^{-4}. An indoor 'degradation' parameter was also included in the calculations. However, a single criterion based on a single set of assumptions cannot, and should not, be universally applied. Reentry criteria that consider a wider range of exposure scenarios, exposure pathways, bioavailability, and behavioral parameters would be very useful to risk managers who may have to address multiple diverse situations in the coming years. This paper describes our recommended reentry building surface criteria for four exposure scenarios: 1) adult occupational, 2) adult residential, 3) childhood 'occupational' (i.e., school), and 4) childhood residential. Using a cancer risk criterion of 1 x 10^{-5}, USEPA's current CSF of 1.56 x 10^5 $(mg/kg\text{-}day)^{-1}$, and updated exposure and bioavailability parameters, we calculated reentry criteria of approximately 100, 50, 10, and 5 ng TEQ/m^2 for the four scenarios, respectively. These criteria result in dose levels of approximately 0.001-0.02 pg TEQ/kg-day and thus could plausibly produce daily intakes in the vicinity of 1/100 to 1/1000 of the 70 pg/kg-month level (based on non-cancer endpoints) considered acceptable by the joint FAO/WHO committee. Therefore, they should be protective for both cancer and non-cancer effects.

753 MALIGNANT TRANSFORMATION OF HUMAN UROTHELIAL CELLS BY ARSENITE AND CADMIUM.

S. Somji[1], V. Gurel[1], S. Park[1], M. Sens[1], S. H. Garrett[1] and D. A. Sens[2]. *[1]Pathology, University of North Dakota, Grand Forks, ND and [2]Surgery, University of North Dakota, Grand Forks, ND.*

Cadmium and arsenite are human carcinogens and exposure to either of them has been associated with the development of bladder cancer. Neither cadmium or arsenite has been shown to elicit the malignant transformation of human urothelial cells under *in vitro* conditions, although such a model would be of value in elucidating the mechanism of carcinogenesis of both compounds. This laboratory has characterized an immortalized cell culture model of human urothelial cells (UROtsa) that does not form colonies in soft agar or produce tumor growth in nude mice. The goal of the present study was to determine if exposure of UROtsa cells to cadmium or arsenite would result in malignant transformation. UROtsa cells were grown on both serum-free and serum-containing growth medium to confluency and then exposed to cadmium and arsenite concentrations that produced greater than 90% cell death over a 30 to 60 day period. Surviving cells were allowed to grow back to confluency in the continued presence of cadmium or arsenite. At passage 4, 8, 12 and 16, the cultures were tested for their ability to form colonies in soft agar. At passage 4 and 8 no colonies were formed, at passage 12 a few colonies were formed, and at passage 16 a large number of colonies were formed in the soft agar assay. Cells at passage 16 from each treatment group were injected into nude mice and all 4 groups formed tumors. Control UROtsa cells of equal passage failed to form colonies in soft agar and did not form tumors in nude mice. Histological examination of the tumors demonstrated characteristics expected of transitional cell carcinoma of the bladder. Tumors produced from cells grown in serum tended to have features associated with high grade tumors while those from serum-free cells had features associated with low grade tumors. These studies show that both cadmium and arsenite can cause malignant transformation of human urothelial cells.

754 ARSENIC TOXICITY IN HUMAN KERATINOCYTES.

M. A. Ngo, T. J. Patterson and R. H. Rice. *Environmental Toxicology, University of California, Davis, CA.*

Arsenic, a human carcinogen and drinking water contaminant, is encountered in the environment in the trivalent (AsIII) and pentavalent (AsV) oxidation states. AsV, the most prevalent form, usually appears much less toxic with its potency being enhanced by reduction to AsIII. To understand the importance of reduction in elucidating arsenic mechanisms we evaluated the responsiveness of human keratinocyte cultures to different oxidation states using Northern and quantitative PCR analysis of heme oxygenase-1 induction. We found AsV to be as efficacious as AsIII; however a longer time was required for AsV to reach maximal effect. These observations were correlated with ICP-MS measurements of cellular uptake and conversion rates. In parallel experiments, we found pentavalent antimony (SbV) to

have limited biological activity, uptake, and conversion compared to the trivalent form (SbIII). These findings emphasize the importance of intracellular reduction of metalloids for biological activity.

755 CYTOTOXICITY OF METALS ON CULTURED MORTAL AND IMMORTAL HUMAN MAMMARY CELLS.

C. M. Schmidt, M. B. Anderson and F. A. Barile. *Pharmaceutical Sciences, St. John's University College of Pharmacy, Jamaica, NY.*

An *in vitro* study was conducted to determine the effects of metals on human cultured mammary cells. Monolayers of human mortal (MCF-12A) and immortal (MDA-MB231) mammary epithelial cells were incubated in the absence or presence of increasing concentrations of arsenic, mercury and copper for 24-hrs, 72-hrs, 4-days, and 7-days. The MTT assay was used to assess cytotoxicity for all time periods and cell proliferation was monitored for 4-day and 7-day studies. The data suggest that both cell lines have similar sensitivities to arsenic and mercury at 24- and 72-hr exposures. Cell *via*bility of MCF-12A cells, however, is stimulated by otherwise equivalent inhibitory concentrations of copper on MDA-MB231 cells at 24-hrs. Similarly, arsenic and mercury suppress proliferation and *via*bility in both cell lines, whereas copper enhances cell proliferation and *via*bility of MCF-12A cells after 4-days and 7-days of exposure. The immortal cells, however, recover better after 4-days of toxic insult to the metals than the finite cells. In combination with previous reports, it appears that a basal cytotoxic phenomenon may not be appropriate to explain differences between finite or continuous cell lines, particularly in response to trace metal exposure. In addition, sensitivity of immortal cells to slightly higher concentrations of copper, a biologically important trace metal, may play an important role in controlling unregulated cellular processes and proliferation. (Supported by NIH/NIEHS/AREA R15 ES012170-01).

756 SODIUM CHROMATE AND CADMIUM CHLORIDE TOXICITY IN STELLER SEA LION CELLS.

C. Goertz[1], S. Wise[1], L. Dunn[3], F. Gulland[4], A. Morin[1], N. Jayasundara[1], M. Bozza[2], S. Atkinson[2] and J. P. Wise[1]. [1]*Wise Laboratory of Environmental and Genetic Toxicology, Center for Integrated and Applied Environmental Toxicology, University of Southern Maine, Portland, ME,* [2]*Alaska Sea Life Center, Seward, AK,* [3]*Mystic Aquarium, Mystic, CT and* [4]*The Marine Mammal Center, Sausalito, CA.*

The Western population of the Steller sea lion is endangered and the cause is unknown. We are investigating the hypothesis that metals might be a factor in this decline. We find that metals are toxic to Steller sea lion cells in an organ and metal specific manner. For example, chromium induced concentration-dependent cytotoxicity in dermal, renal, bronchial and testicular fibroblasts. 2.5, 5, 10, and 25 uM sodium chromate induced 92, 73, 29, and 1% relative survival in kidney cells; 76, 64, 37, and 24% relative survival in dermal fibroblasts; and 54, 41, 25, and 1% relative survival in bronchial cells. Testicular cells had 22 and 1% relative survival at 2.5 and 5uM, and no survival at higher doses. Thus, testicular cells were the most sensitive, dermis most resistant, and bronchial and renal cells were intermediate. Cadmium was generally less toxic than chromium at all doses studied in these tissues. Cadmium chloride concentrations of 2.5, 5, 10, and 25 uM induced 77, 53, 40, and 30% relative survival in kidney cells; 121, 98, 82, and 81% relative survival in dermal fibroblasts; 84, 76, 57, and 52% relative survival in bronchial cells; and 65, 63, 54, and 27% survival in testicular cells. It is noteworthy that testicular cells were the most sensitive to both of these metals which has implications concerning possible transgenerational effects of metal exposure. Current efforts include evaluating tissue loads of metals in Steller sea lion tissue to compare with the intracellular loads in cell lines at toxic concentrations, and understanding the genotoxic effects of metals. Further research is aimed at testing additional metals and extending the lifespan of these cells in culture with telomerase. This work was supported by a grant from National Oceanic and Atmospheric Administration, NA16FX1412 (JPW).

757 METAL TOXICITY OF SODIUM CHROMATE IN STELLER SEA LION BRONCHUS AND DERMIS COMPARED TO HUMANS.

A. Morin[1], C. Goertz[1], S. Wise[1], L. Dunn[3], F. Gulland[4], N. Jayasundara[1], M. Bozza[2], S. Atkinson[2] and J. P. Wise[1]. [1]*Wise Laboratory of Environmental and Genetic Toxicology, Center for Integrated and Applied Environmental Toxicology, University of Southern Maine, Portland, ME,* [2]*Alaska Sea Life Center, Seward, AK,* [3]*Mystic Aquarium, Mystic, CT and* [4]*The Marine Mammal Center, Sausalito, CA.*

Hexavalent chromium (Cr(VI)) is a known carcinogen to humans. Some species of marine mammals are known to be more resistant to the effects of this and other xenobiotics than humans. For example, Cr(VI) has been shown to cause cancer in humans, but relatively higher levels of chromium and other metals have been found in tissues from many populations of marine mammals without increasing incidences of pathologies. Therefore, the focus of this investigation was to explore the possibility that sea lion cells respond differently than human cells to the cytotoxic effect of Cr(VI). We found that concentrations of 1, 2.5, 5, 10, and 25 uM sodium chromate induced 63, 42, 8, 0, and 0% relative survival in human bronchial fibroblasts (WTHBF-6) when compared to untreated controls, and that these same concentrations induced 67, 54, 41, 25 and 1% relative survival in Steller sea lion bronchial fibroblasts. Similarly, concentrations of 1, 2.5, 5, 10, and 25 uM sodium chromate induced 56, 28, 11, 0, and 0% relative survival in human dermal fibroblasts (BJ-htert) when compared to untreated controls; whereas the same concentrations induced 89, 76, 64, 37, and 24% relative survival in Steller sea lion dermal fibroblasts. This suggests that at low and moderate concentrations Sea lion cells may have protective mechanisms for the cytotoxic effects of Cr(VI). Further research is aimed at understanding the mechanism of this.

758 CYTOTOXICITY OF ORGANO-TIN COMPOUNDS IN DIFFERENT CULTURED CELL LINES.

C. Siegers[2], A. Hoth[2], S. Syed Ali[2] and J. B. Schulze[1]. [1]*Office of the Dean, Frankfurt/Main, Germany and* [2]*Inst. Exp. Clin. Pharmacology Toxicol., Frankfurt/Main, Germany.*

Organo-tin as monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT) are used as fungizides and antifouling compounds. Little is known about their toxicological profile. We therefore studied the cytotoxicity of these agents in different human and animal cell lines. As a measure of cell death and cell growth the neutral red assay and the release of LDH into the medium was used. The hepatoma-derived cell line HepG2, the renal tubular cell line LLC-MK2 from monkey kidney and the cornea epithelial cell line (CE cells) was investigated. As a measure of growth inhibition the IC50-value were calculated for the different tin compounds: TBT-chloride 160 nmol/L in LLC-MK2, 150 nmol/L in HepG2 and 180 nmol/L in CE cells; for DBT the corresponding values were higher, i.e 500 nmol/L DBT for LLC-MK2 cells and 220 nmol/l for CE cells. ED50 values for LDH release indicating disturbances of the outer cell membrane was higher than 250 nmol/l in LLC MK2 cells for both TBT acetate and TBT chloride addition. We conclude that all three organo-tin compounds tested are equally effective. Significant cytotoxicity (LDH release) was not observed in the concentration range tested, whereas growth inhibition as indicated by decreased neutral red uptake was affected at organo tin concentrations between 150 and 500 nmol/l. This concentration is appr. 70 to 200 fold higher than values observed in environmental samples.

759 COMPARATIVE CHROMIUM TOXICITY IN CULTURED BOWHEAD WHALE AND HUMAN LUNG CELLS.

S. S. Wise[1], A. Holmes[1], M. Thompson[1], B. Smith[1], T. O'Hara[2] and J. P. Wise[1]. [1]*Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME and* [2]*North Slope Borough Department of Wildlife Management, Barrow, AK.*

Bowhead whales live 150-200 years and yet do not appear to get cancer. This is remarkable given that bowhead whales are routinely exposed to, and bioaccumulate, high levels of metals. We have established bowhead whale lung cell lines to compare their cellular responses to metals to those of human cells. Hexavalent chromium (Cr(VI)) is a metal of particular concern and has long been recognized to cause human lung cancer. Studies have shown that it is the insoluble forms of Cr(VI) that are the most potent carcinogens. In humans, these particles dissolve outside the cell. We found that the particulate form of Cr(VI) was more cytotoxic to bowhead cells than to human cells. For example, 0.1, 0.5, 1 and 5 ug/cm^2 lead chromate induced 39, 20, 7 and 0% relative survival in bowhead cells and 90, 71, 43 and 15% relative survival in human cells at the same concentrations. By contrast, we found that the soluble form of Cr(VI) was more cytotoxic to human cells than to bowhead cells. 1, 2.5, 5 and 10 uM sodium chromate (SC) induced 63, 42, 8 and 0% relative survival in human cells and 98, 96, 62 and 3% relative survival in bowhead cells. Human cells treated with SC had intracellular chromium concentrations of 1.81, 3.84, 7.71 and 15.41 uM at treatment concentrations of 1, 2.5, 5 and 10uM, while bowhead cells had intracellular concentrations of 3.54, 9.16, 14.99 and 29.25 uM at the same treatment concentrations. By measuring the toxicity of chromium in human and bowhead lung cells we can start to understand the differences in these species biological response to environmental insult. Further research is needed to elucidate the differences in the cellular responses to soluble versus insoluble chromium and to look at the biochemical mechanisms in the responses of both cell types to chromium. This work was supported by NIEHS grant 1R01 ES10838-01 (J.P.W.) and NOAA grant NA16FX1412.

760 LEAD CHROMATE-INDUCED CYTOTOXICITY IN HUMAN BRONCHIAL CELLS IS MEDIATED BY EXTRACELLULAR CHROMIUM.

A. Holmes[1], N. Gordon[2] and J. P. Wise[1]. *[1]Wise Laboratory of Environmental and Genetic Toxicology, Center for Integrated and Applied Environmental Toxicology, University of Southern Maine, Portland, ME and [2]Department of Chemistry, University of Southern Maine, Portland, ME.*

Hexavalent chromium (Cr(VI)) compounds are established human lung carcinogens and solubility plays a key role in the carcinogenicity of Cr(VI), with the most potent carcinogens being the particulate Cr(VI) compounds. Lead chromate (LC) is often used as a prototypical particulate Cr(VI) compound since it is the most insoluble of these compounds; however, it is unknown whether chromium (Cr), lead (Pb), or a combination is responsible for the cytotoxic effects of LC. We compared the cytotoxicity and ion uptake of LC with sodium chromate (SC) and lead glutamate (LG) in WTHBF-6 cells, and the effects of co-treating LC, SC and LG with vitamin C to evaluate changes in cytotoxicity and ion uptake since vitamin C is known to reduce Cr(IV) to Cr(III) and prevent chromium uptake. LC and SC both induced concentration-dependent increases in cytotoxicity at similar intracellular Cr concentrations. LC at 0.1 and 5 ug/cm^2 induced 85 and 3% relative survival at intracellular concentrations of 1.3 and 16.7 uM respectively, while 1 and 5 uM SC induced 75 and 3% relative survival with intracellular concentrations of 1.7 and 15.2 uM respectively. Co-treating LC or SC with vitamin C dramatically reduced intracellular Cr concentrations and cytotoxicity. LG also induced a concentration-dependent increase in cytotoxicity, but 200 and 1000 uM LG induced 86 and 62% relative survival at an intracellular Pb concentration of 4.4 and 48.3 uM respectively, while 5 ug/cm^2 LC induced 3% relative survival with an intracellular Pb concentration of only 2.9 uM. Co-treating LG with vitamin C had no effect on the cytotoxicity or intracellular Pb concentrations. Cytotoxicity and intracellular Pb concentrations decreased when LC was co-treated with vitamin C 5 ug/cm^2. These data indicate that Cr and not Pb mediates LC-induced cytotoxicity in human bronchial cells. This work was supported by NIEHS grant 1R01 ES10838-01

761 BRONCHIOLAR EPITHELIUM CHANGES AFTER PB, CD OR ITS MIXTURE INHALATION.

T. I. FORTOUL[1], L. Saldivar[3], G. Espejel[3], L. Colin-Barenque[2], A. Zepeda[1], F. Pasos[1] and M. Avila-Costa[2]. *[1]Biologia Celular Y Tisular, Universidad Nacional Autonoma De Mexico, Mexico City, Mexico, [2]Fes Iztacala, Universidad Nacional Autonoma De Mexico, Tlalnepantla, Mexico and [3]Facultad De Quimica, Universidad Nacional Autonoma De Mexico, Mexico, Mexico.*

Pollutants are present as mixtures and limited information about its behavior is available. Pb and Cd are some of the most frequently metals found in the air and both enter together into the respiratory tract. The objective of this study was to identify the interaction that the inhalation of Pb, Cd and its mixture has on the bronchiolar epithelium evaluated by scanning electron microscopy (SEM) and its correlation with metal's tissue concentration. CD-1 male mice were exposed by inhalation to Pb (0.01M), Cd (0.006M) or its mixture twice a week, four weeks. Animals were sacrificed each week; the right lung was processed for SEM and the left for metal determination by atomic absorption spectrometry. Pb maintained its concentration, while Cd increased abruptly, while in the mixture, Pb was notoriously decreased, and Cd had values like those found in Cd single inhalation. Changes in morphology indicated that Cd was more aggressive than Pb alone, and in the mixture the predominant changes were similar to those observed with single Cd inhalation. It is very difficult to predict what the behaviour of a mixture might be, because of the interactions with enzymes, receptors, other metals, etc., but it is urgent to increase studies of this type because in real world exposures to mixtures predominate. P.O.S. Institute

762 THROMBOCYTOSIS INDUCED IN MICE AFTER ACUTE AND SUBACUTE V205 INHALATION.

A. GONZALEZ-VILLALVA[1], I. LOPEZ[1], I. SANCHEZ[1], L. COLIN-BARENQUE[3], S. ACEVEDO-NAVA[1], P. BIZARRO[1], G. NINO-CABRERA[1], E. TOVAR-SANCHEZ[4], P. MUSSALI-GALANTE[1], M. AVILA-COSTA[3] and T. I. FORTOUL[1]. *[1]BIOLOGIA CELULAR Y TISULAR, UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, MEXICO CITY, Mexico, [2]FACULTAD DE MEDICINA, UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, MEXICO CITY, Mexico, [3]FES IZTACALA, UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, TLALNEPANTLA, Mexico and [4]INSTITUTO DE ECOLOGIA, UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, MORELIA MICH, Mexico.*

Reports about vanadium (V) inhalation toxicity on the coagulation system are limited, so we decided to evaluate the effects of this element on this system through complete blood count and morphologic analysis of platelets in blood smears. CD-1? mice inhaled V2O5 0.02M one hour, twice a week during 6 weeks. Blood samples were obtained by direct heart puncture, Wright stained smears were used to platelet quantification. At 5th and 6th weeks thrombocytosis with megaplatelets were found and correlation of platelets counts and time of exposure was positive (r=0.45; r2=0.20). Hypercoagulability is associated with metal exposure and this is linked with an increased risk of thromboembolic diseases. Until now no reports were found of thrombocytosis associatiated with metal exposures. Further analysis is needed to evaluate the functionality of these platelets as well as the cause of its increase. PAPIIT 210301

763 UPREGULATION OF CELLULAR THIOLS BUT DOWNREGULATION OF LYSYL OXIDASE IN LONG TERM CADMIUM (CD) EXPOSED LUNG FIBROBLASTS.

Y. Zhao[1], I. Chou[1], P. Toselli[2], P. Stone[2], H. Kagan[2] and W. Li[2]. *[1]Microbiology, Boston University School of Medicine, Boston, MA and [2]Biochemistry, Boston University School of Medicine, Boston, MA.*

Lysyl oxidase (LO), a Cu-dependent enzyme, oxidizes lysine residues in collagen and elastin leading to crosslinking of these proteins, thus stabilizing the extracellular matrix (ECM). Chronic inhalation of Cd, a toxic metal for humans, induces emphysema. To probe mechanisms of Cd injury to the lung we developed Cd-resistant (CdR) cells from rat fetal lung fibroblasts (RFL6) by long-term Cd exposure and further examined their relative capacities to synthesize LO and Cu scavenging thiol components, e.g., metallothionein (MT) and glutathione (GSH). Cellular levels of MT and GSH in CdR cells were elevated to 8- and 2.5-fold of the parental control, respectively, whereas LO activity in the CdR cell-conditioned medium was much less than that in parental cells amounting to 12% of the latter. Addition of Cu into CdR cell cultures restored LO activity suggesting limitation of Cu bioavailability for LO occurred in these cells. Immunoprecipitation assays indicated that in addition to a decrease in the 46 kD preproenzyme of LO in the cell extract, both the 50 kD proenzyme and 32 kD mature enzyme of LO were essentially undetectable in the CdR cell extract and conditioned medium. Instead, a conspicuous 52 kD band appeared in the CdR cell extract but not in the conditioned medium. Immunofluorescent staining revealed the 52 kD species colocalized with the ER marker [DiOC5(3)] within the cell. These results suggest that the Cd-induced 52 kD protein is a product of abnormal LO processing which was not secreted extracellularly. In view of the roles of MT and GSH in Cu metabolism and the function of LO in ECM stability, downregulation of LO by Cd due to limited Cu bioavailability following elevated cellular thiol levels coupled with perturbed LO processing may weaken the deposition of collagen and elastin, the substrates of LO, destabilizing the lung ECM, a critical event to emphysema development (supported in part by NIH grants R01-ES 11340 and R01-ES 11164 and Philip Morris foundation).

764 MORPHOLOGICAL CHANGES IN TESTES. AFTER MANGANESE INHALATION. STUDY IN MICE.

P. BIZARRO[1], I. SANCHEZ[1], I. LOPEZ[1], F. PASOS[1], V. DELGADO[1], A. GONZALEZ-VILLALVA[1], L. COLIN-BARENQUE[2], S. ACEVEDO[1], G. NINO-CABRERA[1], P. MUSSALI-GALANTE[1], M. AVILA-COSTA[2] and T. I. FORTOUL[1]. *[1]BIOLOGIA CELULAR Y TISULAR, UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, MEXICO CITY, Mexico and [2]NEUROCIENCIAS, FES IZTACALA UNAM, TLALNEPANTLA, Mexico.*

Manganese (Mn) has been used as an antiknocking agent in gasoline. Its increase in the atmosphere enhances the risk of its inhalation and the induction of systemic damage. Some reports mention that oral administration of MnCl2 induces reproductive delay in male mice. Prostatic cancer has been identified among exposed workers. The objective of this study was to identify in a murine inhalation model in CD-1 male mice. Animals inhaled MnCl2 0.02M, 1h, twice a week, for 4 weeks, sacrificed once a week and processed for light and electron microscopy. Light changes evidenced necrosis of stem cells, binucleated spermatocytes and dense nuclear structures. Ultrastructural changes in Leydig cells consisted in hyperplastic endoplasmic reticulum forming whorl-like structures. As a consequence of these modifications the function of the testes might be altered, as well as its endocrine function.

765 ADVERSE EFFECT OF COPPER TOXICITY ON SOME BLOOD PICTURE AND CELLULAR CONSTITUENTS OF A TELEOST.

A. Panigrahi, A. K. Dasmahapatra and A. K. Medda. *Department of Physiology, Basirhat College, West Bengal, India.* Sponsor: M. Ehrich.

The heavy metal toxicity is the inherent capacity of the metal to affect adversely any biological activity due to the non-degradation of metals leading to accumulation in tissue and an interaction of the metal with a protein or enzyme leading to changes in physiologic and metabolic processes. Copper if the most potent toxic and heavy metal in trace amounts. It was evident from results that a single injection of cupric acetate at the dose of 5.10 and 20x10^{-9} mole/g decreased RBC count, Hb content

and the PCV in an Indian Teleost (Clairas batrachus L.) on days 7, 14, 28, 42. In cupric acetate injected fish MCV increased but MCHC decreased with some exceptions. The MCH decreased only on day 14 with 10 and $20x10^{-9}$ mole/g but increased on days 28 and 42 with the said dose. It was also evident from the results that a single injection of cupric acetate at the dose of 5, 10 and $20x10^{-9}$ mole/g exerted reducing influence on protein, RNA and DNA metabolism of the liver of the said fish on different days of the experiment, i.e., on days 7, 14, 28, and 42. Liver protein content was significantly reduced on day 14 only compared to control but no change was observed on the other days of the experiment. Liver RNA content was found to be reduced on all the days, except with the lower dose ($5x10^{-9}$ mole/g) of the cupric acetate which reduced the RNA content from day 7 to day 28 only. The higher dose ($20x10^{-9}$ mole/g) caused maximum reduction in liver DNA on day 7 and day 14, particularly with medium and the higher dose. No significant change in DNA content was observed on days 28 and 42.

766 A JUVENILE SWINE MODEL FOR COPPER OVERLOAD.

T. J. Evans[1], S. W. Casteel[1] and K. V. Katti[2]. *[1]Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO and [2]Radiopharmaceutical Sciences Institute, University of Missouri, Columbia, MO.*

Cu overload occurs in various human disease conditions, including Wilson's disease, and often results in liver damage. Novel chelating agents and animal models for testing the efficacy of these compounds are needed since current chelation regimens cause adverse health effects. Although there are many similarities between the metabolism of metals in humans and swine, there have previously not been any experimental protocols which consistently induce Cu overload in pigs. In the first of two studies, 6 to 8-week-old, male swine were assigned to one of three treatment groups. Pigs received no IV Cu (n=4) or were given 1 mg Cu/kg BW IV (as $CuCl_2$ in a solution containing 0.1% Cu), either once daily (n=3) or twice daily (n=3), for 3 consecutive days. Twenty-four hours after the final treatment, pigs were sacrificed and samples of liver and kidney were collected for Cu analysis by AAS. Statistical comparisons were performed using ANOVA. Cu-treated pigs had 3-fold higher mean hepatic concentrations of Cu than negative controls. Twice daily administration of Cu did not result in higher mean liver concentrations of Cu than once daily Cu administration, but Cu given twice daily did result in mean renal Cu concentrations that were greater than those of the other two treatment groups. In a second study, similarly aged, male pigs were administered either saline (n=3) or 1 mg Cu/kg BW (n=4) IV, once daily, for 5 consecutive days. Beginning 48 hours after the final treatment, urine was collected from each of the pigs for 96 hours and analyzed for Cu using AAS. Following urine collection, pigs were sacrificed and samples of liver and kidney were collected and analyzed for Cu as before. Comparisons were performed using the *t*-test. There were no differences in the urinary excretion of Cu between groups, but the mean concentrations and total amounts of Cu were 6-fold greater in the liver and 2-fold greater in the kidney in Cu-treated pigs than in pigs treated with saline. These results form the basis for the development of reliable swine models for Cu overload.

767 TOXICOLOGICAL EVALUATION OF EXPANDED SHREDDED TOBACCO STEMS.

E. H. Theophilus, D. R. Meckley, M. A. Higuchi, B. R. Bombick, M. F. Borgerding, P. H. Ayres, D. H. Pence and J. E. Swauger. *R&D, R. J. Reynolds Tobacco Company, Winston-Salem, NC.*

A tiered testing strategy has been developed to evaluate the potential of tobacco processes, ingredients, or technological developments to change the biological activity resulting from burning tobacco. The strategy is based on comparative chemical and biological testing. Expanded shredded tobacco stems (ESS) constitute an example of a common tobacco components expansion process currently used in the manufacture of cigarettes to increase the tobacco blend filling capacity. As part of the toxicological evaluation of ESS, test cigarettes containing 9.5%, 18.5%, and 25% ESS were compared to control cigarettes containing 0% ESS. Testing included mainstream cigarette smoke chemistry studies, genotoxicity studies (Ames and sister chromatid exchange), a 13-week inhalation study in Sprague-Dawley rats, and a 30-week dermal tumor promotion study in SENCAR mice. Although statistically significant differences in several smoke constituent yields were observed, the yields of most constituents from cigarettes containing ESS were within the range observed for cigarettes in the US market. Collectively, data indicated that cigarettes with and without ESS had a similar toxicological profile in this test battery.

768 TOXICOLOGICAL EVALUATION OF PROPANE EXPANDED TOBACCO.

J. E. Swauger, E. H. Theophilus, B. R. Bombick, D. R. Meckley, M. A. Higuchi, M. F. Borgerding, M. J. Morton and P. H. Ayres. *R&D, R. J. Reynolds Tobacco Company, Winston-Salem, NC.*

A tiered testing strategy has been developed to evaluate the potential for tobacco processes, ingredients, and other technological developments to increase or decrease the biological activity resulting from burning tobacco. The strategy is based on comparative chemical and biological testing. Propane expanded tobacco is an example of a processed tobacco that may be used in the manufacture of cigarettes to increase the tobacco blend filling capacity. Test cigarettes containing propane expanded tobacco were compared to control cigarettes containing tobacco expanded with a previous expansion agent (Freon-11). The toxicological evaluation included chemistry studies with mainstream cigarette smoke (determination of selected constituent yields), *in vitro* studies with cigarette smoke condensate (Ames study in Salmonella typhimurium and sister chromatid exchange study in Chinese hamster ovary cells) and *in vivo* studies (13-week inhalation study of mainstream cigarette smoke in Sprague-Dawley rats and 30-week dermal tumor promotion study of cigarette smoke condensate in SENCAR mice). Although statistically significant differences in several smoke constituent yields were observed, the yields of most constituents from cigarettes containing 100% propane expanded tobacco were within the range observed for cigarettes in the US market. Collectively, biological tests indicated that the cigarettes containing propane or Freon-11 expanded tobacco had a similar toxicological profile in this test battery.

769 TESTING INGREDIENTS ADDED TO CIGARETTES.

D. M. Byrd, M. C. Falk, R. S. Feldman, K. D. Lewis, P. Nixon and N. P. Royaee. *Life Sciences Research Office, Bethesda, MD.*

The Life Sciences Research Office (LSRO), a nonprofit, biological research organization, has embarked on a three-phase project to assess the health risks of smoking cigarettes with added ingredients relative to smoking comparable cigarettes without added ingredients. The project includes an evaluation of the feasibility of testing (Phase One), development of the scientific criteria to apply in testing (Phase Two), and assessments of specific ingredients (Phase Three). LSRO assembled a multidisciplinary committee of experts, which has met eleven times. LSRO staff and the advisory committee reviewed more than 3, 000 scientific publications, including books, clinical studies, and governmental reports. Phase One comprised an analysis of the relevant scientific literature about cigarette smoke, including its chemistry and physics, exposures, morbidity and premature mortality, issues in testing, and potential testing programs. LSRO concluded that although challenging, testing is feasible and worthwhile. Work on Phase Two (Scientific Criteria) is underway. LSRO is developing a framework to guide the process of testing the ingredients added to cigarettes. LSRO will make recommendations about pyrolysis, smokers' exposures, pharmacokinetics, biological effects, research needs, and human smoking behaviors. The Phase Two report will discuss the data necessary to evaluate an added ingredient and the limitations of reviews. LSRO has held two open meetings, the second one on November 5, 2003 in Bethesda, MD about Phase Two. The meetings provide public fora, encourage inputs from different organizations and individuals, maintain transparency during a long process, and facilitate discussion of the relative health risks, if any, attributable to ingredients. LSRO advertised the meetings in Science magazine and invited external attendees. For additional information or to request LSRO reports, go to www.LSRO.org. Philip Morris USA sponsored this work.

770 EFFECT OF 4-DAY FOOD RESTRICTION AND CORN OIL STIMULATION ON SERUM ALKALINE PHOSPHATASE ACTIVITY IN THE FISCHER 344 RAT.

D. K. Ness, J. M. Sullivan and W. P. Hoffman. *Eli Lilly and Company, Greenfield, IN.*

Rationale: Toxicity to the gastrointestinal (GI) mucosa is difficult to monitor. This study begins examining the potential use of serum intestinal alkaline phosphatase (iALP) as an indicator of mucosal injury by studying the effect of decreased food intake, a common confounding toxicity. Methods: Female Fischer 344 rats (10/group) were assigned to four treatment conditions: ad libitum (AD)-fed (Group 1); AD-fed, corn oil stimulated (CO) (Group 2); food restricted (FR) (Group 3); and FR, CO (Group 4). The treatment period was 4 days, animals were fasted overnight, and euthanasia and necropsy occurred on the 5th day. Food was restricted to 50% of baseline consumption during the treatment phase for the two FR groups. One mL of corn oil was given orally 5 hours prior to necropsy for the two CO groups. Blood was collected at termination for ALP fractionation (intestine, liver, bone isozymes). Liver and the GI tract were weighed at necropsy. Intestine, liver, and bone were evaluated histologically. Results: Rats in the FR groups (Groups 3 and 4) lost approximately 15% of their pre-study body weight during the treatment period. Body weights increased 8% in AD-fed rats (Groups 1 and 2) during this period. FR significantly decreased total ALP (-17%), decreased iALP (-43%), and increased liver ALP (+23%) relative to AD-fed rats. Predictably, CO significantly increased total ALP (+32%) and iALP (+118%) relative to unstimulated groups. FR resulted in a decrease, compared to AD-fed groups, in both GI tract and liver weights, both on an absolute basis (39-40%) and relative to body weight (21-22%). Microscopic changes in the GI mucosa included an increased in-

cidence of villous atrophy in FR animals. Liver effects in these rats consisted of centrilobular hepatocellular atrophy attributed to decreased glycogen. Conclusions: 1) Serum concentrations of iALP decrease with the development of villous atrophy in the GI tract. 2) Total ALP measurements may be difficult to interpret accurately if effects, in opposite direction, are observed in multiple isozymes.

771 TOXICITY EVALUATION OF A FLUORINATED NORBORNENE COMPOUND.

M. DeLorme[1], G. S. Ladics[1], M. Donner[1], V. O. Wagner[2], C. Finlay[1] and S. E. Loveless[1]. *[1]DuPont Haskell Laboratory, Newark, DE and [2]BioReliance, Rockville, MD.*

The object of this study was to evaluate the toxicity of the fluorinated norbornene compound 2[(bicyclo[2.2.1]hept-5-en-2-yloxy)methyl]-1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (NBFOH), which is used as an intermediate in the production of fluorinated monomers and polymers. NBFOH was evaluated acutely for dermal sensitization using the LLNA, for mutagenesis by the Ames assay and for subchronic toxicity in a 4wk inhalation rat study. NBFOH produced dermal sensitization but did not cause bacterial mutagenicity either in the presence or absence of S9 activation. Male and female rats were exposed nose only to airborne NBFOH at levels of 0, 410, 1400 and 1500 mg/m^3, 6hr/day, 5days/wk for 4wk with clinical and histopathology specimens collected 1d following the final exposure. Due to the vapor pressure of NBFOH the 1500 mg/m^3 atmosphere was 27% aerosol and 73% vapor, the 1400 mg/m^3 atmosphere was 5% aerosol and 95% vapor, while the 410 mg/m^3 level was pure vapor. No test substance-related mortality or clinical signs of toxicity were observed over the course of the study. However, male rats demonstrated significant weight loss and decreased food consumption at 1400 mg/m^3 or greater during the exposure period. Male rats from the 1500 mg/m^3 group demonstrated an 11% increase in prothrombin time that was significantly higher than the control value of 15.7 sec. Urine fluoride did not demonstrate a concentration-response relationship, with minimal elevations observed in male rats at all exposure levels and sporadic increases in females. Both male and female rats exposed to 1400 mg/m^3 or greater had squamous metaplasia of the laryngeal mucosa and degeneration of the nasal olfactory and respiratory mucosa. The severity of the laryngeal squamous cell metaplasia correlated with the aerosol concentration of NBFOH while the nasal and respiratory lesions did not. Therefore, NBFOH demonstrates the potential to produce allergic dermatitis and subchronic studies indicate a no-observed-effect-level (NOEL) of 410 mg/m^3.

772 SAFETY OF TINOSORB® S, A NEW ORGANIC SUNSCREEN FOR BROAD SPECTRUM UV PROTECTION.

J. R. Plautz[2] and W. F. Salminen[1]. *[1]Product Safety and Regulatory, Ciba Specialty Chemicals Corporation, High Point, NC and [2]PSR, Ciba Specialty Chemicals, Basel, Switzerland.*

Tinosorb® S (CASRN 187393-00-6: Phenol, 2, 2'-[6-(4-methoxyphenyl)-1, 3, 5-triazine-2, 4-diyl]bis[5-[(2-ethylhexyl)oxy]-) is a new active sunscreen ingredient that provides broad spectrum UVA and UVB protection and is highly photostabile. Tinosorb S is currently approved for use as an active sunscreen ingredient in the European Union, Switzerland, and many other countries with the major exception being the US. We present here the results showing Tinosorb S to be safe as an active sunscreen ingredient when tested in standard (OECD) protocols. Tinosorb S is of low acute toxicity with rat dermal and oral LD50 values >2, 000 mg/kg. Tinosorb S caused none to minimal irritation when applied to rabbit eyes and skin. Tinosorb S did not cause skin sensitization, photoirritation, or photosensitization when applied to the skin of guinea pigs or humans. Tinosorb S was not genotoxic or mutagenic in standard *in vitro* and *in vivo* assays and in tests using UV exposure protocols. In a percutaneous absorption assay with *ex vivo* human skin, Tinosorb S exhibited low penetration across the skin (<0.1%). Rats given doses of radiolabelled Tinosorb S showed very low systemic uptake after a single oral dose (<1% of dose) or after dermal dosing (<1% of dose). In a 90-day subchronic oral gavage study in the rat, the NOEL was 1, 000 mg/kg/day, which was the highest dose tested (HDT). In a developmental toxicity study, the maternal and fetal NOEL was 1, 000 mg/kg (HDT). Reproductive effects did not occur in Segments I and III studies. Tinosorb S did not show binding affinity or competitive inhibition of natural hormone binding to either estrogen or androgen receptors *in vitro* and effects did not occur in the uterotrophic assay with immature rats given Tinosorb S orally. Additional safety tests are underway to assess the long-term photo(co)carcinogenicity and dermal carcinogenicity of Tinosorb S. In conclusion, Tinosorb S is a safe UV filter sunscreen ingredient that can be used to make highly photostabile, broad-spectrum sunscreens.

773 PHYSIOLOGICAL PARAMETERS AND BACKGROUND HISTOPATHOLOGY FINDINGS FROM CHRONIC (2 YEAR) CARCINOGENICITY STUDIES IN THE HAN WISTAR RAT.

C. Barton and C. Springall. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: D. Everett.

The HAN Wistar rat is becoming increasingly popular for use on regulatory toxicology studies. This strain tends to have a lower incidence of spontaneous background histopathology, when compared with some other rat strains; most notably those tumours involving endocrine and hormone-associated tissues. The HAN Wistar rat generally has a low mortality rate, with good survival over the course of a 2 year study and the potential for reducing group sizes. In addition, the small adult body weight can result in considerable reductions in bulk drug requirement. There are few publications detailing basic physiological data: bodyweight gain, food consumption, survival data and spontaneous tumour incidences for the HAN Wistar rat in chronic (2 year) carcinogenicity studies. The control data in this poster presentation are from carcinogenicity studies conducted recently at Covance Laboratories, Harrogate, England

774 TOXICOLOGICAL EVALUATION OF LEACHABLES AND EXTRACTABLES IN INHALATION DRUG PRODUCTS: RISK ASSESSMENT OF DI(2-ETHYLHEXYL)PHTHALATE (DEHP).

L. A. Haighton[1], K. L. Bibeau[1], N. N. Kim[2] and J. M. Daniels[1]. *[1]CANTOX HEALTH SCIENCES INTERNATIONAL, Mississauga, ON, ON, Canada and [2]Sepracor Inc., Marlborough, MA.*

Container closure systems may be used for nasal spray and inhalation solutions, suspension, and spray drug products. The US Food and Drug Administration (FDA) requires that a toxicological evaluation be completed for chemicals that can be extracted from the container components. DEHP, a commonly used plasticizer, is an example of an extractable that was identified in extraction studies of a novel container closure system. Although the level extracted was very low (approximately 0.0088 mg per container closure system) there is currently no regulatory threshold for extractables. Therefore, a detailed toxicological evaluation of DEHP was conducted including consideration of liver tumor mechanistic data (peroxisome proliferation) and potential Sertoli cell vacuolation. Exposure to DEHP was calculated using the highest concentration determined from the extraction testing and assuming the maximum dosing regimen for the drug product. This is very conservative, since extraction testing conditions do not reflect normal use and storage conditions. As the majority of toxicology data were generated using the oral route of administration, the exposure limit derived from oral studies was adjusted to account for differences in absorption. The results of the evaluation showed that the maximum theoretical exposure to DEHP through use of the inhalation spray drug product of 0.0037 micrograms/kg bodyweight/day was approximately 400 times lower than the inhalation exposure limit of 1.5 micrograms/kg bodyweight/day. Thus, no adverse effects attributable to exposure to DEHP from use of the drug product would be expected to occur. Furthermore, the results of this assessment indicate that, as long as future extraction testing indicated that the concentration of DEHP was below 3.6 mg per closure system, there would be no concern with regard to potential health effects.

775 DIETARY INCLUSION OF NOVASIL: SUBCHRONIC TOXICITY EVALUATION IN SPRAGUE-DAWLEY RATS.

E. Afriyie-Gyawu[1], J. Mackie[2], B. Dash[1], M. Wiles[1], H. J. Huebner[1], K. E. Lee[3] and T. D. Phillips[1]. *[1]Faculty of Toxicology (VAPH), Texas A&M University (TAMU), College Station, TX, [2]Veterinary Pathobiology, TAMU, College Station, TX and [3]Statistics, TAMU, College Station, TX.*

NOVASIL (NS) clay, commonly added to animal feeds, has been reported to diminish the bioavailability and toxicity of aflatoxins. To investigate the potential toxicity of subchronic exposure to NS, a 90-day study was conducted using male and female Sprague-Dawley rats (4-5 week old) fed rations containing 0, 0.25, 0.5, 1.0, and 2.0 % levels of NS. Doses were based on the lowest and highest levels recommended in animal feeds for the prevention of aflatoxicosis. Treatments consisted of 3 females and 3 males in five groups. Body weights, body weight gain, organ weights, histopathology, plasma biochemistry, serum vitamins A and E and micronutrients (Fe and Zn) were measured, standardized and compared to determine toxicity and any interactions of NS with critical nutrients. No morbidity or mortality was observed among treatment groups throughout the duration of the study. Among the males there were no significant differences in feed intake (FI), body weight gain (BWG), and FI/BWG. In females, FI/BWG was significantly higher at the 2.0% level suggesting impaired feed utilization. At necropsy, no changes in the major organs were observed. The ratios of organ weight to final body weight for the

liver, kidneys, lungs, heart, brain, spleen, and tibia among the treatment groups in each sex were not significantly different. Histopathological analysis of the liver and kidneys indicated no differences between controls and clay treatments. No significant reductions were observed in Zn and Fe. Other serum biochemical parameters were also unaffected. However, serum vitamin A and E levels in females were reduced at 2.0% clay, which may be associated with an increase in FI/BWG. Long-term studies (using larger sample sizes) are ongoing to confirm these preliminary findings. Results suggest that inclusion of NS at levels less than 2.0 % (w/w) in the diet should not result in overt toxicity (Supported by USAID LAG-G-00-96-90013-00, NIEHS P42-ES04917, and NIEHS Center Grant ES09106).

776 QUANTITATIVE CELL CYCLE INFORMATION COMPARED TO CYTOCHALASIN B BLOCKAGE IN CELL LINE MICRONUCLEUS ASSAYS.

E. Luther and M. Lee. *Strategic Research Development, CompuCyte Corp., Cambridge, MA.* Sponsor: S. Zhao.

The *in vitro* micronucleus assay is routinely performed to detect clastogenic and other chromosomal damage as a result of test samples being exposed to toxic substances. Typically it is performed by manual scoring of the test slides by microscope observation, often using cytochalasin B to identify binucleate cells that have completed DNA replication and are thus able to allow drug effects to manifest themselves. We investigated if DNA content per cell, the nuclear area, the number of cells per test well, expression of apoptotic markers, combined with the micronucleus count would be a suitable replacement for the cytochalasin B cell blockage method. CHO cells were seeded in microtiter wells, incubated with test substances (etoposide, mitoycin C,) in increasing dosages, with and without cytochalasin B, fixed in situ, and stained with DNA specific dyes, and a protein marker. The plates were analyzed with the iCyte Automated Imaging Cytometer (CompuCyte, Cambridge, MA). Dosage response curves were obtained for multiple cellular factors. In experiments without cytochalasin B, control wells are characterized by an exponential cell cycle distribution, less than .5% micronuclei, and normal nuclear morphology. With an increase in drug concentration we saw a progression of formed micronuclei (up to 12%), blockage of cells in S-phase in the cell cycle, and a decrease in cell count. An increase in nuclear area was observed at the highest dosages. In cytochalasin B treated experiments, control wells exhibited a large proportion of binucleate cells. Increases in drug dosages were accompanied by increased amounts of micronuclei, a decrease in the percentage of binucleate and total number of and the appearance of sub-G2 phase cells in the cell cycle distributions. A moderate increase in nuclear area was registered. Both methods gave equivalent results in terms of the dosage presenting the maximum micronuclei, however, the non-cytochalasin B treated sets had a higher signal to noise level, indicating greater sensitivity.

777 SAFETY OF GLYCOLIC ACID IN CLEANING PRODUCTS.

A. W. Hayes[1] and J. C. Stadler[2]. *[1]Department of Environmental Health, Harvard School of Public Health, Boston, MA and [2]DuPont Haskell Laboratory, Newark, DE.*

Glycolic acid occurs widely in nature, and is in a number of edible fruits, vegetables and meat products. It is also manufactured by several processes and used in a broad range of industrial and consumer products. The concentrated acid produced industrially is significantly diluted in end use products. Calculated estimates indicate that exposure to glycolic acid through household products is less than 1/10 the naturally occurring amount typically ingested on a daily basis. It has been used by dermatologists for years to treat skin disorders and is a component of many over-the-counter personal care products. No systemic toxicity has been noted as a result of these uses. Toxicity results indicate that glycolic acid (70%) causes effects that are typical of a strong acid, such as dermal and eye irritation; however, concentrations of < 5%, typically used in cleaning formulations, are not irritating to the skin. Extremely high doses given orally (-3000 mg/kg) will cause liver and kidney damage. In repeated dose testing, a no-effect level (NOEL) of 150 mg/kg/day was established for subchronic toxicity and for developmental effects. No effects to the immune system, neurological function or to reproductive parameters were observed. Genetic toxicology studies were negative. A safety margin may be calculated based on the repeated dose NOEL and estimated daily exposures during institutional cleaning. Glycolic acid biodegrades quickly and, therefore, does not persist or bioaccumulate in the environment. When chemicals containing glycolic acid are used on a daily basis, protection for the skin and eyes is advised to prevent localized irritation. Child-proof packaging is available to prevent children from ingesting these products. Overall, the evidence indicates there is minimal risk of adverse health effects from glycolic acid during the normal use of commercially available cleaning products.

778 REPEATED DOSE ORAL TOXICITY OF 8-2 TELOMER B ALCOHOL RANGE-FINDING STUDY IN RATS.

G. L. Kennedy[1], G. S. Ladics[1], J. O'Connor[1], S. Gannon[1], R. Jung[2], H. Iwai[3] and S. Shin-ya[4]. *[1]DuPont Haskell Laboratory, Newark, DE, [2]Clariant, GmbH, Sulzbach, Germany, [3]Daikin Industries, Ltd.,, Osaka, Japan and [4]Asahi Glass Co., Ltd.,, Tokyo, Japan.*

8-2 Telomer B Alcohol is a fluorinated chemical intermediate used to manufacture specialty polymers and surfactants. Preliminary to a repeated dose toxicity assessment, a range-finder study involving repeated oral doses of 8-2 Telomer B Alcohol was conducted in rats to determine the time needed to reach steady-state as determined by total organofluorine blood fluoride levels. Groups of 10 male and 5 female Crl:CD®(SD)IGS BR rats were each dosed daily by gavage with either 0 (control), 5, 25, or 125 mg 8-2 Telomer B Alcohol/kg/day. A number of toxicology end-points, such as body weights, liver and kidney weights were recorded during and at the end of the study. Blood samples were analyzed for total fluorine levels weekly. Steady-state concentrations were reached between test days 60 and 84. At steady-state, pooled plasma samples obtained from males and females over the duration of the study were analyzed for 8-2 Telomer B Alcohol (parent compound), 8-2 Telomer B acid, perfluorooctanoate acid (PFOA), perfluorononanoic acid (PFNA), and total fluorine levels. The level of parent compound detected did not exceed 0.074 ppm in either males or females. In males, PFOA represented approximately 85% of the total plasma fluorine. In females, PFOA represented approximately 5%. The 8-2 acid of Telomer B Alcohol and PFNA were also found in plasma of both males and females. Significantly lower mean body weights and increased liver weights compared to control were observed in male rats administered 125 mg/kg/day. Based on these data, the following doses were chosen for the 90-day oral toxicity study with the 8-2 Telomer B Alcohol: 0, 1, 5, 25, and 125 mg/kg/day. This study was sponsored by the Telomer Research Program.

779 A SUBCHRONIC TOXICITY STUDY IN RATS AND GENOTOXICITY TESTS WITH SURELEASE® AQUEOUS ETHYLCELLULOSE DISPERSION.

C. C. DeMerlis[1], D. R. Schoneker[1], C. Kelly[2] and J. Borzelleca[3]. *[1]Colorcon, West Point, PA, [2]Huntingdon Life Sciences, East Millston, NJ and [3]Medical College of Virgina, Richmond, VA.*

C C DeMerlis, D R Schoneker, C M Kelly and J F Borzelleca. 1Colorcon, West Point, PA, USA, 2Huntingdon Life Sciences, E. Millstone, NJ, USA; 3Medical College of Virginia, Richmond, VA, USA. Surelease® is an excipient used as a modified release coating for beads, granules, non-pariels, drug crystals and tablets and for taste masking applications for drug products and dietary supplement products. A study was conducted to assess the toxicity of spray dried Surelease when administered orally, *via* dietary admixture, to Sprague-Dawley CD® rats (20/sex/group) at dose levels of 0, 2000, 3500, and 5000 mg/kg/day for a period of at least 3 months. After 3 months of treatment, all survivors were sacrificed and selected organs were weighed. Complete macroscopic examinations and histopathological evaluation of selected tissues were conducted on all animals. Neuropathological evaluations were performed on 5 animals/sex/group. No mortality occurred during the study. Clinical observations, ophthalmology, body weight and food consumption, hematology, coagulation, clinical chemistry, urinalysis, functional observational assessments, motor activity, organ weights and ratios and macroscopic and microscopic observations did not reveal any test article-related effects. The NOAEL (no-observable-adverse-effect-level) is 5000 mg/kg/day, the highest dose tested. A series of genotoxicity tests were conducted with Surelease. Surelease showed no evidence of mutagenic activity in the bacterial reverse mutation test with and without metabolic activation and in the *in vitro* cell mutation assay under the experimental conditions employed. Surelease did not show any evidence of causing chromosome damage or bone marrow cell toxicity when administered orally by gavage in the mouse micronucleus *in vivo* test procedure. These findings support the safety of Surelease for use as an excipient.

780 P450-GLO™LUMINESCENT ASSAYS DETECT CYP450 ACTIVITIES IN RECOMBINANT FRACTIONS, LIVER MICROSOMES AND CULTURED HEPATOCYTES.

J. J. Cali, S. Ho, D. Ma and R. Bulleit. *Research and Development, Promega Corporation, Madison, WI.*

P450-Glo™ is a luminescence-based technology for assaying cytochrome P450 (CYP450) activities. We used P450-Glo™ to measure CYP450 activities in recombinant fractions, liver microsomes and cultured hepatocytes. The approach is based on a series of D-luciferin derivatives that are not substrates for firefly luciferase but are metabolized by CYP450s to the firefly luciferase substrate D-luciferin. When

CYP450 reactions with the derivatives are combined with a luciferase reaction an amount of light is produced that is directly proportional to the amount of CYP450-generated D-luciferin. Light is therefore used to measure CYP450 activity. Luminogenic substrates reacted with recombinant human CYP1A1, 1A2, 1B1, 2C8, 2C9, 3A4 and 3A7 and detected known CYP450 inhibitors of these activities with IC50s similar to those reported using conventional assays. The assays also detected CYP450 activities in liver microsomes. Two substrates, luciferin benzyl ether and deoxyluciferin were highly specific for CYP3A4 and CYP2C9, respectively. The luminogenic CYP450 substrates are cell permeable and therefore provided a basis for luminescent assays that measured CYP450 induction in cultured hepatocytes. Because P450-Glo™ Assays do not rely on fluorescent substrates data analysis was not confounded by overlap of the fluorescent properties of probe and analyte. P450-Glo™ Assays were homogenous, highly sensitive and robust. The assays were readily configured in multi-well plate formats for rapid screening of multiple compounds against CYP450 activities.

781 EVALUATION OF THE SUBCHRONIC, REPRODUCTIVE, AND DEVELOPMENTAL TOXICITY OF A FLUORINATED ACRYLIC COPOLYMER.

N. E. Everds, J. C. Stadler, S. A. MacKenzie, L. A. Malley, S. M. Munley and G. P. Sykes. *DuPont Haskell Laboratory, Newark, DE.*

The objective of these studies was to evaluate the subchronic, reproductive, and developmental toxicity of a fluorinated acrylic copolymer in rats. The test substance was suspended in methylcellulose and administered by oral gavage at dosages of 0, 10, 100, or 1000 mg/kg/day (90-day subchronic toxicity and one-generation reproduction), and at the same dosages on gestation days 6-20 (developmental toxicity). The NOEL for subchronic toxicity was 100 mg/kg/day in males and females, based on reversible, minimal to mild thyroid follicular hypertrophy observed at 1000 mg/kg/day. Other non-adverse, test substance-related effects, observed in males and/or females at 1000 mg/kg/day, were mild clinical pathology alterations, increased liver and kidney weights, minimal liver hypertrophy, and increased hepatic b-oxidation activity. All effects were reversible after a one-month recovery, except the thyroid hypertrophy, which was still present but at reduced incidence and severity. No test substance-related effects were observed on in-life, neurobehavioral, ophthalmological, or anatomic pathology parameters, or on any reproductive parameter in P1 or F1 rats dosed up to 1000 mg/kg/day. In the developmental toxicity study, the NOEL for both maternal and fetal toxicity was 1000 mg/kg/day, based on a lack of test substance-related effects on any maternal or fetal parameter. No fetal malformations were observed. Overall this polymer, which is used in stain and soil-resistance applications, is considered to have low potential to produce human health effects.

782 DOSE RANGE-FINDING STUDY OF HALOFUGINONE (NSC-713205) IN BEAGLE DOGS.

K. Veley[1], B. Sparrow[1], J. W. Merrill[1], I. M. Grossi[1], J. Tomaszewski[2] and J. O. Peggins[2]. *[1]Toxicology, Battelle, Columbus, OH and [2]NCI, NIH, Bethesda, MD.*

Halofuginone is a botanical quinazolinone alkaloid capable of inhibiting tumor progression through its ability to suppress extracellular matrix deposition, cell proliferation and angiogenesis. Beagle dogs were utilized to determine the maximally tolerated dose and relative drug toxicity of halofuginone when administered as a bi-weekly 1hr infusion for 4 consecutive weeks. Dose concentrations delivered over the 1hr period were 0, 0.025, 0.05, 0.1, 0.5 and 1.0mg/kg. Following the completion of the first infusion dose on Day 1, animals in the 0.5 and 1.0mg/kg dose groups displayed signs of toxicity in the form of extreme emesis and diarrhea. On Day 2, emesis and diarrhea continued in conjunction with altered hematology and serum chemistry parameters. Dehydration subsequent to the diarrhea was considered responsible for increases in RBC, HEM and HCT levels. Increased AST, ALT and CK values may have been related to cardiac or skeletal muscle damage, however hepatic alteration could not be ruled out. Elevated ALP may have been a secondary response to either the stress of diarrhea or possibly to increased mineral resorption from bones, which invariably accompanies renal dysfunction. Additionally, elevated BUN and CREA values indicated renal dysfunction with dehydration/poor renal perfusion and was proposed to have contributed to the moribundity of these animals. The affected animals in the 0.5 and 1.0mg/kg dose groups were euthanized moribund on Day 2, and additional infusions at these levels were suspended. With the exception of elevated nRBC on Day 9 in the 0.05 and 0.1mg/kg groups, there were no other effects noted at the 0.1mg/kg dosage and lower. Based on these results, the maximum tolerated dose for intravenously dosed halofuginone when administered bi-weekly to beagle dogs appears to be between 0.1mg/kg and 0.5mg/kg.

783 NOGATOXIN ISOLATED FROM NON-AXENIC CULTURES OF PFIESTERIA.

D. Baden[1], E. Noga[3], K. Rein[2], J. Benson[5], W. Abraham[4], R. Belas[6] and C. Tomas[1]. *[1]UNCW, Wilmington, NC, [2]FIU, Miami, FL, [3]NCSU, Raleigh, NC, [4]Mt Sinai Medical Center, Miami, FL, [5]Lovelace Respiratory Research Institute, Albuquerque, NM and [6]UMd, Baltimore, MD.*

An ichthyotoxic lipid-soluble extract has been isolated from mixed cultures of fish-killing Pfiesteria spp. Nogatoxin was purified by column and thin-layer chromatography (TLC), using fish bioassay-guided fractionation. Crystalline material was subjected to NMR and toxicologic testing methods. MW was estimated at <500 amu and a cyclopropane ring is suggested. Gambusia fish exposed to TLC fractions developed obvious skin damage after 4 hrs of exposure, indicated grossly by cloudiness which was prominent on the fins. Sometimes, fish behaved normally and in other cases they died after developing the skin damage. Histological examination of fish revealed skin erosion and ulceration. There were no deaths in mice by oral or ip injection (up to 1.26 mg/kg body weight) and no clinical signs of toxicity were noted. Some organ lesions were noted, but did not correlate with dose. Organ weights between control and experimental mice did not differ. Airway responsiveness after Nogatoxin challenge (20 breaths at 100 pg/mL), measured in naive sheep, was 107 +/- 27% (mean +/- sem). There was no induced hyper-responsiveness and no changes in inflammatory response. Extracted cultures were a taxonomically diverse microbial community. Denaturing gradient gel electrophoresis and quantitative real-time PCR methods were used to identify and quantify microorganisms. Multiple ciliate species and one species of rotifer were detected in addition to Pfiesteria piscicida and P. shumwayae. The ciliate Chilodonella, a fish gill parasite, was also found. The complex eukaryotic microbial community may have influenced both Pfiesteria dinoflagellate abundance and the occurrence of fish deaths due to the extracted toxic fraction. Nogatoxin is not present in our clonal cultures of Pfiesteria. The lack of potency in mammalian toxicological assays may reflect our use of fish bioassay for purification. Supported by NIH ES05705 and ES09563, Sea Grant NA46-RG-0087, and Saltonstall-Kennedy NA67FD00500.

784 UP-REGULATION OF CYCLOOXYGENASE-2 EXPRESSION BY GELATIN IN MURINE MACROPHAGES.

J. Kim[1, 2] and H. Jeong[1, 2]. *[1]Pharmacy, Chosun University, Kwangju, Kwangju, South Korea and [2]Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea.*

Gelatin is derived from animal collagen tissues and is therefore present in many kinds of animal protein food. The biological origin and biocompatibility of gelatin has led to wide-ranging applications in the pharmaceutical and medical fields; for example, as sealants for vascular prostheses, bone-repairing materials, wound healing agents and scaffolds for tissue engineering purposes. In the present study, we investigated the effects of gelatin and collagen on the cyclooxygenase-2 (COX-2) gene expression in the mouse macrophage cell line RAW 264.7. Gelatin and gelatin significantly increased the prostaglandin E2 production and the expression of COX-2 mRNA and COX-2 protein in a dose-dependent manner. To investigate the significant cis-acting regions which COX-2 promoter, transient transfection experiments were carried out using reporter vectors harboring deleted COX-2 promoters. The transcriptional factor binding sites for activator protein 1 (AP-1) and NF-kB between -574 and -51 could be important for the induction of COX-2 by gelatin and collagen. The results of these studies suggest that induction of transcriptional activation of COX-2 by gelatin and collagen might be mediated through the AP-1 and NF-kB activation.

785 INDUCTION OF INDUCIBLE NITRIC OXIDE SYNTHASE AND TUMOR NECROSIS FACTOR-A EXPRESSION BY GELATIN VIA NUCLEAR FACTOR-KB TRANSACTIVATION IN MACROPHAGES.

D. Oh[1, 2], J. Kim[1, 2] and H. Jeong[1, 2]. *[1]Pharmacy, Chosun University, Kwangju, Kwangju, South Korea and [2]Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea.*

As biological polymer, gelatin employed as backbones in the construction of hydrogels or cell/tissue scaffoldings for various biomedical applications. In the present study, we investigated the effect of collagen and gelatin on the inducible nitric oxide synthase (iNOS) gene expression in the mouse macrophage cell line RAW 264.7. The nitric oxide production and iNOS expression were induced by both of gelatin and collagen in dose-dependent manner. Gelatin and collagen also increased the production of TNF-a and its expression level in a dose-dependent manner. The effects of gelatin and collagen on the transcriptional activity of NF-kB were examined, since the transcriptional regulation of iNOS has been shown to be under the control of the NF-kB. Transient expression assay with the luciferase reporter vector

containing three-copies of NF-kB binding site revealed that the increased level of iNOS mRNA induced by collagen or gelatin could be mediated by the NF-kB transcription factor complex. Furthermore, gelatin and collagen activates the DNA binding ability of NF-kB, in the result of electrophoretic mobility shift analyses using the NF-kB binding sequence within iNOS promoter as a probe. These results demonstrated that nitric oxide and TNF-a production in the gelatin- and collagen-stimulated macrophages might be induced by the up-regulation of these genes expression through NF-kB transactivation.

786 GROWTH INHIBITION AND INDUCTION OF APOPTOSIS BY DIHYDRO-N-CAFFEOYLTYRAMINE ON HUMAN LEUKEMIA CELLS.

E. Woo[2, 3], C. Choi[1] and H. Jeong[2, 3]. [1]*Food Science, Jinju International University, Jinju, South Korea,* [2]*Pharmacy, Chosun University, Kwangju, Kwangju, South Korea and* [3]*Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea.*

Lycii Radicis Cortex, the root bark of Lycium chinense Miller (Solanaceae) is used in oriental medicine as a tonic and is reported to exhibit hypotensive, hypoglycemic, and antipyretic activity. Recently, we have isolated dihydro-N-caffeoyltyramine, a phenolic amide, from the Lycii Radicis Cortex. Treatment with dihydro-N-caffeoyltyramine significantly inhibited the proliferation of human leukemia cell lines HL-60 in a dose-dependent manner. We found also that the growth inhibition of HL-60 by dihydro-N-caffeoyltyramine is associated with induction of apoptosis of cells. Dihydro-N-caffeoyltyramine induced the apoptosis of HL-60 such as apoptotic body and DNA fragmentation. In addition, the flow cytometric analysis revealed dihydro-N-caffeoyltyramine dose-dependently increased apoptotic cells with hypodiploid DNA contents. These results indicate that dihydro-N-caffeoyltyramine can control growth of leukemic HL-60 cells through apoptosis and may have a possibility of potential anticancer activities.

787 PLATYCODON GRANDIFLORUM SUPPRESSED PDGF-DRIVEN PROLIFERATION AND COLLAGEN SYNTHESIS IN HEPATIC STELLATE CELLS.

K. Jung[1, 2], K. Lee[1, 2], Y. Chung[3], C. Choi[3], S. Roh[4], Y. Cho[4] and H. Jeong[1, 2]. [1]*Pharmacy, Chosun University, Kwangju, Kwangju, South Korea,* [2]*Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea,* [3]*Food Science, Chinju International University, Jinju, South Korea and* [4]*R&D, Jangsaeng Doraji Co., Ltd.,, Jinju, South Korea.*

Activation of hepatic stellate cells has been identified as a critical step in hepatic fibrogenesis and is regulated by several factors including cytokines and oxidative stress. Although the underlying mechanisms remain incompletely understood, it is widely accepted that oxidative stress plays a critical role in liver fibrogenesis. We investigated an aqueous extract from the roots of Platycodon grandiflorum A. DC (Campanulaceae), Changkil (CK)-mediated signaling pathway involved in hepatic stellate cell line (HSC-T6) inactivation. CK significantly inhibited in PDGF-driven HSC-T6 cell growth by inducing cell cycle arrest in a dose- and time-dependent manner. CK suppressed the expression of collagen and alpha-smooth muscle actin expression in PDGF-driven HSC-T6 cell. Furthermore, CK induced cell arrest at G1 *via* a relative protein of cell arrest pathway, p53 and a cell cycle-dependent kinase inhibitor, p21Cip1/WAF1 in PDGF-driven HSC-T6 cell. These results suggested that the protective effects of CK on the hepatic fibrosis in stellate cells may, at least in part, be due to its ability to reduce proliferation and collagen synthesis.

788 MATRIX METALLOPROTEINASE GENE EXPRESSION IN RAT MICROGLIA EXPOSED TO THE MARINE TOXIN DOMOIC ACID.

A. M. Mayer[1], M. J. Fay[1] and A. M. Romanic[2]. [1]*Pharmacology, Midwestern University, Downers Grove, IL and* [2]*Cardiovascular Pharmacology, Glaxo SmithKline, King of Prussia, PA.* Sponsor: W. Prozialeck.

The pathology of Amnesic Shellfish Poisoning, one of the shellfish poisoning syndromes in the United States caused by the marine glutamate analog domoic acid (DOM), is incompletely understood. Our working hypothesis is that DOM may activate rat neonatal microglia (BMϕ), cause generation of matrix metalloproteinases (MMP), and toxicity to the nervous system. We have reported that *in vitro treatment of BMϕwith DOM lead to MMP-9 protein release (Mayer et al.* BioMedCentral Pharmacology *1:7-19, 2001). The purpose of our study was to investigate MMP gene expression in 4-24 hour DOM [1mM]-treated BMϕusing a MMP-specific cDNA array (SuperArray Inc., Bethesda, MD). Through side-by-side hybridization with cDNA probes prepared from mRNAs of control or 4, 8, 16 and 24 hour DOM-treated BMϕ, the expression of 18 MMP and 4 tissue inhibitor of metalloproteinase (TIMP) genes was determined. Control BMϕ expressed 4 MMP and 2 TIMP genes constitutively at high levels* in vitro*: collagenase-like A (MMP-1), MMP-2, -9, -19 and TIMP-2 and -4. Over a 24-hour observation period, DOM* increased *expression of MMP-2, -9, TIMP-2 and -4, by 242, 226, 144 and 163 % of control expression, respectively. Concomitantly, DOM* decreased *expression of MMP-1 and -19 in a time-dependent manner. Our current data, while providing additional experimental evidence to support of our working hypothesis, also extends our recent observations (Mayer A. et al.* The Toxicologist *72(S1): 346, 2003) by generating new evidence that suggests that DOM has a time-dependent effect on BMϕ MMP and TIMP gene expression* in vitro*. Supported by Midwestern University (AMM) and Glaxo SmithKline (AMR).*

789 14-DAY AND 90-DAY MELATONIN TOXICITY STUDIES IN FISCHER 344(F344) AND LONG-EVANS(LE) RATS.

D. Gerken[1], M. Ryan[1], M. Hejtmancik[1], A. Wiechmann[3], G. Boorman[2], M. Vallant[2], J. Roberts[4] and R. Chhabra[2]. [1]*Battelle Science and Technology, Inc., Columbus, OH,* [2]*NIEHS, Research Triangle Park, NC,* [3]*University of Oklahoma Health Sciences Center, Oklahoma City, OK and* [4]*Fordham University, New York, NY.*

Melatonin(MEL), a pineal gland hormone and "nutritional supplement", was administered by gavage in 0.5% methylcellulose containing 0.25% ethanol to male(M) and female(F) LE and F344 rats at dosages of 0, 5, 50, 5000, 50, 000, and 200, 000 ug/kg in both studies. In the 14-day study, 10/sex/dosage/strain were administered 12 daily doses (weekdays only) and subjected to one of three lighting and dosing regimes. Group I rats were exposed to full-spectrum fluorescent lighting (F-SFL) during the 12-hour light cycle (10-40 lux at cage level) and were dosed during the 4th and 5th hour of the light cycle. Group II rats were exposed to F-SFL during the 12-hour light cycle (10-40 lux at cage level) and were dosed during the 3rd and 4th hour of the 12-hour dark cycle. Group III rats were exposed to F-SFL during the 12-hour light cycle (10-40 lux at cage level) plus either 1000 lux(F344) or 1500 lux(LE) during the 6th and 7th hour of the light cycle approximately every other day and were dosed during the 4th and 5th hour of the light cycle. Weekly body weights, weekly clinical observations, organ weights, morphometric measurements of the outer nuclear layer (ONL) retinal thickness and histopathology were collected. No MEL related effects were noted in this study. In the 90-day study, 10/sex/dosage/strain were administered 68 daily doses (excluding weekends). Weekly body weights, weekly clinical observations, clinical pathology assessments including thyroid hormone analyses, organ weights, morphometric measurements of the ONL retinal thickness and histopathology were performed. MEL administration resulted in a mean weight decrease (10-15%) in F344 M and LE F compared to control, dark colored feces (200, 000 and 50, 000 ug/kg M and F LE and F344), increases in serum thyroid hormones (200, 000 ug/kg LE and F344 M and F, 50, 5, 000, 50, 000 ug/kg F344 F) and cystic uterine endometrial hyperplasia (all dosages in LE F).

790 CYTOTOXIC AND APOPTOSIS-INDUCING PROPERTIES OF *GUAIACUM SANCTUM L.* (ZYGOPHYLLACCEAE) ON BREAST CANCER CELL LINES.

K. J. Chavez[1], I. Delgado[3], M. T. Laux[2], J. A. Flanders[2] and E. Rodriguez[3]. [1]*Institute for Comparative and Environmental Toxicology, Cornell University, Ithaca, NY,* [2]*Department of Clinical Sciences and Molecular Medicine, Cornell University, Ithaca, NY and* [3]*Department of Plant Biology, Cornell University, Ithaca, NY.*

Guaiacum sanctum L. (Zygophylacceae) is a medicinal and ornamental plant from tropical America and the West Indies. The heartwood and bark is widely used in South American folk medicine for the treatment of rheumatism, gout, boils, and gonorrhea. Extracts of the plant have been shown to possess anti-inflammatory activity, including immunomodulatory and analgesic properties. However, to date no studies have been carried out to verify the direct antitumor activity of the extracts. The present investigation was initiated to study the direct effect of organic extracts from the root and bark of *Guaiacum sanctum L.* on the growth of human breast cancer cell lines (SKBR-3, BT20, MB-MDA-468, MB-MDA-453, and MB-MDA-231) in addition to cervical cancer cell line (A-431) and simian kidney cell line (COS). Antiproliferative *in vitro* assays were based on both metabolic activity (Alamar Blue Assay) and determination of bound protein [Sulforhodamine B Assay (SRB)]. Indication of apoptosis was studied utilizing fluorescence microscopy "double stained" with propidium iodide and Hoechst 33342. Our results indicate that *Guaiacum sanctum L.* exerts a direct antiproliferative effect on the growth of human breast cancer cell lines with inhibition ranging from 1000 ppm-15.6 ppm with Alamar Blue Assay and IC50 ranging between 200-20 μg/ml as determined by SRB, but had no effect on cell line A-431 and showed inhibition of (COS) only at the highest concentration. Apoptosis was induced in a dose-dependent manner.

IC50 for cell death was detected at 50μg/ml for cell lines MB-MDA-231 and SKBR-3. Morphologic features (chromatin condensation and apoptotic bodies formation) were seen as low as 12.5 μg/ml as early as 6 hrs. The study provides preliminary information that *Guaiacum sanctum L.* contains chemicals with chemotherapeutic potential. The study was supported by MIRT/NIH T37TW00076.

791 THE USE OF ELISA IN DIFFERENTIAL DIAGNOSIS OF THE GENUS TRIMERESURUS SNAKE BITES IN TAIWAN.

D. Hung[1] and M. Liau[2]. *[1]Toxicology Center, Taichung Veterans General Hospital, Taichung City, Taiwan and [2]Department of Biotechnology, Fooyin University, Kaohsiung Hsien, Taiwan.* Sponsor: S. Lin-Shiau.

In Taiwan, Trimeresurus mucrosquamutus (Taiwan habu) and T. stejnegeri (green bamboo habu) are the two most important poisonous snakes and half of the snakebite cases are afflicted by these Trimeresurus snakes. Their venoms are thought to be similar in toxicity despite of outstanding differences in their appearances. As reported, some distinct clinical pictures have been noted with more bleeding diathesis in T. stejnegeri snakebites. However, in many cases the afflicting snake is not witnessed. Here, we developed the method of Sandwich enzyme-linked immunosorbent assay (Sandwich-ELISA) to differentiate and detect snake venom in biological samples with the 1ng/ml of detection limit. In cases of definite T. stejnegeri or T. mucrosquamutus snakebite (snakes witnessed or caught), the cross reactivity of these two venoms and antivenoms was found to be only 1.8-13.5% by ELISA, which proved that Sandwich-ELISA method is useful to differentiate these two snake bites clinically. Key Words: ELISA, snake bites, Trimeresurus

792 TOXICITY EVALUATION OF KAVA KAVA EXTRACT IN FISHER 344 RATS AND B6C3F$_1$ MICE FOLLOWING REPEAT DOSING BY ORAL GAVAGE.

B. Sparrow[1], M. Hejtmancik[1], M. Ryan[1], A. Skowronek[1], P. Chan[2] and D. Orzech[2]. *[1]Battelle, Columbus, OH and [2]NIEHS, Research Triangle Park, NC.*

Kava kava extract is a nutraceutical promoted as a natural relaxant to treat nervous anxiety, stress and restlessness. Recently, severe hepatotoxicity including hepatitis, cirrhosis and liver failure was associated with kava kava products. This study investigated the toxicological effects of kava kava extract in rats and mice. Kava kava extract in corn oil was administered by oral gavage to Fischer 344 rats and B6C3F$_1$ mice at dosages of 0, 0.125, 0.25, 0.5, 1.0 or 2.0 g/kg. Doses were administered daily (excluding weekends) for up to 13 doses. Clinical observations were recorded daily and food and water consumption monitored. Body weights were recorded weekly. At study termination, animals were humanely euthanized and necropsied. Liver, heart, lung, kidney, thymus and testis weights were recorded. Brain, liver, kidney and lung tissues were processed for microscopic examination. One 2.0 g/kg male mouse was moribund on Day 2 and was euthanized. One 2.0 g/kg female rat and male mouse died on Day 3. Treatment-related clinical observations included abnormal breathing, ataxia and lethargy, which occurred in both the rat and mouse 1.0 and 2.0 g/kg groups. No notable body weight changes occurred. Food and water consumption for the 2.0 g/kg groups were initially low, but eventually recovered to control values. For rats, organ-to-body weight changes attributed to kava kava extract included increased liver-to-body weight ratio for the 1.0 and 2.0 g/kg male rats and the 0.5, 1.0 and 2.0 g/kg female rats. Similarly, liver-to-body weight ratio changes attributed to kava kava extract occurred in mice, with increases noted in 1.0 and 2.0 g/kg male mice and 0.25, 0.5, 1.0 and 2.0 g/kg female mice. Microscopically, hepatic cellular hypertrophy was noted for the 2.0 g/kg male rats, the 0.25, 0.5, 1.0 and 2.0 g/kg female rats and the 2.0 g/kg male and female mice. Based on this study, further investigation of kava kava extract toxicology is recommended. (Supported by NIEHS Contract NO1-ES-25484).

793 USE OF THE AFRICAN GREEN MONKEY (CHLOROCEBUS AETHIOPS) MODEL TO DETERMINE PATHOPHYSIOLOGICAL RESPONSES TO INHALED RICIN TOXIN AND EFFICACY OF RICIN VACCINES.

R. W. Wannemacher, R. Dinterman, J. Hewetson, M. Pitt, R. Tammariello, R. Rietcheck, C. Klages and C. Millard. *USAMRIID, Frederick, MD.*

In rodent models, inhaled ricin (R) causes severe lung injury that can result in death. Preliminary observations in the rhesus macaque indicated that inhaled doses of R that resulted in death were associated with severe lung damage. In this study, the African green monkey (AGM) was chosen because of freedom from Cercopitheane herpes virus 1 infection, non-aggressive behavior, and more uniform in weight and size as adults. To date, 5 monkeys have been exposed to various doses of aerosolized R with 3 deaths and 2 survivors. The median lethal inhaled dose (MLID) appears to be ~ 7 μg/kg. Death occurred in to 2 to 3 days (D), with a gross pathology of moderate pulmonary edema and hemorrhage. Lung histology included inflammation, fibrin, edema, and hemorrhage. Surviving monkeys had persistent lung damage as measured by chest X-ray. They had elevated body temperature on D 1, which became subnormal for the next 5 to 6 D. The monkeys became anorexic for 10 to 14 D and on D 14 had lost weight; had an increased white blood, neutrophil, eosinophil, basophile, and platelet count; decreased total serum protein and albumin; and elevated serum LDH. The survivors will be monitored for 10 weeks (W). Twenty-two additional AGM are being used in an efficacy study for 2 vaccine candidates, deglycosylated R A-chain (dgRTA), and recombinant truncated (1-198) deletion (34-43) mutation of R A-chain (33/198 rRTA), plus 2 controls. At 0, 4, & 8 W, 10 monkeys in each vaccine group were vaccinated IM with 20 μg of antigen protein + 0.2% Alhydrogel. The dgRTA group developed serum anti-R IgG titers of 1:40, 000 by 4 W after the first vaccination, while it took 2 W after the second vaccination for the 33/198 rRTA group to develop titers of 1:4, 000. Six W after the third vaccination the monkeys will be exposed to 5 MLID of R. The AGM appears to be an excellent model to study the effects of inhaled R toxicity and efficacy of R vaccines.

794 DOWN-REGULATION OF CYCLOOXYGENASE-2 EXPRESSION BY CAFFEOYL-4-DIHYDROCAFFEOYL QUINIC ACID IN MACROPHAGES.

Y. Chung[1], C. Choi[1] and H. Jeong[2, 3]. *[1]Food Science, Jinju International University, Jinju, South Korea, [2]Pharmacy, Chosun University, Kwangju, Kwangju, South Korea and [3]Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea.*

Inducible cyclooxygenase-2 (COX-2) has been implicated in the processes of inflammation and carcinogenesis. Thus, the potential COX-2 inhibitors have been considered as anti-inflammatory or cancer chemopreventive agents. In this study, we investigated the effect of Caffeoyl-4-dihydrocaffeoyl quinic acid (CDCQ) isolated from Salicornia herbacea on the expression of cyclooxygenase (COX-2) in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. When CDCQ was treated with LPS, the prostaglandin E2 production and COX-2 gene expression induced by LPS were markedly reduced in a dose-dependent manner. Transient transfection experiments showed that LPS-induced increase in COX-2 promoter activities was suppressed by CDCQ. Moreover, transient transfection experiments using reporter vectors harboring deleted COX-2 promoters revealed that the transcriptional factor AP-1, but not NF-kB, between -574 and -51 in COX-2 promoter could be important for the inhibition of LPS-induced COX-2 mRNA by CDCQ. This study suggests that modulation of COX-2 by CDCQ may be important in the prevention of carcinogenesis and inflammation.

795 SUPPRESSION OF LIPOPOLYSACCHARIDE-ACTIVATED CYCLOOXYGENASE-2 EXPRESSION BY DIHYDRO-N-CAFFEOYLTYRAMINE IN MURINE MACROPHAGE RAW 264.7 CELLS.

H. Kim[1, 3], J. Kim[1, 3], C. Choi[2], K. Jung[1, 3], E. Woo[1, 3], S. Han[1, 3] and H. Jeong[1, 3]. *[1]Pharmacy, Chosun University, Kwangju, Kwangju, South Korea, [2]Food Science, Jinju International University, Jinju, South Korea and [3]Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea.*

Lycii Radicis Cortex, the root bark of Lycium chinense Miller (Solanaceae) is used in oriental medicine as a tonic and is reported to exhibit hypotensive, hypoglycemic, and antipyretic activity. A phenolic amide, dihydro-N-caffeoyltyramine was isolated from the Lycii Radicis Cortex. In the present study, we investigated the effects of dihydro-N-caffeoyltyramine on the cyclooxygenase-2 (COX-2) gene expression, which plays a crucial role in many physiological and pathological processes in macrophages. Treatment with dihydro-N-caffeoyltyramine significantly decreased the production of LPS-induced prostaglandin E2 and the expression of COX-2 mRNA in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages in a dose-dependent manner. Transient transfection experiments also showed that LPS-induced increase in COX-2 promoter activities was suppressed by dihydro-N-caffeoyltyramine. This study suggests that modulation of COX-2 expression by dihydro-N-caffeoyltyramine may be important in the prevention of inflammation.

796 AN AQUEOUS EXTRACT ISOLATED FROM PLATYCODON GRANDIFLORUM SUPPRESSED IN B16F10 MELANOMA CELL METASTASIS.

K. Lee[1, 2] and H. Jeong[1, 2]. *[1]Pharmacy, Chosun University, Kwangju, Kwangju, South Korea and [2]Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea.*

Metastasis of cancer cells to distant sites is one of the major deciding factors in cancer outcome. In fact, prognosis of cancer is mainly determined by the invasiveness of the tumor and its ability to metastasize. In this study, we assayed the preventive

and therapeutic effects of aqueous extract from the roots of Platycodon grandilflorum A. DC, Changil (CK) on the experimental lung metastasis induced by melanoma cell (B16F10) in C56BL6 mice. CK clearly inhibited both B16F10 melanoma cell adhesion to the extracellular matrix proteins as well as invasion through Matrigel-coated filter. In addition, CK significantly inhibited the proliferation of B16F10 melanoma cells in a dose-dependent manner. In B16F10 melanoma lung metastasis experiment, CK showed remarkable inhibitory effects on the lung tumor colonization in a dose-dependent manner. These results demonstrate that anti-metastatic and anti-angiogenetic activity of CK resulted from blocking of proliferation and invasion of the melanoma cells. Taken together, the results of our study provide evidence that CK possess an anti-metastatic activity.

797 PLATYCODON GRANDIFLORUM SUPPRESSED INVASION AND ANGIOGENESIS.

D. Shin[1, 2], K. Lee[1, 2] and H. Jeong[1, 2]. [1]*Pharmacy, Chosun University, Kwangju, Kwangju, South Korea and* [2]*Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea.*

Medical plants contain pharmacological substances including flavonoids and saponin, and their extracts have been therapeutically administered for cancer therapy *in vitro* and *in vivo*. In this study, we assayed the preventive and therapeutic effects of aqueous extract from the roots of Platycodon grandiflorum A. DC, Changil (CK) on the *in vitro* angiogenesis using human microvessel endothelial cell-1 (HMEC-1). CK inhibited cell migration and proliferation of HMEC-1 in a dose-dependent manner. CK also inhibited the tube formation of HMEC-1 in a dose-dependent manner. Furthermore, in an *in vivo* matrigel plug assay, CK reduced B16F10 tumor melanoma cell-induced angiogenesis compare with control. These results demonstrated that prevention of angiogenesis by CK was mediated by inhibition of proliferation and migration of endothelial cells. Taken together, the results of our study provide evidence that CK possess an anti- angiogenetic potential that is a new insights and previously unrecognized biologic activity.

798 PROTECTIVE EFFECT OF PLATYCODON GRANDIFLORUM ON THE ACETALDEHYDE-INDUCED ACTIVATION OF HEPATIC STELLATE CELLS.

H. Jeong[1, 2] and K. Lee[1, 2]. [1]*Pharmacy, Chosun University, Kwangju, Kwangju, South Korea and* [2]*Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea.*

The increased deposition of extracellular matrix by hepatic stellate cells following liver injury in a process known as activation is considered a key mechanism for increased collagen content of liver during the development of liver fibrosis. In this study, we investigated the protective effects of an aqueous extract from the roots of Platycodon grandiflorum A. DC (Campanulaceae), Changkil (CK), on hepatic fibrosis in hepatic stellate cells. We report that CK reduces the accumulation of collagen in acetaldehyde-induced hepatic stellate cells. The accumulation and synthesis of collagen were measured by Marson-Trichrom stain and pulse-labeling with [3H]-proline, respectively. As the results, CK inhibited collagen accumulation and incorporation of proline in a dose dependent manner. Furthermore, the effects of CK on expression of alpha-smooth muscle actin (a-SMA) and collagen type I were evaluated utilizing immunocytochemistry. CK reduced a-SMA and collagen type I expressions compared with acetaldehyde-induced hepatic stellate cells. These results suggested that the protective effects of CK on the hepatic fibrosis in stellate cells might, at least in part, be due to its ability to reduce the accumulation of collagen and blocked activation of hepatic stellate cells by acetaldehyde.

799 STUDY OF THE MUTAGENIC ACTIVITY OF *LUPINUS termis* ALCOHOLIC EXTRACT IN *SALMONELLA typhimurium* STRAINS.

M. R. Santiago[1], M. D. Antoun[2] and D. Herreno-Saenz[1]. [1]*Pharmacology and Toxicology, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico and* [2]*School of Pharmacy, University of Puerto Rico, San Juan, Puerto Rico.*

Higher plants that have served as source of several lead compounds for drugs are used in modern medicine. The genus Lupinus (Leguminosae) is represented by about two hundred species in the New World and a smaller in the Mediterranean region and Africa. *Lupinus termis* Forsk, also known as Tirmus or Turmuz, is widely cultivated in Sudan and has been widely used as an aqueous extract in folklore medicine due to its beneficial effects on a variety of dermatological disorders. Previous studies have shown the efficacy of the alcoholic extract of *Lupinus termis* seeds in the treatment of chronic atopic dermatitis. The study of the genotoxic potential of *Lupinus termis* alcoholic extract is important to determine if this extract is a safe natural product, which could have a potential of being developed as a new treatment for chronic atopic dermatitis in United States. The mutagenic potential of this alcoholic extract was studied using the *Salmonella typhimurium* reverse mutation assay (Ames assay). The study was performed using six *Salmonella typhimurium* strains, TA97a, TA98, TA100, TA102, TA1535 and TA1538. Three concentrations (5 μg/plate, 20 μg/plate and 100 μg/plate) of the alcoholic extract of Lupinus termis, as well as its main alkaloid, Lupanine, were studied using the plate incorporation method in the presence and absence of metabolic activation (S9). Three replicates were done for each concentration, and the assay was performed in triplicate. 2-Aminofluorene (20 μg/plate) and Benzo(a)pyrene (5 μg/plate) were used as positive controls and DMSO was used as the solvent and as the negative control. For each concentration treatment, the revertants per plate obtained in the presence and absence of metabolic activation (S9), were statistically lower compared with controls. The results obtained show that at these concentrations, the alcoholic extract of *Lupinus termis* and Lupanine, do not have any mutagenic activity in the *Salmonella typhimurium* tester strains.

800 MALDI-TOF/MS IN THE CHARACTERIZATION OF NUTRACEUTICALS.

J. R. Guthrie[1], H. A. Weber[1], D. E. Gray[1], R. K. Harris[1], A. P. Clark[1], J. W. Algaier[1] and C. S. Smith[2]. [1]*Midwest Research Institute, Kansas City, MO and* [2]*NIEHS, Research Triangle Park, NC.*

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS), traditionally used in the study of large biomolecules and polymers, was used in the chemical characterization of various nutraceuticals. Powdered kava kava extract (KKE), goldenseal root powder (GSRP), comfrey root (CR), powdered *Ginkgo biloba* extract (GbE), grape seed extract (GSE), and pine bark extract (PBE) have been selected for toxicological evaluation by NIEHS. The analysis of these herbal products, which contain a wide variety of chemical constituents, poses a number of significant analytical challenges. An overall investigative approach to MALDI-TOF/MS analysis of nutraceuticals was developed, including evaluation of different sample preparation procedures, various MALDI matrices, different matrix/sample ratios, and the use of reflector- and linear-mode TOF/MS. The developed approach was applied to the above nutraceuticals. During analysis of KKE, 20 kava-related ions were observed, all of which were $\langle$ 360 Da. Alkaloids, including berberine and hydrastine, were observed during analysis of GSRP using 2, 5-DHB and CHCA as MALDI matrices. Analysis of CR extract led to the observation of polysaccharides $\rangle$ 3, 900 Da. Ginkgolides and flavonol glycosides were observed during MALDI-TOF/MS analysis of GbE. Linear- and reflector-mode methods were developed for the analysis of GSE and PBE, which led to the identification of up to tridecamer proanthocyanidins.

801 CHEMICAL CHARACTERIZATION OF *GINKGO BILOBA* EXTRACT.

J. E. McClintock[1], H. A. Weber[1], M. D. Armstrong[1], B. M. O'Brien[1], D. Logan[1], R. A. Swaney[1], J. R. Guthrie[1], R. K. Harris[1], A. P. Clark[1], J. W. Algaier[1] and C. S. Smith[2]. [1]*Midwest Research Institute, Kansas City, MO and* [2]*NIEHS, Research Triangle Park, NC.*

Ginkgo biloba L. leaf extract, which is an extremely popular dietary supplement and is consumed to improve memory and mental alertness, has been selected for toxicological evaluation by the NIEHS. Millions of consumers use powdered *Ginkgo biloba* extract (GbE), which is standardized to contain $\backsim$ 24% flavonol glycosides (mainly based on kaempferol, quercetin, and isorhamnetin) and $\backsim$ 6% terpene lactones (ginkgolides and bilobalide). A strategy, which included HPTLC, HPLC, GC/MS, LC/MS, and MALDI-TOF/MS analyses, was developed for overall characterization of GbE. Two HPTLC methods were used to analyze GbE; one was used to identify terpene lactones and the other to identify flavonol glycosides. After GbE was hydrolyzed with HCl at 90°C for 1 hr, the resulting solutions were analyzed using gradient RPHPLC with UV and ELSD detection to determine aglycone and terpene lactone content, respectively. Quercetin dihydrate standards were used to quantitate the aglycones, while bilobalide standards were used to quantitate terpene lactones. Using UV detection, quercetin, kaempferol, and isorhamnetin glycosides were observed at 16.71%, 12.20% and 2.37%, respectively. Using ELSD detection, bilobalide was observed at 6.94%, while ginkgolides A, B, and C were observed at 3.74%, 1.62%, and 3.06%, respectively. The HPLC method was transferred to positive electrospray LC/MS, which was used to identify 18 components in nonhydrolyzed GbE and 10 components in hydrolyzed GbE. LC/MS analyses verified that GbE did not contain colchicine, a reported *Ginkgo* contaminant, and that ginkgolic acids were present at 10.45 ± 2.40 μg/g. Ten volatile components were identified during GC/MS analyses. Reflector mode MALDI-TOF/MS analyses were conducted using CHCA as the matrix and led to the identification of 22 GbE components.

802 NEUROPROTECTION BY *GINKGO BILOBA* EXTRACT EGB761.

J. T. Kern, A. D. Kraft and J. A. Johnson. *Department of Pharmaceutical Sciences, University of Wisconsin-Madison, Madison, WI.*

Oxidative stress plays an important role in cell death and has been implicated in the development of a number of neurological diseases, including Parkinsons and Alzheimers (AD). The plant extract *Ginkgo biloba* EGb761 contains a number of chemical constituents that counteract these oxidative-stress induced effects in standardized neurophysiological tests. The cellular mechanisms underlying the beneficial effects, however, remain unclear. Here, we demonstrate that ginkgo treatment induces two distinct neuroprotective pathways, including activation of the antioxidant response element (ARE) and increasing transthyretin (TTR) levels. The ARE is an enhancer element commonly found in the flanking region of a number of phase II detoxifying enzymes, including heme oxygenase I and NAD(P)H:quinone reductase I. Induction of the ARE protects cells from glutamate, dopamine, and hydrogen peroxide induced cytotoxicity. TTR is a protein involved in the transport of retinol-binding protein in the cerebrospinal fluid. *In vitro*, TTR has been shown to bind beta amyloid and prevent the formation of fibrils, a species of beta amyloid found in human AD plaques. Utilizing chemical and genetic inhibitors against phosphatidylinositol 3-kinase, as well as other known enzymes in the ARE signaling cascade, we demonstrate that the signaling events leading to ARE activation and increased TTR levels are distinct from one another. Interestingly, in an ARE-luciferase reporter assay comparing ginkgo treatment (50 micrograms/mL) to treatment of the ARE-activating antioxidant *tert*-butylhydroquinone (10 micromolar) in IMR-32 human neuroblastoma cells, we observe that ginkgo induces the ARE only one-fourth as well as tBHQ but affords similar, if not better, protection from 250 or 500 micromolar hydrogen peroxide-induced cytotoxicity. Overall, these data suggest that *Ginkgo biloba* extract EGb761 activates at least two neuroprotective pathways and paves the way for future studies into the neuroprotective role of the constituents of the extract and a detailed examination of the signaling events involved in ARE and TTR activation.

803 THE BRAIN HOMEOSTASIS IS AFFECTED BY INTRACEREBRAL BUT NOT SUBCUTANEOUS INFUSION WITH FUMONISIN B_1 IN BALB/C MICE.

M. F. Osuchowski and R. P. Sharma. *Physiology and Pharmacology, The University of Georgia, Athens, GA.*

Fumonisin B_1 (FB_1) is a mycotoxin produced by *Fusarium verticillioides*, a widespread contaminant of corn. FB_1 is a causative agent of leukoencephalomalacia, a fatal neurodegenerative condition affecting equide. This study investigated the neurotoxicity of FB_1 in a mouse model after its intracerebral or systemic administration. Female BALB/c mice (5/group) were continuously infused (0.5 $^{\mu l/hr)}$ with the total doses of 10 or 100 μg FB_1 or saline over 7 days *via* osmotic pumps implanted either subcutaneously or *via* intracerebral cannulation of the lateral ventricle in brain bypassing the blood-brain barrier. One day after the treatment mice were killed; their brains dissected fresh or after intracardiac fixation with paraformaldehyde. High performance liquid chromatography indicated increased free sphinganine in both intracerebral FB_1-treated groups in cortex, cerebellum and midbrain (also free sphingosine in the cortex after 100 $^{\mu g)}$ but not in medulla oblongata. Increase of cerebellar sphinganine in the 100 μg group was substantially greater compared to other two regions. Real-time polymerase chain reaction assessed the expression of proinflammatory cytokines in brain. Intracerebral administration of FB_1 induced expression of tumor necrosis factor $2^{\alpha,\ interleukin\text{-}1}\beta$, interleukin-6 and interferon γ in the whole brain. FluoroJade B staining detected neuronal degeneration in hippocampus of mice treated intracerebrally with high dose. The subcutaneous administration of FB_1 had no effect on the brain sphingolipid metabolism, cytokine gene expression or morphology. Results indicated the intracerebral FB_1 treatment inhibited de novo synthesis of sphingolipids, stimulated inflammatory cytokines and inflicted neuronal damage in the murine brain. Results indicate the lack of fumonisin permeability into brain is responsible for the absence of its neurotoxicity in mouse. (Partially supported by ES09403).

804 CHEMICAL CHARACTERIZATION OF POWDERED KAVA KAVA EXTRACT.

H. A. Weber[1], A. E. Hodges[1], S. M. Barnes[1], L. A. Moody[1], R. A. Swaney[1], J. R. Guthrie[1], R. K. Harris[1], A. P. Clark[1], J. W. Algaier[1] and C. S. Smith[2]. *[1]Midwest Research Institute, Kansas City, MO and [2]NIEHS, Research Triangle Park, NC.*

Powdered kava kava (*Piper methysticum*) extract (KKE) is an herbal supplement that has been selected for toxicological evaluation by the NIEHS. Kava, which is a traditional beverage of various Pacific Basin countries, has psychoactive properties and is used as a treatment for anxiety. A strategy, which included extraction, HPLC, GC/MS, LC/MS, and MALDI-TOF/MS was developed for overall characterization of KKE. KKE was extracted under ambient conditions and the extracts were analyzed using an isocratic HPLC method with UV detection at 220 nm to determine the weight percentages of six kavalactones. Samples containing between ∽ 5 and 500 mg of KKE were extracted by sonicating for 60 minutes at ambient temperature with 50 mL of methanol. All samples were diluted to a final analytical concentration of ∽ 0.1 mg KKE/mL methanol in order to obtain more reproducible results. Kavain, a major kavalactone, was used to prepare quantitation standards (∽ 3 – 393 μg/mL) to quantitate weight percentages of kavain and five other kavalactones in KKE. The extraction of methysticin, dihydromethysticin (DHM), kavain, dihydrokavain (DHK), yangonin, and desmethoxyyangonin (DMY) from KKE proved to be linear ($r \rangle 0.9999$) and precise, with RSDs $\langle$ 1.9% for 10 replicates. The HPLC method was transferred to positive electrospray LC/MS, which was used to identify 14 KKE components. Twenty KKE components were identified using GC/MS analyses. MALDI-TOF/MS analyses were conducted using three matrices, which led to the observation of 20 kava-related ions. Contaminant testing indicated that *N*-nitrosopyrrolidine was present at 30.9 ppb; all other nitrosamines were less than the detection limit of 1.0 ppb. All organophosphate and organochlorine pesticide levels, except for pentachloronitrobenzene (PCNB) which was present at 16.6 ppb, were below the detection limit. Aflatoxins, heavy metals, and microbial contaminants were all below the reported detection limits.

805 POLYSACCHARIDE ISOLATED FROM PORIA COCOS SCLEROTIUM INDUCES INOS EXPRESSION THROUGH THE ACTIVATION OF NF-KB/REL IN MURINE MACROPHAGES.

Y. J. Jeon. *Pharmacology, Chosun University, Kwangju, South Korea.* Sponsor: H. Kim.

We show that PCSC, a polysaccharide isolated from the sclerotium of Poria cocos with one percent sodium carbonate, significantly induces nitric oxide (NO) production and inducible NO synthase (iNOS) transcription through the activation of nuclear factor-kB/Rel (NF-kB/Rel). *In vivo* administration of PCSC induced NO production by peritoneal macrophages of B6C3F1 mice. PCSC also dose-dependently induced the production of NO in isolated mouse peritoneal macrophages and RAW 264.7, a murine macrophage-like cell line. Moreover, iNOS protein and mRNA transcription were strongly induced by PCSC in RAW 264.7 cells. To further investigate the mechanism responsible for the induction of iNOS gene expression, we investigated the effect of PCSC on the activation of transcription factors including NF-kB/Rel and Oct, whose binding sites were located in the promoter of iNOS gene. Treatment of RAW 264.7 cells with PCSC produced strong induction of NF-kB/Rel-dependent reporter gene expression, whereas Oct-dependent gene expression was not affected by PCSC. DNA binding activity of NF-kB/Rel was significantly induced by PCSC, and this effect was mediated through the degradation of IkB . In conclusion, we demonstrate that PCSC stimulates macrophages to express iNOS gene through the activation of NF-kB/Rel.

806 SYNERGISTIC EFFECTS OF OCHRATOXIN A AND FUMONISIN B1 IN C6 GLIOMA CELLS: CYTOTOXICITY AND DNA SYNTHESIS INHIBITION.

E. E. CREPPY[1], M. CARRATU[2], P. CHIARAPPA[2], I. BAUDRIMONT[1], P. BORRACCI[2], T. A. MOBIO[1] and S. M. MOUKHA[1]. *[1]Toxicology, University Bordeaux 2, Bordeaux, France and [2]Pharmacology and Human Physiology, University of Bari, Medical School, Bari, Italy.*

Ochratoxin A (OTA) and Fumonisin B1 (FB1) are mycotoxins detected as contaminants in animal feed and food. These mycotoxins are produced by Aspergillus and/or Penicillium and by Fusarium species respectively. *In vitro* studies, so far available, focusing on the effects of each toxin, show that both are cytotoxic and inhibit the synthesis of cellular macromolecules 1-2. Since multi-mycotoxins contamination of feed and foodstuffs occurs more frequently than a single mycotoxin contamination, the aim of the present study was to investigate possible synergistic or antagonistic effects of these two mycotoxins on both cell *via*bility and DNA synthesis in the rat glioma cells, C6. Cell *via*bility and DNA synthesis were studied by the methods previously described 1-2. The results show that OTA inhibits cell *via*bility (IC50 ~18 microM, for 48h incubation) and DNA synthesis (IC50 ~8 microM, for 24h incubation) in C6 glioma cells. The combination of OTA with different concentrations of FB1 significantly enhances OTA-induced inhibition of both cell *via*bility and DNA synthesis. DNA synthesis inhibition was increased from <5% for 2.5 microM of FB1 to >50% when combined to 5 microM OTA while this OTA concentration induces solely less than 20% of inhibition. The present findings underline that multi-mycotoxins contamination must be carefully considered when assessing the risk for humans exposed to mycotoxins. References [1] Creppy EE, Baudrimont I, Betbeder AM, How aspartame prevents the toxicity of

ochratoxin A. J. Toxicol. Sciences. 23, 165-172, 1998. [2] Mobio TA, Anane R, Baudrimont I, Carratu MR, Shier TW, Dano SD, Ueno Y, Creppy EE., Epigenetic properties of fumonisin B1: cell cycle arrest and DNA base modification in C6 glioma cells. Toxicol. Appl. Pharmacology 164, 91-96, 2000

807 TOXIC BREVETOXIN COMPLEXES ARE IN AQUEOUS SOLUTIONS.

A. J. bourdelais and D. G. Baden. *Center for Marine Science, University of North Carolina at Wilmington, Wilmington, NC.*

Florida red tides are caused by blooms of the marine dinoflagellate *Karenia brevis* which produce brevetoxins. These toxins are associated with massive fish kills, marine mammal poisoning and human health problems. There are 9 natural brevetoxins with two different polyether backbones. This study presents the structure and characterization of three new compounds (AJB29.0psp, AJB29.5psp and AJB31.5psp) produced by *K. brevis*. These compounds are believed to be complexes of brevetoxins and are more toxic to fish. The brevetoxin-complexes were purified from cultures of *K. brevis*. Structure elucidation was perfrormed using LC-Mass Spectrometry and NMR. 1-D and 2-D NMR experiments were run. Fish bioassays using mosquito fish were run as follows: fish were placed in 100 ml beakers containing 20 ml tank water. The fish received one of the following: 200 ml of ethanol, 20 µg PbTx-2 in 200 ml ethanol or 20 µg of one of the new complexes in 200 ml ethanol. Fish were then observed until death. NMR spectra suggest a polyether ladder structure for all three compounds. The AJB29.0psp 1H-spectrum contains an aldehyde moiety . The AJB29.5psp 1H-spectrum contains no aldehyde or exomethylene and the LC-MS showed masses of 866, 868, 892, 894, 898 and 910. The AJB31.5psp NMR spectrum shows and aldehyde and exomethylene with 50 carbons, LC-MS data showed masses of 876, 892, 894, and 910. The easiest explanation for the brevetoxin-complexes is that they are mixtures. However, after the NMR runs in CDCl3 the complexes were dried and rerun on the HPLC. The resulting chromatograph indicated several different peaks. When collected separately and rerun on the HPLC these peaks eluted as individual peaks which eluted at retention times for known brevetoxins. However, if the peaks were collected together, dried and rerun on the HPLC only one peak would appear. These results suggest that the new compounds are complexes in aqueous solutions and dissociate in non-aqueous solutions. This work was supported by NIEHS grants ES05853 and ES10594

808 THE EFFECTS OF (-)-HYDROXYCITRIC ACID EXTRACT AND FLUOXETINE ON RAT BRAIN CORTEX NEUROTRANSMITTERS AFTER 30, 60 AND 90 DAYS TREATMENT.

M. Shara[1], S. J. Stohs[1], D. Bagchi[1], M. Bagchi[3] and S. Ohia[2]. *[1]Pharmacy Science, Creighton University, Omaha, NE, [2]Pharmacology, University of Houston, Houston, TX and [3]InterHealth USA, Benicia, CA.*

This study examined the effects of a novel (-)-hydroxycitric acid (HCA) extract and fluoxetine on rat brain cortex neurotransmitters. Previous studies have demonstrated that HCA use is associated with significant decreases in food consumption and body weight in animals and humans. The effects of HCA on brain cortex serotonin (5-HT), its metabolite 5-hydroxyindoleacetic acid (HIAA), dopamine (DA) and its metabolites 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) have not been examined. Methods: HCA was administered as 0.2, 2 and 5% of the diet and 15 mg/kg/day of fluoxetine was administered orally. The 5% HCA dose is 25 fold the recommended dose. Animals were killed after 30, 60 and 90 days. Brain cortices were analyzed for 5-HT, HIAA, DA, DOPAC and HVA content utilizing reverse phase HPLC with electrochemical detection. Results: HCA (0.2, 2, and 5%) increased cortex 5-HT by 11, 9, and 12% after 90 days ($p < 0.05$). In contrast, fluoxetine treatment decreased cortex 5-HT by 9, 7 and 8% on days 30, 60 and 90, respectively ($p < 0.05$). Fluoxetine also decreased cortex HIAA by 19, 15 and 17% ($p<0.05$) 30, 60 and 90 days post-treatment, respectively. No changes in HIAA were observed in any of the HCA treatment groups. After 90 days, HCA (0.2, 2 and 5%) increased cortex DA by 10, 15 and 18 % ($p < 0.05$) and increased DOPAC by 23, 26 and 29% ($p<0.05$), respectively. In contrast, fluoxetine decreased DA by 18, 15 and 19% ($p<0.05$), respectively. No changes in cortex DOPAC were observed in all fluoxetine groups. On day 90, HCA (0.2, 2 and 5%) increased cortex HVA by 12, 15 and 13% ($p<0.05$), respectively. Fluoxetine decreased cortex HVA by 17, 13 and 14% ($p < 0.05$), respectively. Conclusions: The results for fluoxetine agree with previous reports. The changes in 5-HT, DA and their metabolites due to HCA have not been previously reported. The changes in these neurotransmitters may play a role in the weight loss and appetite suppressing effects of HCA.

809 EFFECTS OF KAVA KAVA EXTRACT ON HEPATIC PHASE I AND PHASE II DRUG METABOLIZING ENZYMES.

A. B. Cadwallader and M. R. Franklin. *Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.*

South Pacific Islanders have long utilized aqueous extracts of kava kava (Piper methysticum) for relaxation and feelings of satisfaction and until recent suspicions of hepatotoxity, its use has been on the upswing in other populations. Kava kava has been shown to both inhibit and induce P450 suggesting the potential for drug interactions with OTC or prescription medicines. Using the rat as an experimental model, we investigated the effects of *in vivo* administration of kava kava extract on hepatic Phase II in addition to Phase I drug metabolizing enzymes. Both enzyme activity and mRNA levels were monitored, and the effects were compared with two known inducing agents, β-naphthoflavone (βNF) and dexamethasone. Following kava kava treatment, increases were seen in the mRNA levels of CYP2B and CYP3A and no changes were seen in CYP1A2. Compared to dexamethasone effects, the increase in the cytochrome P450 concentration and CYP3A mRNA was smaller and the increase in CYP2B mRNA was greater. Increases in UDP-glucuronosyltransferases (UGT), glutathione S-transferases, quinone oxidoreductase, and microsomal epoxide hydrolase were also seen. Microsomal p-nitrophenol glucuronidation activity and UGT1A6 and UGT2B1 mRNAs were all increased following kava kava treatment but UGT1A1 mRNA was not. The UGT2B1 increase was similar to that elicited by dexamethasone treatment and the UGT1A6 increase was less than elicited by βNF. The increases in glutathione S-transferase (CDNB) activity and GSTA2 mRNA and quinone oxidoreductase activity and its mRNA, were not as large as elicited by βNF treatment. The results indicate that the effects of kava kava on drug metabolizing enzymes extend beyond P450 and include many of the Phase II enzymes.

810 KAVA EXTRACT MODULATES EXPRESSION OF DRUG-METABOLIZING ENZYMES AND TRANSPORTERS IN MICE AND HEPA-1 CELLS.

M. Z. Dieter, J. M. Maher, A. L. Slitt and C. D. Klaassen. *University of Kansas Medical Center, Kansas City, KS.*

Severe hepatic toxicity in humans has been associated with consumption of dietary supplements containing Piper methysticum (kava), prompting a consumer advisory by the USFDA. To explore the possibility that kava may alter the expression of genes involved in drug metabolism and transport, mRNA levels of several genes were examined in male mice treated with kava extract. A single 300 mg/kg intraperitoneal dose of kava extract (standardized to 30 mg kavalactones/ml) caused no significant alterations in liver mRNA levels of any of the genes examined. In male mice treated for 1 week with an oral dose of 300 mg/kg kava twice a day, significant increases in liver NQO1 (2.1 fold), MRP3 (2.3 fold), and MRP4 (4 fold) mRNA were observed, whereas CYP1A1/2, CYP3A11, MRP2, UGT1A6, and OATP2 were unchanged. Oral gavage of kava also increased CYP1A1/2 mRNA expression in mouse duodenum more than 15 fold, with minor changes in other genes. In mouse hepatoma (Hepa-1) cells, kava extract caused concentration-dependent increases in mRNA levels of NQO1, CYP1A1/2, MRP3, and MRP4, with maximal inductions of 7.6, 26, 2.4, and 4.3 fold, respectively, at 90 ng/ml for 12 hours. These results indicate that repeated administration of kava can cause significant increases in gene expression in both liver and duodenum of mice. The more marked induction of CYP1A1/2 in duodenum compared to liver suggests that metabolism of kavalactones may occur in intestinal mucosa. The gene expression changes observed in mice and cells suggest that a kava component could mediate its effects through activation of the aromatic hydrocarbon receptor- and/or Nrf2- responsive drug metabolism pathways. Changes in expression of drug-metabolizing enzymes and transporters could result in changes in metabolism and disposition in humans regularly consuming kava-containing supplements, possibly contributing to drug-drug interactions and/or hepatotoxicty. (Supported by NIH grants ES-09716 and ES-07079)

811 IMMUNOMODULATORY EFFECTS OF NUTRACEUTICALS ON MOUSE PHAGOCYTIC CELLS.

S. Navarro[2, 1], A. Rhule[1], J. M. Wilham[2, 1], B. Seaver[1], J. R. Smith[1] and D. M. Shepherd[2, 1]. *[1]Department of Biomedical & Pharmaceutical Sciences, University of Montana, Missoula, MT and [2]Center for Environmental Health Sciences, University of Montana, Missoula, MT.*

Herbals have been used by many ancient civilizations to treat illness and boost general well-being. Furthermore, phytomedicines have been reported to enhance the function of the immune system. However, information to support the efficacy and/or toxicity of herbal extracts is severely lacking. To address this deficiency, we have evaluated the effects of a select group of nutraceuticals on the activity of cul-

tured dendritic cells (DC2.4 cells) and macrophages (RAW264.7 cells). Based on the selective action of a panel of herbals on the enzymatic activity of COX-2, but not COX-1, we chose to evaluate the immunomodulatory effects of andrographis, amentoflavone, notoginseng, ostivone, thunder god vine and white willow bark. In a concentration-dependent manner, notoginseng and thunder god vine suppressed the LPS-induced production of TNF-alpha by DC2.4 and RAW264.7 cells. Conversely, white willow bark and andrographis increased TNF-alpha production by the dendritic cells, and ostivone increased the production of this pro-inflammatory cytokine by the macrophages. The LPS-stimulated induction of both COX-2 and IL-1B mRNA in RAW264.7 cells was inhibited by thunder god vine, amentoflavone, and notoginseng. Delayed exposure of DC2.4 cells to notoginseng failed to suppress LPS-induced TNF-alpha production whereas exposure at or before LPS activation resulted in significant decreases. Similar effects were observed following the LPS activation of macrophages that had been treated with thunder god vine. These results demonstrate that herbal extracts can modulate the inflammatory responsiveness of cultured immune cells and suggest that these phytochemicals may be useful for the treatment of inflammatory diseases. Future investigations will assess the potential immunomodulatory effects of these nutraceuticals on *ex vivo* immune cells and *in vivo* models of inflammatory disease.

812 HISTOPATHOLOGICAL CHANGES OCCURRING IN THE ORGANS OF RATS FOLLOWING SUBCHRONIC TOXICITY OF ZEARALENONE.

R. M. kadry, M. Abdel-Wahab and N. A. El-Danaf. *Animal Reproduction Research Institute, ARC, Giza, Egypt.* Sponsor: A. Kadry.

Contamination of the food supply by drugs, synthetic chemical and naturally occurring toxins, poses a threat to public health. Although toxicosis resulting from ingestion of residue-contaminated food products is a relatively rare occurrence, the long-term health effects from repeated exposure to low concentrations of certain chemicals may be of much greater concern. Zearalenone is a mycotoxin that exhibits strong estrogenic properties when fed to domestic animals. When present in sufficient quantities in the ruminant diet, this toxin may cause infertility, vaginal prolapse, enlargement of the uterus and mammary glands and could lead to abortion. Anabolic agents such as zearalenone are suspected to cause morphologic tissue response. In the current study, Zearalenone was orally administered daily to SD rats at three dose levels 0, 1 and 10 mg/kg B.W. for 14 days. Gross signs of toxicity or mortality were recorded five days/ week. Following the conclusion of the exposure period, the liver, kidney, lung, uterus and ovaries were examined for tissue distribution and for histopathological changes. Tissues slides from all animals were prepared by routine procedure and stained with hematoxylin and eosin. High dose exposure to Zearalenone caused hydropic degeneration, sinusoidal congestion and lymphocyte infiltration in liver. Marked cloudy swilling and hydropic degeneration were observed in the kidney tissues. Focal aggregation of lymphocytes and macrophages were observed in lung tissues. Examination of the heart showed subendocardial infiltration with lymphocytes and macrophages. These data suggest that subchronic exposure results in deleterious effects from zearalenone toxin.

813 PROTECTION BY METALLOTHIONEIN INDUCTION FROM CYTOTOXICITY INDUCED BY HIGH LEVELS OF GLUCOSE AND TRIGLYCERIDE.

J. Wang, Y. Jiang, Y. Kang and L. Cai. *Medicine, University of Louisville, Louisville, KY.*

Previous studies have shown that the cardiac damage caused by diabetes was significantly inhibited in a cardiac-specific metallothionein (MT)-overexpressing transgenic mouse model. The present study was undertaken to investigate the determinant factor of MT protection; MT itself versus the metal species bound to MT. Cardiac myoblast cell line (H9C2) was treated with high levels of glucose and triglyceride (Tg) to mimic the model of diabetes *in vitro*. Before incubated with 50 μg/ml of Tg and 22 mM of glucose in culture media for 60 h, the cell line was pretreated with 50 μM of $ZnSO_4$ or 1.0 μM of $CdCl_2$ for 24 hours. The cell *via*bility was measured by a colorimetric MTT. Compared with controls in the presence of 5.5 mM of glucose, cells without Zn-pretreatment exposed to both high levels of glucose and Tg survived only 42%, but cells with Zn-pretreatment survived 70% ($P<0.01$). The survival rate of Cd-pretreated cells (80%) was also significantly higher than that of cells without Cd-pretreatment (46%) ($p<0.01$). Western blot revealed that both Zn and Cd pretreated groups had a significant high level of MT. We concluded that MT induction, regardless of the metal species, provides protection from myocardial injury by high levels of glucose and Tg. (Supported in part by American Diabetes Association, Philip Morris USA Inc. and Jewish Hospital Research Foundation, NIH grant 59225).

814 DIABETES-INDUCED CARDIAC MT SYNTHESIS: INDEPENDENT OF METAL ALTERATIONS OR OXIDATIVE DAMAGE, BUT LIKELY RELEVANT TO INFLAMMATORY FACTORS.

L. Cai[1], Y. Song[1], L. Zhang[1], Y. Jiang[1], J. T. Saari[2] and Y. Kang[1]. *[1]Medicine, University of Louisville, Louisville, KY and [2]Grand Forks Human Nutrition Research Center, Grand Forks, ND.*

Diabetic complications are associated with oxidative stress. We have shown that both hepatic and renal MT expression increased in the diabetic rats, independent of metal alterations, suggesting that MT induction in different organs is a diabetes-related response. In this study, we investigated cardiac MT induction in streptozotocin (STZ)-induced diabetic mice. We found a significant increase in cardiac MT content without significant changes of cardiac metals including zinc, copper, iron, calcium and magnesium at 2 months after STZ-treatment. We also found that the cardiac MT induction did not resulted from cardiac oxidative damage (lipid peroxidation measured by TBARS), but was correlated with an increase in systemic inflammation such as marked increases in serum endotoxin and TNF-α levels. This study suggests that diabetes-induced MT in the heart is not related to metal alterations, but may be a systemic response to diabetic changes such as hyperglycemia, hyperlipidemia and systemic inflammation. (Supported in part by American Diabetes Association, Philip Morris USA, Inc., Jewish Hospital Research Foundation and NIH grant 59225).

815 PREVENTION OF DIABETIC CARDIOMYOPATHY BY ZINC SUPPLEMENTATION CORRELATES WITH METALLOTHIONEIN INDUCTION.

Y. Song, J. Wilkerson, Y. Kang and L. Cai. *Medicine, University of Louisville, Louisville, KY.*

Using a cardiac-specific metallothionein (MT)-overexpressing transgenic mouse model, we have demonstrated that MT significantly prevented cardiomyopathy in diabetic mice. The present study was undertaken to determine whether zinc can induce MT production in the heart to an efficient level for pretection against diabetic cardiomyopathy in C57BL/6J mice. Diabetic mice, induced by streptozotocin (STZ), were treated intraperitoneally with zinc sulfate (5mg/kg) 3 times a week over a period of 3 months. At 3 month after the last injection, cardiac function was examined and myocardial tissue was collected for morphological study along with serum cardiac enzyme analysis. Diabetic mice exhibited cardiomyopathy characterized by significant increases in serum creatine kinase, morphological abnormalities, and reduced contractility. These changes were significantly inhibited by zinc-treatment. Immunohistological staining showed an increased expression of cardiac MT in zinc-treated mice. These results suggest that zinc supplementation significantly suppresses diabetic cardiomyopathy, probably through MT induction. (Supported in part by American Diabetes Association, Philip Morris USA, Inc., Jewish Hospital Research Foundation and NIH Grant 59225).

816 ALCOHOL-INDUCED MYOCARDIAL FIBROSIS IN A METALLOTHIONEIN-NULL MOUSE MODEL.

L. Wang, Z. Zhou and Y. Kang. *Medicine, University of Louisville School of Medicine, Louisville, KY.*

Alcohol-induced cardiomyopathy is both exposure time- and dose-dependent and myocardial fibrosis has been found in alcoholic patients but seldom reported in animal models. The present study was undertaken (1) to develop a mouse model resembling clinical observation of alcohol-induced myocardial fibrosis and (2) to define the role of zinc depletion in the pathogenesis. Because metallothionein (MT) may participate in the regulation of cellular zinc homeostasis, MT-knockout (MT-KO) mice along with their wild-type (WT) 129/Sv controls were used and fed alcohol-containing Lieber-DeCarli liquid diet following a 2 X 2 factorial design: Ethanol versus zinc. An improved alcohol feeding protocol was established as follows: 1) The alcohol content (%, w/v) in the diet started from 3.2, with an increment of 0.2 every week, reached 5.0 at the end, and 2) an one-day-stop of alcohol feeding on the last day of each week was introduced. Zinc was supplemented at a level of 100 mg zinc ion/l. Both WT and MT-KO mice fed ad libitum for 10 weeks showed significant cardiac injury, as indicated by increased serum creatine phosphokinase activity, myocardial histopathological and ultrastructural alterations, and disturbance in signaling pathways. However, a severe and massive collagen deposition was observed only in the MT-KO myocardium along with increased ventricular volume. Zinc supplementation significantly attenuated alcohol-induced myocardial injury in both WT and MT-KO mice and inhibited alcohol-induced fibrosis in the MT-KO myocardium. These results thus demonstrate that (1) alcohol-induced myocardial fibrosis can be produced in the MT-KO mouse model, providing a valuable tool for understanding alcoholic cardiomyopathy, and (2) zinc is an important modulator in alcoholic myocardial pathogenesis. (Supported in part by NIH grants AA13601 and HL59225).

817 DOSE AND TIME RESPONSE OF ALCOHOL-INDUCED HYPERTENSION AND CARDIOVASCULAR INJURIES IN RATS.

K. Husain. *Pharmacology and Toxicology, Ponce School of Medicine, Ponce, Puerto Rico.*

Alcoholic beverages (ethanol) are consumed by most human societies regularly or at least occasionally. Low dose of ethanol ingestion has been shown to be beneficial but chronic high dose of ethanol is harmful to the cardiovascular system. Therefore, the aim of this study was to investigate the dose and time response of alcohol-induced hypertension and associated cardiovascular injuries in a rat model. Male Fisher 344 rats were divided into different groups of 24 rats each and treated as follows: 1) Control (5% sucrose, p.o.) daily for 12 weeks), 2) Ethanol (1g/kg, p.o.) daily for 12 weeks, 3) Ethanol (2g/kg, p.o.) for 12 weeks, 4) Ethanol (4g/kg, p.o.) for 12 weeks, 5) Ethanol (6g/kg, p.o.) for 12 weeks. The blood pressure (BP) was recorded weekly by tail-cuff method and rats were sacrificed at 6, 8, 10 and 12 weeks post-treatment. The heart and thoracic aorta were isolated and analyzed. Ethanol at a dose of 1 g/kg did not alter BP, nitric oxide (NO), glutathione (GSH), antioxidant enzymes: superoxide dismutase SOD), catalase (CAT), and glutathione peroxidase (GSH-Px); lipid peroxidation (malondialdehyde and 8-isoprostane levels) and protein oxidation (carbonyl contents) in the heart and aorta of rats at 6, 8, 10 and 12 weeks post-treatment compared to control. Ethanol at a dose of 2g/kg caused mild hypertension (BP=140-145 mm of Hg), depletion of cardiovascular NO, GSH and antioxidant enzymes (SOD, CAT, and GSH-Px) as well as augmentation of lipid peroxidation and protein oxidation at 8, 10 and 12 weeks post-treatment. Higher doses of ethanol (4 and 6 g/kg) significantly elevated BP (145-155 mm of Hg), depleted cardiovascular NO, GSH and antioxidant enzymes (SOD, CAT, and GSH-Px) and enhanced lipid peroxidation and protein oxidation at 8, 10 and 12 weeks post-treatment. The data suggest that alcohol-induced hypertension is related to the down regulation of NO and antioxidant systems and oxidative injuries to the cardiac and vascular tissues of rats in both dose and time dependent manner (supported in part by NIH grant #2 G12 RR03050-19).

818 CHEMICAL INDUCTION OF ENDOGENOUS ANTIOXIDANTS AFFORDS PROTECTION AGAINST OXIDATIVE AND ELECTROPHILIC INJURY IN CARDIOVASCULAR CELLS.

Z. Cao, M. Tsang and Y. Li. *Pharmaceutical Sciences, St.John's University, Jamaica, NY.*

Reactive oxygen species (ROS) and electrophiles are critically involved in the pathogenesis of various forms of cardiovascular diseases, including atherosclerosis, ischemia-reprfusion injury, and drug-induced cardiomyopathy. Accumulating evidence suggests that exogenous antioxidative compounds provide protection against oxidative cardiovascular injury. However, whether induction of endogenous antioxidants by pharmacological agents can also exert protective effects on ROS- and electrophile-mediated cardiovascular injury has not been carefully investigated. In this study, using rat aortic smooth muscle A10 cells and rat cardiomyocyte H9C2 cells as the *in vitro* systems, we have characterized the induction of cellular antioxidants by the chemical inducers, 3H-1, 2-dithiole-3-thione (D3T), resveratorl, and alpha-lipoic acid (LA), and determined the cytoprotection of the chemically-elevated endogenous antioxidative defenses against oxidative and electrophilic cardiovascular cell injury. Incubation of cardiovascular cells with the above chemical inducers results in a remarkable induction of a battery of cellular antioxidants, including reduced glutathione, glutathione reductase, glutathione S-transferase, NAD(P)H:quinone oxidoresuctase 1, catalase, and/or superoxide dismutase. Moreover, pretreatment of cardiovascular cells with these chemical inducers leads to a marked protection against the cytotoxicity elicited by various oxidative and electrophilic insults, including xanthine oxidase-derived ROS, peroxynitrite, acrolein, 4-hydroxy-2-nonenal, and doxorubicin. Taken together, this study demonstrates for the first time that a scope of endogenous antioxidants in cardiovascular cells can be induced by exposure to the chemical inducers, D3T, resveratrol and LA, and such chemically-induced cellular antioxidative defenses are accompanied by a significantly increased resistance to oxidative and electrophilic cardiovascular cell injury. The results of this study may have important implications for chemical protection of oxidative cardiovascular disorders.

819 CHEMICAL INDUCTION OF ENDOGENOUS ANTIOXIDANTS IN MOUSE CARDIAC TISSUE: IMPLICATIONS FOR CARDIOPROTECTION.

Y. Li and Z. Cao. *Pharmaceutical Sciences, St. John, Jamaica, NY.*

The critical involvement of reactive oxygen in cardiac disorders has led to extensive investigation on the protective effects of exogenous antioxidants on oxidative cardiac injury. On the other hand, another strategy for protecting against oxidative cardiac injury may be through induction of endogenous antioxidants in myocardium. However, our understanding of the chemical inducibility of endogenous cardiac antioxidants *in vivo* is limited. In addition, studies on the basal levels of a scope of antioxidants in myocardium as compared with other tissues, such as liver are lacking. Accordingly, this study was undertaken to determine the basal levels of endogenous antioxidants, including superoxide dismutase (SOD), catalase, glutathione (GSH), GSH peroxidase (GPx), glutathione reductase (GR), GSH S-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1), and investigate the inducibility of endogenous antioxidants by 3H-1, 2-dithiole-3-thione (D3T) and resveratrol in mouse heart as well as liver. Our results demonstrated that the levels of catalase, GSH, GPx, GR and GST were significantly lower in cardiac tissue than in hepatic tissue. Total SOD activity did not differ between mouse heart and liver. Notably, heart contained a much higher NQO1 activity than liver. Imuunobloting and RT-PCR analyses further confirmed the high expression of NQO1 protein and mRNA in the heart. Oral administration of D3T resulted in a significant induction of cardiac SOD, catalase, GR, GST and NQO1. Only GR, GST and NQO1 in the liver were induced by the D3T treatment. Oral administration of resveratrol also caused a significant induction of catalase and NQO1 in mouse cardiac and hepatic tissues. Taken together, this study demonstrates that expression of NQO1 is remarkably high in the heart though other cardiac antioxidants are relatively lower as compared with liver, and a number of antioxidants in mouse heart can be induced by D3T and/or resveratrol. This study provides a basis for future investigation of the cardioprotection of chemically-induced antioxidants in myocardium in animal models of oxidative cardiac disorders.

820 INCREASED FORMATION OF MITOCHONDRIAL REACTIVE OXYGEN SPECIES CAUSES MITOCHONDRIA PERMEABILITY TRANSITION-DEPENDENT KILLING OF CULTURED ADULT RAT MYOCYTES AFTER ISCHEMIA/REPERFUSION.

J. Kim, Y. Jin and J. J. Lemasters. *UNC-Chapel Hill, Chapel Hill, NC.*

BACKGROUND: Opening of high conductance permeability transition pores in the mitochondrial inner membrane is a causative event precipitating ischemic damage to heart and other tissues upon reperfusion. Cellular factors contributing to onset of the mitochondrial permeability transition (MPT) in myocardium are unknown. The **AIM** of this study was to characterize the role of mitochondrial reactive oxygen species (ROS) in the MPT after ischemia/reperfusion of myocytes. **METHODS:** Cultured adult rat ventricular myocytes were incubated in anoxic Krebs-Ringer-HEPES buffer at pH 6.2 for 3 h to simulate tissue ischemia. Reperfusion was then simulated by reoxygenating cells at 7.4 for 2 h. In some experiments, myocytes were treated with 1 μM cyclosporin A (CsA), a MPT blocker, and antioxidants including 0.5 mM desferal, 1 μM diphenylphenylene diamine (DPPD), and 5 mM 2-mercaptopropionyl glycine (2-MPG), beginning 20 min prior to reperfusion. Cell killing was assessed by propidium iodide fluorometry. To monitor formation of ROS, cells were labeled with 10 μM chloromethyl dichlorodihydrofluorescein (cmH_2DCF) diacetate for 30 min at 37 °C before reperfusion. cmDCF fluorescence was also assessed by a multiwell fluorometry. Confocal images of green fluorescence (cmDCF) were collected with a Zeiss LSM 510 microscope. **RESULTS:** Cell killing increased to 74.5% after 2 h of reoxygenation at pH 7.4, which was blocked by CsA, desferal, and 2-MPG. cmDCF fluorometry showed 1.7-fold increase in ROS formation after 30 min of reperfusion, compared to normoxia. All three antioxidants blocked the reperfusion-induced increase in ROS formation, whereas CsA did not. Confocal microscopy revealed that reperfusion caused hypercontracture and subsequent cell death after a substantial increase in ROS formation. CsA did not block increased ROS generation, but prevented hypercontracture and cell death. **CONCLUSION:** Mitochondrial ROS generation is a key event promoting the MPT and subsequent cell killing after reperfusion.

821 OPENING OF MITOCHONDRIAL PERMEABILITY TRANSITION PORES PRIOR TO REPERFUSION DURING PROLONGED ISCHEMIA TO CARDIAC MYOCYTES.

J. R. Blattner and J. J. Lemasters. *Curriculum in Toxicology and Department of Cell and Developmental Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Opening of non-specific high conductance pores in the mitochondrial inner membrane causes the mitochondrial permeability transition (MPT). Cyclosporin A (CsA) blocks MPT pores in their regulated state but not in their unregulated state. In ischemia/reperfusion injury, controversy exists whether PT pores open during ischemia or only during reperfusion. Here, our aim was to assess regulated and unregulated pore opening during I/R stress to neonatal rat cardiomyocytes. Myocytes were exposed to 2 or 3 h of anoxia at pH 6.2 in Krebs-Ringers-HEPES buffer con-

taining 5 mM 2-deoxyglucose to simulate ischemia. Subsequently, myocytes were re-oxygenated with minimal essential media at pH 7.4 to simulate reperfusion. Cell killing determined by propidium iodide fluorimetry did not increase during either 2 or 3 h of ischemia, but progressively increased to 48 ± 2% and 75 ± 1%, respectively, during 2 h of reperfusion. After 2 h of ischemia, the non-immunosuppressive analog of CsA, NIM811 (5 μM), added 15 min prior to and during reperfusion suppressed cell killing to 26 ± 2%. NIM811 added at reperfusion did not protect after 3 h of ischemia (73 ± 2%). Protection did occur after 3 h ischemia when NIM811 or the unregulated pore inhibitor, 2-aminoethoxydiphenyl borate (2-APB), was added from the beginning of ischemia (47 ± 6% and 43 ± 4%). Confocal microscopy of MitoTracker Red (500 nM)-labeled myocytes revealed mitochondrial swelling during ischemia. Moreover, mitochondrial repolarization after reperfusion assessed by tetramethylrhodamine methyl ester (200 nM) became increasing less frequent as the time of ischemia increased. These results suggest that MPT pore opening may occur before reperfusion after longer periods of ischemia, possibly developing to an unregulated state causing irreversible mitochondrial injury.

822 INDUCTION OF P21$^{WAF1/CIP1/SDI1}$ BY DOXORUBICIN IN CARDIOMYOCYTES: FUNCTIONAL IMPLICATIONS OF P21$^{WAF1/CIP1/SDI1}$ IN POSTMITOTIC CELLS.

J. Terrand[1], S. K. Williams[2] and Q. M. Chen[1]. *[1]Pharmacology, University of Arizona, Tucson, AZ and [2]Biomedical Engineering, University of Arizona, Tucson, AZ.*

The p21$^{WAF1/Cip1/Sdi1}$ (p21) protein is known as a cyclin dependent kinase inhibitor. Chemical and physical stresses can induce the expression of the p21 gene in proliferating cells as well as in non-proliferating cells. The biological function of p21 induction in non-proliferating cells remains unclear. Cardiomyocytes cease proliferation in most species of mammals after birth. During the process of heart failure, cardiomyocytes often develop hypertrophy to compensate for the loss of cardiomyocytes. When treated with doxorubicin, a cancer chemotherapy drug known to induce dilated cardiomyopathy, cardiomyocytes show an elevation of p21 protein. Time course studies indicate that the elevation of p21 is delayed but sustained, following the expression of the hypertrophy biomarker Atrial Natriuretic Factor. Hypertrophy inducers angiotensin II (50-200 nM), phenylephrine (100-200 nM) and endothelin-1 (100-200 nM) can also induce the expression of p21, although the induction is transient by angiotensin II and is less pronounced with all the hypertrophy inducers as compared to doxorubicin. While the mechanism of p21 induction by hypertrophy inducers is under investigation, we have used p21 gene knockout (p21KO) mice to study the function of p21 in heart failure induced by doxorubicin. Doxorubicin treated wild type mice showed a reduction of cardiac contractility and an increase in the ratio of heart weight over body weight. In contrast, the p21KO mice showed little alterations in cardiac contractility and in heart-to-body weight ratios with doxorubicin administration. These data suggest the possibility that the p21 gene exhibits biological functions irrelevant to cell cycle regulation.

823 BOTH SPHINGANINE AND SPHINGOSINE ARE CYTOTOXIC TO H9C2[2-1] CARDIOMYOCYTES AND HEPG2 HEPATOCYTES.

S. V. Hsiao[1], P. D. Constable[2], M. Tumbleson[3] and W. M. Haschek[1]. *[1]Veterinary Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, [2]Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana, IL and [3]Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL.*

Cardiotoxicity and cell death induced by doxorubicin and TNF-α have been linked to increased concentrations of sphingolipid molecules such as ceramide and sphingosine. In fumonisin B_1 toxicity, sphinganine (another sphingoid base in *de novo* sphingolipid synthesis) concentration increases in parallel with sphingosine. However, the role of sphinganine in such cytotoxic events remains poorly understood. Here we compared the cytotoxic effects of exogenous sphinganine and sphingosine in rat embryonic cardiomyocytes (H9C2[2-1]) and hepatocellular carcinoma cells (HepG2), using the MTT assay. Sphinganine was a more potent cytotoxic agent than sphingosine for both H9C2[2-1] and HepG2 cells. The ED_{50} in H9C2[2-1] and HepG2 cells for sphinganine was 35.2 and 45.1 μM and for sphingosine was 50.3 and 64.4 μM, respectively. Cytotoxicity was detectable at 1 hour after exposure with a plateau after 12 hours. In addition, cells in suspension were more susceptible to sphinganine and sphingosine than cells in adherent monolayers. The ED_{50} in suspended H9C2[2-1] and HepG2 cells for sphinganine was 24.8 and 11.9 μM, and for sphingosine was 37.5 and 26.5 μM, respectively. These findings suggest a possible role for both sphinganine and sphingosine in doxorubicin, fumonisin B_1 and TNF-α-induced cardiotoxicity and cell death. Additionally, cell adhesion may be one of several targets for the cytotoxic effects of sphinganine and sphingosine. Supported by Society of Toxicologic Pathology/Abbott Laboratories Clinical Research Award and C-FAR (02E-082-4VR).

824 MITOCHONDRIAL AND CARDIOVASCULAR PATHOLOGY IN MICE EXPOSED TRANSPLACENTALLY TO ZIDOVUDINE (AZT) ALONE OR WITH LAMUVIDINE (3TC).

V. E. Walker[1], D. M. Walker[1], M. J. Campen[1], R. L. Divi[2], O. A. Olivero[2] and M. C. Poirier[2]. *[1]Lovelace Respiratory Research Institute, Albuquerque, NM and [2]NCI, NIH, Bethesda, MD.*

Antiretroviral drug combinations including nucleoside analogues are remarkably effective in preventing vertical transmission of HIV-1, but accumulating evidence suggests long-term risks for mitochondrial dysfunction in children exposed *in utero*. Our research team is investigating the temporal relationships between mitochondrial damage in mice exposed *in utero* to AZT or AZT-3TC and the onset of cardiomyopathy. During transplacental cancer bioassays of AZT in B6C3F1 mice and F344 rats (0, 80, 240, or 480 mg AZT/kg bw/day by gavage to dams during the last 7 days of gestation), non-cancerous lesions included a significant dose-dependent cardiac enlargement that was most pronounced in AZT-exposed female mice (when, at 2 years-of-age, heart weight was normalized to body weight and compared to control animals). Studies are in progress to determine the nature and extent of AZT- and AZT-3TC-induced mitochondrial damage in transplacentally exposed female mice by evaluating levels of mtDNA incorporation of these drugs, mtDNA depletion, ultrastructural pathology, and change in cardiac function. Transmission electron microscopy (EM) evaluation of hearts from newborn mice following *in utero* exposure to 100 mg AZT + 50 mg 3TC/day during the last 7 days gestation showed substantial damage to mitochondria (characterized by swelling, fragmentation of membranes, loss of normal central architecture, and occurrence of occasional inclusion bodies), with the loss of mitochondria ranging from 20% to 50% in cardiomyocytes of different animals. EMs of hearts from control mice showed normal appearing densely packed clusters of mitochondria between cardiac myofibrils. Our initial results demonstrated clear mitochondrial insult in cardiac tissue of newborn mice exposed transplacentally to AZT-3TC, while data from echocardiographic screening of (AZT-3TC-exposed) litter mates at 7 weeks of age were consistent with our findings of cardiomyopathy in adult mice following *in utero* exposure to AZT.

825 ALLYLAMINE-INDUCED VASOSPASM *IN VITRO*: ROLE OF ACROLEIN, HYDROGEN PEROXIDE, SEMICARBAZIDE-SENSITIVE AMINE OXIDASE ACTIVITY, AND EXTRACELLULAR Ca^{++}.

D. J. Conklin[1], H. R. Cowley[2], N. Xiong[2], G. H. Johnson[3], R. J. Wiechmann[3], L. M. Sayre[4], M. B. Trent[5] and P. J. Boor[5]. *[1]Department of Medicine, Division of Cardiology, University of Louisville, Louisville, KY, [2]Department of Biology, University of Wisconsin-Eau Claire, Eau Claire, WI, [3]Department of Cardiothoracic Surgery, Luther Hospital/Midelfort Clinic, Eau Claire, WI, [4]Department of Chemistry, Case Western Reserve University, Cleveland, OH and [5]Department of Pathology, University of Texas Medical Branch, Galveston, TX.*

Allylamine (AA; 3-aminopropene) is a cardiovascular toxicant that induces vasospasm *in vitro* and hypothetically *in vivo*. To understand the mechanism of AA-induced vasospasm we determined: 1) the sensitivity of isolated human blood vessels (internal mammary artery, IMA; saphenous vein, SV) to AA, acrolein, and hydrogen peroxide (1-1, 000 μM; H_2O_2), 2) the dependence of AA-induced vasospasm on semicarbazide-sensitive amine oxidase activity (SSAO) using SSAO inhibitors (semicarbazide, 1 mM; MDL 72274-E, active isomer; MDL 72274-Z, inactive isomer; 100 μM), and 3) the role of extracellular Ca^{++}. Vasospasm (sustained, intractable hypercontraction) was evidenced by maintenance of elevated tension resistant to sodium nitroprusside addition (100 μM) and AA removal. AA, acrolein, and H_2O_2 similarly altered tension in norepinephrine-precontracted (1 μM) vessels in a concentration-dependent manner but only AA and acrolein led to vasospasm. AA-induced vasospasm and vessel SSAO activity were inhibited by semicarbazide or MDL 72274-E but not by MDL 72274-Z pretreatment. AA-induced responses were significantly attenuated in the absence of extracellular Ca^{++} but not overall AA toxicity. The return of normal [Ca^{++}] buffer gradually increased tension that was sustained in AA-treated but not in untreated vessels. Thus, AA-induced vasospasm in human IMA and SV was dependent on SSAO activity, acrolein formation, and extracellular Ca^{++} influx. We propose that AA metabolism by SSAO triggers vasospasm *via* a Ca^{++} overload mechanism. This work supported by the Ronald McNair Program at UWEC and NIEHS AREA #1 R15 ES011141-01.

826 *IN VITRO* CHARACTERIZATION OF THE MECHANISMS INVOLVED IN CARDIOVASCULAR TOXICITY OF ULTRAFINE PARTICULATE.

M. Marinovich[1], E. Corsini[1], C. L. Galli[1], R. Pieters[3], S. Bellosta[2], K. Remedios[2] and A. Corsini[2]. *[1]Department Pharmacological Sciences, University of Milan, Milan, Italy, [2]Laboratory of Cellular Pharmacology of Atherosclerosis, University of Milan, Milano, Italy and [3]IRAS, University of Utrecht, Utrecht, Netherlands.*

A major issue in modern environmental toxicology is to find a scientific explanation for the epidemiological effects of air pollution. A particularly puzzling point is that fine particulate matter affects not only respiratory but also cardiovascular morbidity and mortality. Most research is centered around the possible consequences of particle-induced pulmonary inflammation on the heart and other systems. Another hypothesis considers the possible "direct" effects of particles that may pass from the lung into the blood stream. The present knowledge of the pathogenesis of the atherosclerotic process allowed the identification of the key processes involved in atherosclerotic plaque formation. Smooth muscle cells (SMC) proliferation and apoptosis in the vessel wall drive the formation of the plaque, while cytokines production and proteolytic enzymes secretion such as the matrix metalloproteinases (MMP) by inflammatory cells like activated macrophages, facilitates plaque fissuration and rupture. Plaque rupture and the subsequent thrombosis cause the fatal complications of atherosclerosis. The purpose of this study was to highlight proatherogenic mechanism/s of action of the ultrafine particulate. To this purpose the proliferation and apoptosis of SMC, and the release of MMP was evaluated. Replication of SMC isolate from rat aorta or human femoral artery was evaluated either by direct cell count or by thymidine incorporation into nuclear DNA. Apoptosis was measured by cytofluorimetry. MMP activity was evaluated by zymography. Our data suggest that a direct effect of ultrafine particulate on SMC vitality is likely to contribute to plaque fissuration and rupture. This study provides an understanding of fine particulate matter effects on cardiovascular morbidity and mortality.

827 ARTERIAL CARCINOGEN METABOLISM COULD INITIATE THE ACCELERATED ATHEROSCLEROSIS SEEN IN SMOKERS.

J. L. Holtzman[1], L. M. Dunning[1], W. Carter[3] and R. J. Edwards[4]. *[1]Pharmacology, University of Minnesota, Minneapolis, MN, [2]Pharmacology, University of Minnesota, Minnapolis, MN, [3]Edina Plastic Surgeions, Edina, MN and [4]Imperial College, London, United Kingdom.*

HYPOTHESIS: The accelerated atherosclerosis seen in smokers may be initiated by the toxic products formed during the arterial metabolism of the 2 major carcinogens of tobacco smoke, the polycyclic aromatic hydrocarbons (PAHs) and N-nitroso-amines (NNAs). BACKGROUND: Numerous laboratories have reported that arteries from various animals metabolize the PAHs to form the ultimate carcinogens. These reactions are catalyzed by cytochrome P450s, CYP1A1 and 1B1. The 1A1 is present in the aortas of various animal after PAH induction. We previously reported the possible presence of the P450s catalyzing NNAs activation, CYP2A6 and 2E1. OBJECTIVES: We have sought to determine whether these P450s are present in human arterial walls. METHODS: We prepared microsomes by standard procedures from the arteries of patients undergoing abdominal plasties. The P450s were determined by immunoblotting by enhanced chemoluminescence detection. RESULTS: We obtained 125 *u*g of microsomal protein from each artery. Three of the 4 CYPs were observed at concentrations comparable to those found in hepatic microsomes after PAH induction. Due to an artifact in our assay procedure, contrary to our previous report, we did not detect any 2A6. CYP 1A1 1B1 2A6 2E1 pmol/mg protein 21.4 2.1 5.2 1.0 N/D 33.7 3.7 SIGNIFICANCE: These data support the hypothesis that the accelerated atherosclerosis seen in smokers is due to the metabolism of the PAHs and possibly the NNAs in the arterial wall leading to toxic injury. Similarly, estradiol is metabolized by 1B1 to toxic catechols, suggesting a mechanism for the increased disease seen in women with postmenopausal hormone replacement. μ

828 CARDIOVASCULAR EFFECTS OF CHRONIC FUMONISIN B1 INGESTION IN SINCLAIR MINIPIGS.

G. W. Smith[1], P. Constable[2], R. L. Fredrickson[1], M. E. Tumbleson[3], R. M. Eppley[4] and W. M. Haschek[1]. *[1]Veterinary Pathobiology, University of Illinois, Urbana, IL, [2]Veterinary Clinical Medicine, University of Illinois, Urbana, IL, [3]Veterinary Biosciences, University of Illinois, Urbana, IL and [4]Center for Food Safety and Applied Nutrition, USFDA, Laurel, MD.*

Fumonisins are a group of mycotoxins primarily produced by the fungus Fusarium verticillioides, one of the most prevalent fungi associated with corn intended for human and animal consumption. Acute ingestion of fumonisin B1 (FB1) induces hypercholesterolemia in many species, including non-human primates, and has caused cardiovascular toxicity in pigs and horses. Chronic ingestion of FB1 has induced right ventricular hypertrophy in pigs and congestive heart failure in a baboon. Because the potential exists for chronic fumonisin ingestion to induce cardiovascular changes in humans, the main purpose of this study was to evaluate the cardiovascular effects of chronic FB1 ingestion in Sinclair minipigs (barrows, 7 to 14 wk of age). Treated pigs (n=7) were fed a diet containing FB1 at 10 ppm for 18 wk followed by a diet containing FB1 at 30 ppm for 8 wk. Control pigs (n=6) were fed a diet free of fumonisins for the 26 wk experimental period; at the end, serum was obtained for biochemical analyses and pigs were anesthetized and instrumented for cardiovascular function studies. Pigs fed FB1 had significant ($P < 0.05$) increases in sphinganine and sphingosine concentrations in serum, heart, and ascending aorta. There were no differences in serum cholesterol, low-density lipoproteins, high-density lipoproteins or triglyceride concentrations between the two groups. The only hemodynamic difference between the 2 groups was a decrease in systolic aortic pressure in FB1 treated pigs. Chronic ingestion of FB1 may increase the risk of cardiovascular disease in humans by increasing serum, heart, and aortic sphinganine and sphingosine concentrations. These effects were seen in pigs ingesting FB1 at 10 to 30 ppm; it is likely that humans consuming a corn-based diet ingest lower amounts of FB1. Supported by USDA-CSREES #928-39453, USFDA, and ILSI.

829 CHRONIC ARSENIC EXPOSURE ENHANCES FGF-2-STIMULATED ANGIOGENESIS *IN VIVO* AND TISSUE EXPRESSION OF ANGIOGENIC GENES.

A. Barchowsky[1, 2], L. R. Klei[1], D. D. Mayka[2], J. C. Davey[2], J. W. Hamilton[2] and N. V. Soucy[2]. *[1]Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA and [2]Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH.*

Chronic exposures to low levels of arsenic are associated with increased incidence of cardiovascular diseases in humans. However, there few studies have examined the effects of these chronic exposures on vascular development, despite evidence that arsenic stimulates proliferative responses in vascular cells. Therefore, the hypothesis that arsenic enhances neovascularization following chronic exposure through drinking water was examined in C57BL/6 mice. Dose-dependent, synergistic enhancement of FGF-2-stimulated angiogenesis in Matrigel implants was observed after 5 wk exposures to 5-500 ppb of arsenite (AsIII). Significant enhancement of angiogenesis was also observed after 10 and 20 wk exposures; however, the responses to 250 and 500 ppb were desensitized relative to responses to the same doses at 5 weeks. Gene screens and RT-PCR in cardiac and lung tissues were used to investigate systemic changes in cardiovascular genes that contribute to enhanced angiogenesis. The mRNA levels for several angiogenic genes increased and significant increases were confirmed for induction of plasminogen activator inhibitor-1 (PAI-1) and the endothelial specific transcription factor, pigpen. Circulating levels of active PAI-1 protein also increased with chronic exposure. The response of primary smooth muscle cells in culture to arsenic were used to demonstrate the dose-dependence for PAI-1 gene induction. Inhibitor studies suggested that this induction required both PI3K and PKC activation. These studies indicated that low levels of arsenic (including the current MCL in drinking water) potentiated angiogenic responses and suggested that this potentiation involved stimulation of specific signaling processes and gene activation. *Supported by NIEHS SBRP grant ES07373.*

830 EXPRESSION OF PROCOAGULANT ACTIVITY BY LYSOPHOSPHATIDIC ACID IN HUMAN ERYTHROCYTES.

S. Chung, M. Lee, O. Bae and J. Chung. *College of Pharmacy, Seoul National University, Seoul, South Korea.*

Lysophosphatidic acid (LPA), an important mediator in blood clotting and wound healing, has been recently reported to induce influx of extracellular calcium into erythrocytes (RBC). This elevation in intracellular calcium level may cause destruction of membrane asymmetry and microvesicle formation, resulting in procoagulant activity of RBC. Thus, we investigated if LPA could induce phosphatidylserine (PS) exposure and microvesicle formation as a result of extracellular calcium influx in human RBC. Treatment with LPA to RBC resulted in microvesicle generation in a time-, and concentration-dependent manner. Consistent with these findings, scanning electron microscopy studies revealed that LPA treatment changed normal discocytic shape into echinocyte followed by spherocyte. Both microvesicles and remnant erythrocytes expressed procoagulant PS on their surface membrane. Chelation of extracellular calcium with EGTA did not affect vesiculation and PS exposure, suggesting that calcium influx into RBC was not responsible for these events. Suramin that can block the interaction of LPA with its receptor did not inhibit microvesicle formation, indicating that this LPA-induced response in RBC might not be mediated by the known receptor. LPA-exposed RBC potentiated the

generation of thrombin by recombinant human thromboplastin in plasma. These results suggest that LPA-exposed erythrocytes can contribute to clot formation or thrombosis by expressing procoagulant activity.

831 ELEVATED MEAN ARTERIAL BLOOD PRESSURE IN ARYL HYDROCARBON RECEPTOR (AHR) NULL MICE IS ASSOCIATED WITH ENDOTHELIN-1.

A. Lund[1], J. L. Born[1] and M. K. Walker[1, 2]. [1]*College of Pharmacy, University of New Mexico, Albuquerque, NM and* [2]*Cell Biology and Physiology, University of New Mexico, Albuquerque, NM.*

AhR is a ligand-activated transcription factor that mediates the toxicity of environmental pollutants such as TCDD. The role of the AhR in physiological homeostasis is still uncertain, however functional inactivation of AhR (AhR null mice) results in profound effects on the cardiovascular system. We have previously reported AhR null mice exhibit cardiac hypertrophy associated with elevated mean arterial blood pressure (MAP) and increased levels of vasoactive peptides endothelin-1 (ET-1) and angiotensin II (Ang II). However, chronic treatment of AhR null mice with an ACE-inhibitor did not normalize either MAP or ET-1, compared to age-matched C57Bl6 mice. To better understand the role of ET-1 in MAP regulation, we tested the hypothesis that the observed MAP results from increased ET-1 and that this increase is age-progressive. Time-course assessment of MAP in AhR null mice, measured by indwelling catheters, showed elevated MAP by age 2 mo (AhR null: 110.0, C57Bl6: 100.6 mmHg), with a progressive increase seen at 5 mo (AhR null: 123.1, C57Bl6: 102.0 mmHg). Corresponding with these findings, time course analysis of plasma ET-1 levels as determined by RIA, showed significantly increased levels in AhR null mice at age 2 mo (AhR null: 9.9, C57Bl6: 7.7 *f*mol/ml) with further elevations at 5 mo (AhR null: 12.3, C57Bl6: 7.7 *f*mol/ml). Real-time PCR of lung tissue showed that ET-1 mRNA levels were approximately 8-fold higher in AhR null mice compared to AhR wildtype. To examine the role of ET-1 in MAP, 5 mo old AhR null and C57Bl6 mice were treated with ET_A receptor antagonist BQ-123, delivered *via* osmotic minipumps for a period of 7 days, and resulting MAP was measured. BQ-123-treated AhR null mice had significantly reduced MAP compared to untreated AhR null mice (treated: 105.1; untreated: 120.8, C57Bl6 controls: 100.8 mmHg). Such findings indicate that MAP in AhR null mice is mediated by ET-1. Supported by ES10433 to MKW, ES12072 (NM NIEHS Center), and EPA fellowship U91621501 to AKL.

832 EFFECTS OF CHLORODIBROMOMETHANE ON THE DEVELOPING HEART OF MEDAKA.

S. Palit[1], W. R. Hartley[1], L. K. Teuschler[2], C. Gennings[3], O. Conerly[4] and A. Thiyagarajah[1]. [1]*Environmental Health Sciences, Tulane University Health Sciences Center, New Orleans, LA,* [2]*NCEA, USEPA/ORD, Cincinnati, OH,* [3]*MCV, VCU, Richmond, VA and* [4]*Office of Water, USEPA, Washington, DC.*

We observed a variety of cardiovascular lesions induced by trihalomethanes, a class of disinfection byproducts, in medaka embryos. The purpose of this presentation is to report the sequential development of the cardiovascular malformations induced by chlorodibromomethane (CDBM), one of the trihalomethanes. Medaka embryos at stage 10 (N= 60 per concentration) were exposed individually in 5 ml of embryo rearing solution to nominal concentrations of 0, 10, 25, 40, 55 or 70 mg/L CDBM for 15 days. The test solutions were renewed daily. Embryos were evaluated daily for mortality and cardiovascular abnormalities. The heartbeat count was made for one full minute from day 3-10. The earliest lesion observed was the tubular heart on day 4 of exposure. Lack or incomplete chambers formation often began on day 5. Diathesis or pooling of blood in various tissues began to appear staring on day 5 of exposure, followed by slowing of blood flow and complete stasis usually on day 10. At this time pericardial edema began to develop and progressed to other organs and interfered with hatching which resulted in partial hatching. In addition, low incidence of the following lesions were observed: non-development of heart, absence or reduced numbers of vitelline vessels, reduced numbers of blood cells, and ruptured blood vessels. We proposed a sequential development of the pericardial edema in the developing medaka embryos caused by CDBM, which lays the foundation for the future mechanistic studies of cardiovascular malformations.

833 SAFETY EVALUATION OF E5564, A TOLL-LIKE RECEPTOR 4 (TLR4) ANTAGONIST, ON CARDIOPULMONARY BYPASS (CPB) SURGERY IN DOGS BY INTRAVENOUS INFUSION.

A. Suganuma[1], H. Mendenhall[2], J. P. Sites[3], K. Kaneko[1], M. Nedelman[2] and W. D. Kerns[4]. [1]*Drug Safety and Disposition, Eisai Co., Ltd.,, Tsukuba, Japan,* [2]*Discovery and Development Services, Charles River Laboratories, Worcester, MA,* [3]*Cardiovascular and Extracorporeal Technologies, Plymouth, MN and* [4]*Pharmacology Consulting Inc., Harvard, MA.*

E5564, a TLR4 antagonist is expected to block LPS-mediated diseases. To support use during CPB surgery, E5564 was evaluated to determine if there was an adverse effect on oxygenation, heparin reversal, and to determine plasma concentrations in mongrel dogs. Blood was collected for 18 days prior to surgery by the Domino method for autotransfusion at surgery. The animals (3 males/group) received either E5564 (1 mg/kg/hr) or its vehicle (2% lactose/4% dextrose-phosphate buffer) by IV infusion at 2 mL/kg/hr, starting at the time surgery commenced. The heart was arrested and the animal's core body temperature was cooled to ~28°C for 60 min, while on bypass. The animal was warmed to 35-38°C, resuscitated and taken off bypass. The dogs were ventilated with room air and their condition monitored for another 30 min. During the surgery, the following parameters were assessed: anesthesia time, surgery time, CPB time, heparin dose, protamine dose, ECG, heart rate, systolic, diastolic and mean arterial pressure, central venous pressure, pulmonary artery pressure, oxygen saturation, end-tidal carbon dioxide, P_{O2}, P_{CO2}, base-excess, HCO_3^-, pH, hematocrit, Na^+, K^+, Ca, activated clotting time, rectal and esophageal body temperature, total volume of lactated Ringer's solution administered, urine output, defibrillation attempts, hematology, coagulation, blood chemistry, and pharmacokinetics. All animals tolerated the cardiopulmonary bypass procedure well. There were no differences observed between E5564 and vehicle groups in all parameters tested. Similar plasma levels of E5564 from all three treated dogs were obtained and comparable to those seen in a 72-hour infusion toxicity study in the dog. In conclusion, E5564 did not compromise any CPB process or measured parameter. CPB did not interfere with the pharmacokinetics of E5564.

834 A UNIQUE CARDIOVASCULAR TOXICITY IN RATS FOLLOWING ADMINISTRATION OF PYRAZOLE KINASE INHIBITORS IN FISCHER 344 RATS.

A. J. Stauber, J. L. Zimmerman, B. W. Main, S. T. Adams and G. Bricker. *Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN.*

Escalating doses of pyrazole kinase inhibitors were administered to Fischer 344 rats to determine their toxicity profile. After 2-3 doses at 100 mg/kg or greater, rats began to show clinical signs of increased and/or labored respiration. Physical examination of the rib cage revealed an indentation of the sternum towards the chest cavity. Mortality occurred in rats with the most severe signs of respiratory distress. These clinical observations became the best indicator of the toxicity and predictor of mortality. Minimal to moderate amounts of fluid in the pleural cavity was found in some, but not all treated rats, and was not sufficient to be considered the cause of death. Analysis of thoracic fluid revealed a modified exudate containing high protein levels and infiltration of inflammatory cells. No morphologic changes were present in the heart, lungs, sternum, rib cage or diaphragm. Cardiovascular effects were determined in conscious Sprague-Dawley rats after a single dose and resulted in acute hypotension followed by reflex tachycardia that was sustained for up to 24 hours. This data does not identify the primary target organ, yet suggests a functional effect on the cardiovascular system may be responsible for the clinical signs and mortality that are observed after multiple doses. Several compounds from the pyrazole class exhibiting structural diversity were evaluated. Based on administered dose, the data indicated a difference in potency (relative to the toxicity) between the molecules. This suggests a structure activity relationship between this unique toxicity and an, as yet, unidentified target.

835 ALPHA GLUTATHIONE S-TRANSFERASE AS A NOVEL BIOMARKER FOR MONITORING CHRONIC METHOTREXATE HEPATOTOXICITY.

M. C. Shaw[1], P. R. Maxwell[2] and D. Burden[3]. [1]*Biotrin International, Dublin, Ireland,* [2]*Biochemistry, Stobhill Hospital, Glasgow, United Kingdom and* [3]*Dermatology, Western Infirmary, Glasgow, United Kingdom.* Sponsor: R. Chandra Gupta.

Methotrexate is a commonly used drug for treating severe psoriasis and other severe immunological diseases. It is a known hepatotoxin and it is recommended that liver function be monitored in patients and that liver biopsies are performed on patients after they have received fixed cumulative doses of 1-1.5g of the drug. Improved monitoring of liver status could reduce the need for routine biopsies, saving costs and reducing patient morbidity while identifying those most at risk Alpha glutathione S-transferase (αGST) is a more sensitive indicator of acute hepatocyte injury than transaminases, but it has not previously been studied as an indicator of chronic toxicity. 44 subjects receiving low dose methotrexate for psoriasis were monitored using αGST, ALT, AST, GGT, ALP and serum albumin. Alpha GST was assayed using the Biotrin HEPKIT-Alpha GST EIA kit. AST, ALT, GGT, ALP, Albumin and total protein were assayed using the Bayer ADVIA 1650. Data were compared with that obtained from 20 healthy controls. Data for αGST and GGT is presented as mean±SEM. αGST µg/L Control 3.6±1.0, Patients 9.0±7.0 $p< 0.01$ GGT, University/L Control 23±7.0 Patients 36±27, $p < 0.05$ Values for AST, ALT, ALP serum albumin and total protein were similar in both patients and controls. Only 5% of patients (2/44) showed elevated ALT levels, but αGST levels were above normal in 25% (11/44) of cases. In the immediate period after a dose of

methotrexate, (24-48 hours) AST and αGST were elevated but αGST was elevated to a greater degree (~5fold compared with ~2fold for AST). Other liver biomarkers were unchanged. αGST is more often elevated and to a greater degree than other biomarkers in subjects receiving chronic methotrexate therapy. Since subjects with normal transaminases would not normally be biopsied, αGST has the potential to identify those most at risk of hepatic injury.

836 BIOSENSOR DETECTION OF BLOOD NTE INHIBITION.

V. V. Malygin[1], G. F. Makhaeva[1], N. N. Strakhova[1], L. V. Sigolaeva[2], L. G. Sokolovskaya[2], A. V. Eremenko[2], I. N. Kurochkin[2] and R. J. Richardson[3]. *[1]Institute of Physiologically Active Compounds, RAS, Chernogolovka, Russian Federation, [2]Faculty of Chemistry, M.V. Lomonosov Moscow State University, Moscow, Russian Federation and [3]Toxicology Program, University of Michigan, Ann Arbor, MI.*

To enable NTE to be assayed in whole blood, an electrochemical method was developed, based on detection of phenol with a tyrosinase carbon paste electrode. The biosensor was used to establish correlations of NTE inhibition in blood with that in lymphocytes and brain 24 hr after dosing hens with di-1-propyl-2, 2-dichlorovinyl phosphate (PrDChVP). To improve sensitivity, the initial electrode was optimized to achieve a detection limit of 25 nM phenol in a flow cell, allowing NTE activity to be measured in blood diluted 1:500. The new electrode had improved operating stability and a working life >12 mo. Using the biosensor, the *in vitro* sensitivity of hen blood and brain NTE to OP compounds was compared. I50 values for inhibition of NTE with $RO(C_6H_5)P(O)ON{=}CCH_3Cl$ (R = Me, Et, n-Pr, n-Bu) were obtained amperometrically for blood and colorimetrically for brain. A good correlation was found between pI50 values for blood and brain NTE inhibition by the phenylphosphonates, as well as 3 other OP compounds (r = 0.988, n = 7). To assess the time dependence of blood NTE inhibition after OP compound exposure, NTE activity was measured in brain and blood 4 hr after dosing hens with PrDChVP (0.32-1.0 mg/kg, im), as well as 4, 24, 48, 72 and 96 hr after 1.0 mg/kg. Brain and blood NTE inhibition was dose related 4 hr after dosing, and highly correlated between brain and blood (r = 0.997). NTE activity in brain and blood of hens killed 4, 24, 48, 72, and 96 hr after dosing with 1 mg/kg PrDChVP differed significantly from respective control values. During all measured times, NTE inhibition relative to controls (mean ± SE, n = 5) was 72 ± 4% in brain and 75 ± 3% in blood. The results demonstrate that whole blood NTE is a reliable biomarker of exposure to neuropathic OP compounds during 96 hr between exposure and measurement. (Supported by CRDF grants #RB2-2035, #RB2-2488).

837 COMPARATIVE GENE EXPRESSION PROFILING OF EPOTHILONE B AND PACLITAXEL TO SEARCH FOR BIOMARKERS OF EFFICACY AND TOXICITY.

M. Saulnier, F. Staedtler, P. McSheehy, A. Mahl, J. Schaffner, D. Roman, P. Ulrich, M. Wartmann, S. Chibout, H. Firat and L. Mueller. *Novartis Pharmacology AG, Basel, Switzerland.* Sponsor: V. Nogues.

Epothilone B (EPO906) is a novel microtubule stabilizer developed for the treatment of solid tumors, including tumors insensitive and refractory to paclitaxel, a P-gp substrate. In preliminary clinical studies designed to define the optimal dose and schedule of the drug, diarrhea represents the most common dose-limiting side-effect of EPO906 so far. In preclinical models, diarrhea occurs in rats treated with 1.75mg/kg EPO906. In the present study, EPO906 was compared to paclitaxel to understand the mechanism of diarrhea after EPO906 treatment, and identify specific biomarkers. Both compounds were injected i.v. into groups of Lewis rats at roughly equipotent doses (EPO906: 1.75 mg/kg, paclitaxel: 10 mg/kg) with regard to suppression of tumor growth in a rat tumor model. Caecum samples were collected after 2, 24, and 48 hours post-injection; RNA was extracted and analyzed using rat DNA microarrays. Caecum transcript profiles showed that both compounds induced dose- and time-dependent changes related to their pharmacological effects on stabilization of microtubules, leading to cell-cycle arrest. With paclitaxel, most of the changes in terms of number and mRNA expression levels were weak and transient. An EPO906-specific expression pattern was observed, which included genes encoding acute phase proteins, coagulation cascades, tissue remodeling, angiogenesis, lipid metabolism and, particularly, the arachidonic acid pathway, indicating a strong inflammatory response. These data suggest that induction of diarrhea by EPO906 may be related to the expected antiproliferative and cytotoxic activity, which may, in turn, be exacerbated by the presence of a strong inflammatory response, leading to the loss of the intestinal electrolyte/water barrier function. In EPO906-treated animals, early, differentially expressed genes were defined as potential biomarkers for the prediction of EPO906-induced diarrhea.

838 ENVIRONMENTAL TOBACCO SMOKE INDUCED REACTIVE OXYGEN SPECIES GENERATION IN MICE BRAIN REGIONS.

K. C. Wise[1], T. Rangasamy[2], S. Biswal[2] and R. Govindarajan[1]. *[1]Department of Biology, Texas Southern University, Houston, TX and [2]Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.*

Environmental tobacco smoke (ETS) is a key culprit in indoor air pollution. Tobacco smoke contains a mixture of over 4700 chemical components many of which are toxic and have been implicated in the etiology of oxidative stress related diseases such as chronic obstructive pulmonary disease, Parkinsons disease, asthma, cancer and cardiovascular disease. However, the mechanism of action of cigarette smoke in the onset of these diseases is still largely unknown. Previous studies have revealed that the free radicals generated by cigarette smoke may contribute to many of these chronic health problems and this study sought to address the role of environmental tobacco smoke in oxidative stress related damage in different brain regions of a mouse model. In this study, male strain A/J mice were exposed for 7 h/day, 7 days/week, for six months to an atmosphere of ETS consisting of 89% side stream smoke and 11% mainstream smoke produced by burning reference cigarettes using a smoke machine. Chamber atmosphere was monitored for total suspended particulates (TSP) and carbon monoxide (CO). Our preliminary findings from this study indicates that exposure to tobacco smoke leads to increased generation of reactive oxygen species with a concomitant decrease in the level of glutathione, a natural antioxidant utilized to combat oxidative stress. Gel shift analysis also revealed the elevated level of the oxidative stress sensitive, proinflammatory transcription factor NF-kappa-B in different regions of the brain of cigarette smoke exposed AJ mice. Research is in progress to determine the cigarette smoke induced oxidative lesions in different regions of the brain of AJ mice. [This work was supported by grant: RCMI/NIH #G12RR03045-16].

839 IDENTIFICATION OF BIOMARKERS FOR OXYCHLORDANE-EXPOSURE IN RODENT LIVER USING MICROARRAYS.

I. Curran, A. Hierlihy, K. Smith, J. Green and G. Bondy. *Toxicology Research Division, Health Canada, Ottawa, ON, Canada.*

Oxychlordane (OXY) is the primary metabolite of the toxic mixture, technical chlordane. Such persistant organo-pollutants were widely used as pesticides until the 80s and thus have accumulated in the food chain. Recent reports indicate that the formation of this metabolite is more toxic and bioaccumulative than the parent contaminants. The goal of this study was to elucidate possible genetic markers that may be used in identifying toxic exposure before overt physiological effects are evident. Female and male rats were gavaged with 2.5mg/kg body weight/day OXY for 28 days; control animals were gavaged with corn-oil vehicle only. Total RNA was isolated from liver and pooled, followed by comparison of gene expression levels using microarray analysis. Three trials using Mergen chips (contain oligos of 1152 known rat genes and ESTs) were performed. Microarray data was verified using semi-quantitative RT-PCR on individual samples. Microarray analysis on female rat liver RNA showed that 31 targets were expressed at >2-fold increase in treated vs. control animals. Classification of these genes identified several membrane transport genes, as well as cytochrome p450 genes. RT-PCR confirmed increased levels of gene expression in treated female rats in such genes as sodium-dicarboxylate cotransporter (SDCT1), Na+, K+ ATPase-gamma subunit. Interestingly, sex-specific differences were detected in gene expression of 4 of the target genes. These included proton-coupled peptide transporter-2, Na+, K+ ATPase- gamma, SDCT1, and putative potassium channel. While female expression levels were shown to increase in these genes, transcript levels were observed to decrease in male OXY-treated livers. Results indicate that OXY elicits an effect on membrane proteins. This corresponds with previous studies that report depressed organic-ion transport to be an effect of chlordane, trans- and cis-nonachlor on membrane channel, ion transport and other membrane-related proteins. Further testing is currently underway to screen larger gene arrays at lower dose groups to identify additional gene targets.

840 MEETING GUIDELINES FOR CHOLINESTERASE MONITORING BY CLINICAL LABORATORIES IN CALIFORNIA.

B. W. Wilson[1], J. D. Henderson[1], D. E. Arrieta[1] and M. A. O'Malley[2, 3]. *[1]Environmental Toxicology, University of California, Davis, CA, [2]Employee Health, University of California, Davis, CA and [3]Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.*

California (CA) is the only state with a long standing formal monitoring program for mixers, loaders and applicators of pesticides. When we found commercial clinical kits were not optimal for assaying blood cholinesterases (ChEs), CA regulations were revised to specify use of optimal Ellman ChE assay conditions. Alternate

methods were allowed if a correlation ($r^{2)}$ of 0.9 was achieved in a comparison with the specified method. We were enlisted to work with the clinical laboratories. Only 2 of 7 participating laboratories achieved an acceptable correlation for red blood cell acetylcholinesterase (AChE) and 4 of 5 laboratories for plasma ChE. When the CA Department of Pesticide Regulation (DPR) reiterated the need to meet this requirement we used bovine ghost RBC AChE as a standard to work with several of the clinical laboratories. Only 3 of 10 participating laboratories had acceptable correlations. Some said their instruments lacked the sensitivity to assay the bovine AChE samples. Next, we provided all interested laboratories with human blood and plasma samples to perform a comparison study outlined in the regulation (Section 6728f). Fourteen laboratories participated; 9 met the criterion for whole blood, 14 for plasma and 6 for RBC AChE. Based on such data, DPR notified the CA Agricultural Commissioners on July 8, 2003 that 9 clinical laboratories were approved for ChE testing. We continue to work with laboratories interested in being on the approved list. The current list may be seen at: www.cdpr.ca.gov/docs/whs/lablist.htm. Supported by NIOSH (#CDC U07/CCU906162-06) and NIEHS (#ESO5707).

841 BIOLOGICAL MONITORING OF BISPHENOL A IN CHILDREN.

S. Park[1], C. Shin[2], S. Kim[3] and M. Yang[1]. *[1]Preventive Medicine, Seoul National University College of Medicine, Seoul, South Korea, [2]Pediatric Endocrinology, Seoul National University Hospital, Seoul, South Korea and [3]Preventive Medicine, Eulji University School of Medicine, Taejon, South Korea.*

Health risk of bisphenol A (BPA) among potential endocrine disrupters (EDs) is not clear, yet. In addition, children are thought to be more sensitive to BPA than adults. Therefore, we studied exposure levels of BPA and association between the exposure levels and endocrine disorders in Korean children (N= 168; 9.1 ± 2.2 yrs; male, 41.1 %) to provide safe exposure levels of BPA. Cases of endocrine disorders included short stature, precocious puberty, hyperthyroidism, etc. Controls were 94 healthy children. Using our established analysis method of BPA in urine (HPLC/FD), we measured conjugated BPA as a biomarker for BPA exposure. As results, range of BPA was 0.04- 83.5 μg/L in detectable urine samples (N=149). When we gave half of the lowest level, 0.02, to the other 19 non-detectible samples, the median of BPA levels was 5.85 μg/L. The level of BPA was higher in cases (N=65) than controls (N=94): geometric mean (geometric standard de*via*tion) in cases and controls, 3.9 μg/L (5.8) and 1.9 μg/L (12.0), respectively: p = 0.13. When we considered age and sex for BPA levels, the odd ratio for case was 4.7 (95% CI, 1.3-19.4). Even though our results should be confirmed in enlarged populations, our results suggest that exposure to BPA may affect endocrine disorder in children. In addition, approximate 3 μg of BPA is thought to be daily exposed to the children. Our results will be useful for establishment of future safe levels of BPA.

842 METABOLOMIC ANALYSIS OF THE MECHANISMS OF ACETAMINOPHEN LIVER TOXICITY IN RATS.

A. J. Higgins, T. J. Colatsky, B. R. Bullard and S. S. Sumner. *Paradigm Genetics, Inc., Research Triangle Park, NC.*

Acetaminophen (APAP) overdose remains one of the most common causes of hospital admissions for acute liver toxicity. The initiating mechanism is believed to involve hepatic metabolism of APAP to a reactive oxidative species, NAPQI, which subjects the liver to oxidative stress, resulting in depletion of glutathione. Oxidative stress is also believed to play a pivotal role in the hepatotoxicities of a wide range of chemical and pharmaceutical agents, and APAP may therefore serve as a useful model agent. Single oral doses of APAP, ranging from 50 mg/kg (no histopathological changes) to 2000 mg/kg (frank necrosis) were administered to rats. Groups of 6 rats were serial sacrificed up to 48h and livers were snap frozen. Samples were extracted and analyzed by LC/MS (ToF) with an ESI source in either positive or negative mode. Mass spectra at each retention time were matched to a library of around 500 known standards using proprietary software and linked to metabolic pathways. Data were reduced and visualized by principle component analysis. As early as 6h after the acute administration, changes in biochemical profiles could be clearly observed with doses of 1500 mg/kg and above. The time-related trajectories were similar for the 1500 and 2000 mg/kg groups. A similar qualitative trend was also apparent at 150 mg/kg, a dose that produced only minimal histopathological changes. Analysis of individual metabolites revealed major perturbations in pathways associated with known injury and repair mechanisms - e.g. depletion of glutathione and cystathionine (oxidative stress), decreases in NAD and various nucleotides (nucleic acid repair), and a decrease in CDP-choline (phospholipid turnover). Several of these decreases in liver metabolites were also reflected in urine, suggesting that they might serve as useful biomarkers for early detection of liver disease. Time and dose-related changes were also observed in various other biochemicals that were not predicted by current knowledge. Samples for this study were kindly provided by NIEHS.

843 METABOLOMICS: URINE AND SERUM BIOMARKERS FOR ACETAMINOPHEN HEPATOTOXICITY IN RATS.

T. J. Colatsky, A. J. Higgins, B. R. Bullard and S. C. Sumner. *Paradigm Genetics, Inc., Research Triangle Park, NC.*

Acetaminophen (APAP) is a common cause of liver toxicity in humans. Standard markers of liver toxicity are relatively insensitive to the effects of APAP and have limited value in predicting outcomes for patients who have overdosed with the drug. Biochemical profiling (metabolomics) is a zero-based diagnostic approach (i.e. requiring no prior assumptions) that has the potential to simultaneously map changes in multiple biochemical processes that serve as novel biomarkers of liver disease. APAP was administered to groups of 6 rats in a single oral dose of either 50 or 1500 mg/kg. Urine was collected during various periods up to 48h and a single serum sample was also obtained at 48h. Biochemical profiles were determined in urine and serum by LC/MS (ToF) with an ESI source in either positive or negative mode. Mass spectra at each retention time were matched to a library of around 500 known standards using proprietary software. Data were reduced and visualized by principle component analysis (PCA). Results from urine showed separation of the 1500 mg/kg group from the control and low dose groups within the first 6h collection period. Progressively greater separation occurred from 6-24h and 24-48h. PCA analysis of serum showed similar classifications at 48h. The mechanisms underlying the changes in urine included decreases in homocysteine and cystathione, reflecting protection against oxidative stress. Amongst the other changes observed, some were representative of effects on phospholipid and nucleic acid turnover. Others mainly involved various pathways of amino acid metabolism. The most striking alteration in urine was a large, rapid increase in cAMP at the high dose and a smaller, transient increase at the low dose. A large increase in cAMP was also observed with the high dose in serum. These results suggest a basis for development of a novel panel of biomarkers for early detection and monitoring of liver toxicity. Samples for this study were kindly provided by NIEHS.

844 GENE EXPRESSION PROFILING IN A RAT CARDIAC ISCHEMIA MODEL.

P. H. Koza-Taylor[1], B. Lu[1], M. Wenfang[3], S. Eustis[2], X. Li[2] and M. Lawton[1]. *[1]Molecular and Investigative Toxicology, Pfizer, Groton, CT, [2]Pathology, Pfizer, Groton, CT and [3]Comparative Medicine, Pfizer, Groton, CT.*

Cardiotoxicity in preclinical animal species is a common finding during development of vasoactive or cardiotonic pharmaceutical agents. There are multiple mechanisms of cardiotoxicity, with cardiac ischemia being the most common cause for cardiac injury. We have utilized a rodent model of cardiac ischemia to search for biomarkers for the early prediction and/or identification of cardiac injury. In this model, male Sprague-Dawley rats were anesthetized with isoflurane, and a left thoracotomy was performed aseptically to expose the heart and the left coronary artery. For ligation, the left anterior coronary artery was tied completely to occlude the blood flow. For sham control, the needle/suture were passed through under the artery, but was not tied. The heart and whole blood samples were collected for gene expression profiling at 3, 6, 9, 24 and 48 hours after coronary artery ligation or sham surgery. Moderate acute cardiomyocyte necrosis at 3-9 hours and necrosis with mixed inflammatory cell infiltration at 24 -48 hours were observed in the left free ventricular wall after ligation. Principal component analysis (PCA) of the global gene expression data showed clear separation between the sham and treated animal groups. An immediate-early stress response was observed starting the early time points and increasing at the later time points. The genes differentially expressed in the ischemia-induced animals fell into distinct clusters: the down-regulated genes included metabolism, glycolysis and catalase pathway members whilst up-regulated genes included acute phase and inflammatory response, heme oxidation pathway members, heat shock proteins, matrix metalloproteinases and fibronectin. Using both hierarchical clustering and Gene Logic's linear discriminant analysis (LDA)-based predictive models, the transcriptional profile in the hearts of ischemic rats was similar to catecholamine- and minoxidil-like responses, suggesting common mechanisms of ischemic cardiac injury.

845 METABOLISM AND HEMOGLOBIN ADDUCTS OF [1, 2, 3-$^{13}C_3$] ACRYLAMIDE IN HUMANS.

T. Fennell[1], R. Snyder[1], J. P. Burgess[1] and M. A. Friedman[2]. *[1]RTI International, Research Triangle Park, NC and [2]UMDNJ, Newark, NJ.*

Acrylamide (AM), which is used in the manufacture of polyacrylamide and grouting agents, is produced during the cooking of foods. Exposure to AM in the workplace can occur through dermal and inhalation exposure. The objectives of this study were to evaluate the metabolism of AM in humans on oral administration, and to compare hemoglobin adduct formation on oral and dermal administration. The study protocol was reviewed and approved by IRBs at both the laboratory performing the analysis of samples, and the clinical research center conducting the

study. 1, 2, 3-$^{13}C_3$ AM was administered in an aqueous solution either orally (single dose of 0.5, 1.0, or 3.0 mg/kg), or dermally (3 daily doses of 3.0 mg/kg) to sterile male volunteers. Blood and urine samples were obtained before and after administration of AM. Urine samples from the 3 mg/kg oral dose group were analyzed for AM metabolites using ^{13}C NMR spectroscopy. Approximately 34 % of the administered dose was recovered in urine by 24 hr, and 75% of the urinary metabolites were derived from direct GSH conjugation. Glycidamide, its hydrolysis product, glyceramide, and one unidentified metabolite were detected in urine. Hemoglobin adducts derived from AM (*N*-(2-carbamoylethyl)valine, AAVal) and glycidamide (*N*-(2-carbamoyl-2-hydroxyethyl)valine, GAVal) were measured by the modified Edman method, with quantitative analysis by LC-MS/MS. On oral administration, a linear dose response was observed for both $^{13}C_3$ AAVal (514, 914, and 2488 fmol/mg globin at 0.5, 1 and 3 mg/kg, respectively) and $^{13}C_3$ GAVal (186, 344, and 1136 fmol/mg). Dermal administration resulted in lower levels of $^{13}C_3$ AAVal (116, 292 and 440 fmol/mg) and $^{13}C_3$ GAVal (55, 167, and 292 fmol/mg) following 1, 2 and 3 days of dosing (3 mg/kg). $^{13}C_3$ AAVal normalized for dose for all of the oral dose groups was 924 fmol/mg globin/mg AM/kg. These data indicate that humans metabolize AM less than rodents *via* glycidamide, that there is a linear dose response for AAVal and GAVal, and that dermal uptake was approximately 5 % of that observed with oral uptake.

846 ANALYSIS OF HEMOGLOBIN N-VALINE ADDUCTS FROM (1-CHLOROETHENYL)OXIRANE, A METABOLITE OF CHLOROPRENE.

H. Hurst and M. Y. Ali. *Pharmacology and Toxicology, University of Louisville, Louisville, KY.*

Chloroprene (2-chloro-1, 3-butadiene, CAS 126-99-8, CP) is a colorless volatile liquid used in manufacture of polychloroprene, a synthetic rubber polymer. National Toxicology Program inhalation studies of CP in rats and mice gave clear evidence of carcinogenic activity in multiple organs including lung, oral cavity, thyroid, mammary glands, and kidney. CP is metabolized by CYP 2E1 to an electrophilic epoxide, (1-chloroethenyl)oxirane (CEO), which forms adducts with nucleic acids and other nucleophiles such as glutathione and hemoglobin (Hb). CEO-Hb adduct measurement may provide a biomarker of exposure to this air pollutant. This study presents GC/MS measurement of N-terminal CEO-valine Hb adducts. Mouse (C57Bl/6) red cells were exposed in pH 7.4 phosphate-saline to 25 or 100 ppm CEO vapor in septum capped *vial*s at 37°C for 1 to 72 hr. Reactions were stopped with ice-cold acidified acetone to precipitate globin containing CEO-adducts. Precipitates were washed with pure acetone, dried, and stored at −20°C. CEO-valine-tyrosine-valine (CEO-VYV) was synthesized and purified by HPLC as a quantitative standard. Precipitated CEO-globin samples or CEO-VYV and CEO-$^{13}C_5$-Val (internal standard, IS) were dissolved in formamide. NaOH and pentafluorophenylisothiocyanate (PFPITC) were added. Samples were shaken overnight at 25°C and incubated for 2 hours at 50°C. Edman derivatives were extracted with ether, dried, re-dissolved with toluene, washed with aqueous sodium carbonate, dried, and incubated with N, O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) in acetonitrile. GC separation used a DB-1 capillary column with temperature programming. An AutoSpec mass spectrometer (70eV ionization energy and 1000 resolving power) detected ions of m/z 465 [M-Cl]+ and 470 (IS). Identical spectra were observed in two resolved GC/MS peaks at 13.6 and 13.7 min. Low - high CEO and Hb reactions followed 1st order kinetics with yields of 2.0 - 6.2, 11 - 27 picomoles adduct/mg globin and formation half-lives of 5.8 - 7.6, 7.2 - 7.8 hr for isomers 1 and 2, respectively.

847 A SIMULTANEOUS DETERMINATION OF THE THREE EPOXYMETABOLITE-HEMOGLOBIN ADDUCTS OF 1, 3-BUADIENE.

K. Peltonen[1] and T. Anttinen-Klemetti[2]. *[1]EELA, Helsinki, Finland and [2]FIOH, Helsinki, Finland.* Sponsor: S. Kai.

1, 3-Butadiene (BD) is produced from petroleum as a co-product of steam cracking. BD is used as a monomer in the manufacturing of a wide range of polymers and copolymers. In 1996 BD was among the 40 most produced chemical in the USA. About 30.000 workers in Europe and 50.000 workers in the USA are potentially exposed to BD. BD is metabolized mainly in the liver to electrophilic epoxymetabolites: monoepoxybutene (BMO) epoxybutanediol (EBD) and diepoxybutane (DEB). In a reaction with N-terminal valine of globine all epoxymetabolites forms adducts. We have prepared and characterized 33 modified peptide standards which we will use in human biomonitoring purposes. These include alkylated hepta and octapeptide (alpha chaing), nona and oktapeptide (beta chaing) and corresponding deuterated analogs. The progress of the reaction was monitored with HPLC, the products were isolated and spectroscopically identified. At present the markers were utilized to look the adducts at animals inhalation exposed to 1, 3-butadiene. The simultaneous measurements of all adducts allow a reliable estimation of the adduct ratio formed *in vivo*

848 DRUG SIGNATURES THAT PREDICT A VARIETY OF PATHOLOGIES.

C. Pearson, A. Roter, A. Tolley, B. Eynon, B. Ganter, G. Natsoulis, G. Day, K. Jarnagin, K. Kolaja, M. Fielden, M. Lee, R. Nair, S. Dunlea, J. Yang, L. Gong, S. Nicholson, S. Tugendreich, S. Fujimoto and S. Baumhueter. *Iconix Pharmaceuticals, Inc., Mountain View, CA.*

To improve compound selection during drug discovery we have developed a large library of biomarkers, Drug Signatures™, for important toxicities and mechanisms of action. These signatures come from a comprehensive database that marries ~13, 000 microarray gene expression results derived from treating rats with 580 drugs at two doses and four time points in triplicate with traditional measurements of toxicity, pathology and pharmacology. Drug Signatures allow rapid, accurate interpretation of a drug's toxicity and mechanism, potentially hastening drug discovery and approval. Iconix has developed about 100 signatures that predict future pathologies based on early gene expression data in liver, kidney and heart. Pathologies that appear between 5 and 7 days after drug administration can be predicted with 40 to 95% accuracy using gene expression results from 6 and/or 24 hours. These pathologies include decreases in serum cholesterol; serum albumin; body weight; the numbers of neutrophils, lymphocytes and platelets; and increases in liver, kidney and heart weights; serum alkaline phosphatase and total bilirubin, markers of cholestasis; serum blood urea nitrogen and creatinine, markers of kidney injury; and liver pathologies such as hypertrophy, bile duct hyperplasia, apoptosis and centrolobular hydropic change. Genes in these signatures include those that encode proteins that act early in a process, such as transcription factors and signaling molecules. Other genes include those that are also in diagnostic signatures that indicate a concurrent pathology. These findings show that gene expression changes associated with a toxic response can be detected early before overt pathology occurs. Thus, predictive Drug Signatures allow identification of those drugs that may be toxic very early in preclinical studies, saving time and expense during drug discovery decision making.

849 THE CONTRIBUTION OF AGE AND GENOTYPE TO SENSITIVITY TO ENVIRONMENTAL GENOTOXINS.

F. M. Williams, E. L. Davis, A. E. Daly and D. Morgan. *Toxicology Unit, The Medical School, University of Newcastle, Newcastle upon Tyne, United Kingdom.*

The aim of this study was to determine the influence of age and exposure to environmental genotoxins on DNA damage measured in freshly isolated human lymphocytes and to determine whether the genotype for xenobiotic metabolism was a factor contributing to susceptibility. Rural young (n=33) and elderly (n=52) and urban young (n=57) and elderly (n=116) volunteers were recruited in rural Northumberland and urban Newcastle upon Tyne. Lymphocytic DNA damage was determined by the COMET assay, genotype by PCR and haemoglobin adducts of arylamines measured as markers of environmental exposure. DNA damage measurements (tail density moment, median, 25th and 75th percentile) were in the elderly rural 28.45(25.53, 30.65), elderly urban 26.78 (23.9, 30.28) compared to the young rural 23.08(21.5, 25, 42)and young urban 25.98 (23.74, 28.59). The levels in the elderly were higher than the young and in the urban compared to the rural volunteers. There was no significant influence of the following genetic polymorphisms (Ah receptor (Lys 554), CYP1A1*2A, epoxide hydrolase His 113 and Arg139, NAT2*5, *6, *7, GSTM1*0, GSTT1*0 and GSTP1*B and *C) on DNA damage. Arylamine levels were highest in the urban population although only 2-naphthylamine was significantly higher. However, there was no relation between haemoglobin arylamine levels and DNA damage between individuals. The greater DNA damage in the elderly was probably due to increased oxidative stress and reduced repair. Environmental genotoxin exposure influenced lymphocyte DNA damage but to a lesser extent than age but the elderly urban population was not more susceptible to DNA damage.

850 NEURO-SPECIFIC PROTEINS IN REPRODUCTIVE ORGANS AS POSSIBLE BIOMARKERS FOR ASSESSING ADVERSE EFFECTS OF 1-BROMOPROPANE.

H. Wang[1], H. Ito[2], K. Kato[2], W. Li[1], Y. Takeuchi[1], T. Nakajima[1] and G. Ichihara[1]. *[1]Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan and [2]Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi, Japan.*

Our previous studies revealed that 1-bromopropane (1-BP), an alternative to ozone-depleting solvents, has reproductive toxicity in male and female rats, in addition to its neurotoxicity. As a considerable amount of neuro-specific proteins also exist in the reproductive organs, we considered that they might be related to a common basis for reproductive toxicity and neurotoxicity. The present study investigated neuro-specific proteins in reproductive organs in rats exposed to 1-BP and evaluated their possibility as markers of adverse effects. Thirty-six Wistar male rats were divided into four groups of twelve each and exposed to 1-BP at 200, 400 and

800 ppm, and fresh air for eight hrs per day for 2 wks. At the end of the experiment, the rats were euthanized, the testes and epididymides were dissected out and the right cauda epididymidis was minced for evaluation of sperm count and motility. The remaining organs were used for determination of CK-B, α-S100, β-S100, α-enolase, and γ-enolase with the sandwich-type enzyme immunoassay method. The sperm count did not change significantly, but sperm motility decreased dose-dependently with a significant change at 800 ppm. γ-enolase (per weight of wet tissue) significantly increased in the epididymides in a dose-dependent manner at 200 ppm or over, but did not change in the testes. In contrast, α-enolase significantly increased in the testes at 400 ppm and 800 ppm, but did not change in the epididymides. α-S100 significantly decreased only in the epididymides at 800 ppm, while β-S100 increased in the epididymides at 800 ppm. γ-enolase in the epididymides and α-enolase in the testes were more sensitive than sperm indices. Neuro-specific proteins might serve as sensitive and organ-specific biomarkers for assessing adverse effects of 1-BP on reproductive organs.

851 EFFECT OF L-CARNITINE PRETREATMENT AGAINST 3-NITROPROPIONIC ACID (3-NPA) INDUCED NEUROTOXICITY.

Z. K. Binienda[1], J. L. Summage[1], S. Dufour[2], B. Przybyla-Zawislak[1], A. Virmani[3], W. Slikker[1] and L. C. Schmued[1]. *[1]Neurotoxicology, NCTR, Jefferson, AR, [2]Milieux et Peuplements Aquatiques, Museum National d, Paris, France and [3]Scientific Affairs, sigma-tau Health Science s.p.a., Rome, Italy.*

A plant and fungal toxin, 3-NPA, acts as an inhibitor of mitochondrial function *via* irreversible inhibition of the mitochondrial inner membrane enzyme succinate dehydrogenase (SDH). Inhibition of SDH disturbs the electron transport and leads to cellular energy deficit and neuronal injury. We have shown that pretreatment with L-carnitine, while not significantly attenuating SDH inhibition, prevented hypothermia and oxidative stress-associated increase in activity of free radical-scavenging enzymes. Here, a neurohistological method was applied to examine the effect of carnitine pretreatment against 3-NPA-induced neurotoxicity. Twenty adult male Sprague-Dawley rats were randomly divided into two groups (n=10/group). Rats in the first group were injected with 3-NPA at 30 mg/kg, s.c. twice, two days apart and the second group of animals received L-carnitine pretreatment at 100 mg/kg 30-40 min before 3-NPA administration. Rats in both groups were perfused seven days later and their brains harvested. Degenerating neurons were identified and localized *via* the fluorescent marker Fluoro-Jade B. Briefly, tissue sections were pretreated with 0.06% potassium permanganate followed by incubation in 0.0001% Fluoro-Jade B in 0.1% acetic acid vehicle. Examination of the stained sections with an epifluorescent microscope revealed the following results: Of the three animals that survived 3-NPA dosing, one exhibited no pathology, one exhibited moderate unilateral damage to the striatum and the third exhibited extensive bilateral neuronal degeneration in multiple forebrain regions. Of the seven-surviving animals that received L-carnitine prior to 3-NPA insult, six exhibited no lesions, while one exhibited a modest unilateral lesion of the striatum. It appears that L-carnitine is protective against 3-NPA-induced toxicity, as reflected by both reduced mortality and significantly reduced neuronal degeneration.

852 ANALYSIS OF MOLECULAR INTERACTIONS FOR PROTEIN-LIGAND COMPLEXES: INVERSE DOCKING AND TARGET IDENTIFICATION.

J. Wang and R. Richardson. *Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

Computational studies of molecular interactions are important for drug design and toxicological research. Docking is widely used in drug discovery to screen small molecule ligands using a target macromolecule as a template. In contrast, inverse docking is designed to identify potential macromolecular targets for a small molecule and has been little studied. The present work used computer experiments to investigate the promise of inverse docking in predictive toxicology by analyzing molecular interactions for 50 protein-ligand complexes. Proteins represented diverse biological functions, and ligands varied in structural flexibility and hydrophobicity. Protein-ligand structures were extracted from the Protein Data Bank (PDB). Ligands and proteins were separated to form a small molecule set and a macromolecular target set. Autodock was used to dock small molecules into macromolecules in the target set to find potential targets. Based on computed interaction energies generated by docking, inferences were drawn about the potential of a protein to be a target for a ligand. The results showed that some small molecules interacted with one or a few targets, while others had the potential to interact with many targets. Nevertheless, in 48 of 50 cases, the computed interaction energies of small molecules with their assumed targets (based on the PDB complexes) ranked within the top 3. Furthermore, for 32 of 50 small molecules, computed interaction energies with their assumed targets were ranked first. Thus, inverse docking was relatively effective for identifying potential targets for small molecules. Moreover, this technique has the potential to discover alternative targets for toxicities and mechanisms beyond those originally assumed for small molecules. Finally, this study suggests that a database of targets could be formed using the records of the PDB. This database could be designed for virtual docking evaluations of small molecules, thereby furnishing a rapid screening approach for high-throughput predictive toxicology. (Supported in part by ARO grant DAAD19-02-1-0388).

853 EFFECT OF PHYSICAL EXERTION ON THE BIOLOGICAL EXPOSURE INDICES OF TOLUENE FOLLOWING EXPOSURE BY INHALATION IN HUMAN VOLUNTEERS.

V. Nadeau[1], G. Truchon[2], M. Brochu[3] and R. Tardif[1]. *[1]Occupational and Environmental Health, University of Montreal, Montreal, QC, Canada, [2]Occupational Health, IRSST, Montreal, QC, Canada and [3]Kinesiology, University of Montreal, Montreal, QC, Canada.*

Physical exertion (workload) has been recognized as one of the several factors that can influence the kinetics of xenobiotics within the human body. This study was undertaken to evaluate the impact of physical exertion on the biological exposure indices (BEIs) of TOL in human volunteers exposed under controlled conditions in an inhalation chamber. A group of 4 volunteers (1 woman, 3 men) were exposed to TOL (50 ppm) according to the following scenarios involving either aerobic (AERO), muscular (MUSC) or both (AERO/MUSC) types of physical exercises (exercise bicycle, treadmills, pulleys): REST, 50W AERO, 100W AERO and 50W AERO/MUSC during 7 h; 50W MUSC and 100W MUSC during 3 and 2½ h, respectively. Alveolar air and urine samples were collected at different time intervals before, during and after exposures for the measurement of unchanged TOL in expired air and of urinary o-cresol. Overall, results showed that unchanged concentrations of TOL in expired air measured during and after all scenarios involving physical activities were higher (approximately 1.4 - 2 fold) compared to exposures at rest. However, there was no difference between levels measured at 50W AERO and 100W AERO. All scenarios involving physical exertion also resulted in increased urinary levels of o-cresol (umol/mmol creatinine) (mean ± standard error): $REST_{0\text{-}3h}$: 0.2 ± 0.05, $REST_{3\text{-}7h}$: 0.6 ± 0.06; 50W $AERO_{0\text{-}3h}$: 0.63 ± 0.09, $50W_{3\text{-}7h}$: 1.32 ± 0.25; 50W AERO/$MUSC_{0\text{-}3h}$: 0.48 ± 0.07, 50W AERO/$MUSC_{3\text{-}7h}$: 0.88 ± 0.22; 50W $MUSC_{0\text{-}3h}$: 0.39 ± 0.06). Muscular activity was less effective compared to aerobic exercise. In conclusion, this study showed that BEI values of TOL vary according to the level (W) and type (AERO or MUSC) of physical exertion. Additional experiments aimed at characterising the impact of workload on the BEIs of 4 other solvents are underway. (Supported by IRSST, Quebec)

854 THE USE OF METABONOMICS TO DIFFERENTIATE THE TOXICITY OF TWO MAPK KINASE INHIBITORS IN MICE.

A. P. Brown[1], L. Robosky[3], C. V. Okerberg[1], R. Merriman[2], C. Howard[2], H. Tecle[4] and M. Reily[3]. *[1]Safety Sciences, Pfizer Global Research and Development, Ann Arbor, MI, [2]Cancer Pharmacology, Pfizer Global Research and Development, Ann Arbor, MI, [3]Discovery Technologies, Pfizer Global Research and Development, Ann Arbor, MI and [4]Chemistry, Pfizer Global Research and Development, Ann Arbor, MI.*

Analysis of biofluids, such as urine, by nuclear magnetic resonance (NMR) spectroscopy (metabonomics) can serve as a tool for identifying drug-induced alterations in homeostasis and provide a mechanism for screening compounds for toxicity. This study was conducted to determine whether metabonomics could detect potential differences in toxicity of the structurally related MAPK kinase (MEK) inhibitors PD 254552 and PD 320125-2, which differ only by a methyl group on the imidazole ring. Female Balb/C mice were dosed by gavage twice daily, approximately 12 hours apart, at 400 mg/kg/day for up to 7 days. The control group received vehicle (0.5% hydroxypropylmethylcellulose/0.2% Tween 80) alone. Urine samples, collected pretest and once daily during the dosing period, were analyzed by 600 MHz 1H NMR spectroscopy. Four mice/group were sacrificed on Days 4 and 8, and serum chemistries were measured. Select tissues collected on Day 8 were examined microscopically. PD 254552 produced one mortality, body weight loss, increased serum AST and ALT levels (4- to 10-fold), decreased serum total protein and albumin levels (24% to 48%), and hepatic (degeneration, necrosis) and GI tract lesions (gastric erosion/ulcers, mucosal changes). These changes were not produced by administration of PD 320125-2. Principle components analysis demonstrated a difference between the NMR spectra of PD 254552-treated mice from the other dose groups, which increased with time. Analysis of urinary endogenous metabolites indicated that PD 254552-dosed mice had decreased levels of hippurate, oxoglutarate, and trimethylamine-N-oxide. NMR data in the control and PD

320125-2 groups were similar. In conclusion, metabonomics data correlated with indices of toxicity and differentiated between exposures to structurally related compounds.

855 ASSESSMENT OF GLOBIN S-PROPYLCYSTEINE ADDUCTS AND URINARY N-ACETYL S-PROPYLCYSTEINE AS INTERNAL EXPOSURE MARKERS OF 1-BROMOPROPANE.

G. Ichihara[1], K. Amarnath[2], V. Amarnath[2], H. I. Valentine[2], W. Li[1], H. Wang[1] and W. M. Valentine[2]. *[1]Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan and [2]Pathology, Vanderbilt University Medical Center, Nashville, TN.*

1-Bromopropane(1-BP), an alternative to ozone-depleting solvents, is neurotoxic and is a reproductive toxicant in animals and humans. We previously reported the detection of N-acety S-propylcysteine in the urine of workers exposed to 1-BP. The present study investigated the dose response of urinary N-acetyl S-propylcysteine and of S-propylcysteine in globin and neurofilament preparations as a function of exposure level and duration. Thirty two Wistar male rats were randomly divided into four groups of eight and exposed to 1-BP at 0, 50, 200, or 800 ppm for eight h/day for two wks. The urine was collected both during and after the exposure on the first and final exposure days. At the end of the experiment, blood and spinal cord were obtained. Secondly, 24 male rats were divided into two groups of twelve and exposed to 1-BP at 50 ppm or filtered air for eight h/day, five days/wk for four wks. The urine was collected for 15 h during the night before the first exposure and after the fifth exposure each week. Blood (0.5 mL) was also obtained after the last exposure in every week. After 4 wks 6 exposed and 6 control rats were euthanized and blood and spinal cord obtained. The remaining rats were euthanized 1 wk after the end of exposure to examine the elimination kinetics of the metabolites and protein adduct. Urinary N-acetyl S-propylcysteine was measured with two methods using GC-MS and LC-MS/MS. S-Propylcysteine in globin and neurofilaments were determined using LC-MS/MS. S-propylcysteine adducts were correlated with both the level and duration of exposure whereas the urinary metabolite was correlated to exposure level but did not increase after 1 wk of exposure. These results support the potential of urinary N-acetyl S-propylcysteine and globin S-propylcysteine adducts as biomarkers of 1-BP exposure and demonstrate the ability of 1-BP to covalently modify proteins within the nervous system.

856 DETERMINATION OF BIOLOGICAL REFERENCE VALUES FOR CHLORPYRIFOS METABOLITES IN HUMAN URINE USING A TOXICOKINETIC APPROACH.

M. Bouchard[1], G. Carrier[1], R. C. Brunet[2], N. H. Gosselin[1] and Y. Bonvalot[1]. *[1]Environmental & Occupational Health, University of Montreal, Montreal, QC, Canada and [2]Mathematics & Statistics, University of Montreal, Montreal, QC, Canada.*

Urinary biomarkers of chlorpyrifos (CPF) exposure are increasingly being measured in field studies although biological guidelines are not yet available to assess health risks in workers. In this study, a toxicokinetic approach is proposed for the determination of biological reference values for CPF metabolites in urine. As a first step, a toxicokinetic model for CPF and its metabolites was developed and validated using the human kinetic time course data available in the literature. The model was built to link the absorbed dose of CPF under a variety of exposure routes and temporal scenarios to the urinary excretion of its major metabolites, 3, 5, 6-trichloro-2-pyridinol (3, 5, 6-TCP) and alkyl phosphates (AP). The model was then used to predict guidance values of urinary biomarkers of CPF exposure below which workers should not experience adverse health effects, whatever their exposure conditions. This was achieved by linking i) a literature reported repeated CPF no-observed-effect level (NOEL) daily dose for the inhibition of red-blood-cell acetylcholinesterase to a corresponding absorbed daily dose on the one hand and ii) the absorbed daily dose to the urinary excretion of CPF metabolites on the other hand. Model simulations under a variety of exposure scenarios showed that the safest biological reference values are obtained from a dermal exposure scenario with the slowest possible absorption rate rather than from respiratory or oral exposure scenarios. Also, model simulations showed that, for a given total absorbed dose, absorption over 8 hours results in smaller 3, 5, 6-TCP and AP urinary excretion rates than those obtained from shorter exposure durations. From these considerations, biological reference values were derived by simulating an 8-h dermal CPF exposure such that the total absorbed daily dose corresponds to the absorbed NOEL. These guidance values are proposed in the form of total amounts of 3, 5, 6-TCP and AP metabolites collected in urine over conveniently chosen time periods.

857 DNA ADDUCT FORMATION AND TOXICITY OF TRANS-2-HEXENAL IN MALE F344 RATS.

M. D. Stout[1], E. Bodes[1], Y. Li[2], P. B. Upton[2], R. Schoonhoven[2], J. Nakamura[2], Y. Jeong[2], R. Sangaiah[2] and J. A. Swenberg[1, 2]. *[1]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC and [2]Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC.*

Trans-2-hexenal (hexenal) has been given generally regarded as safe (GRAS) status as a flavoring agent by the Flavor and Extracts Manufacturers Association (FEMA) expert panel, even though it is genotoxic *in vitro*. Hexenal also forms exocyclic 1, N^2-propanodeoxyguanosine adducts (H-dGuo) upon reaction with DNA. Since hexenal exerts its biological effects without metabolic activation, we hypothesized that the dose-response of both H-dGuo formation and its toxicity would be sub linear, due to saturation of detoxification. We have developed a highly sensitive and specific mass spectrometry based method for quantitating H-dGuo in DNA. Eight-week old male F344 rats were exposed to single doses of 0, 50, 200, or 500 mg hexenal/kg b.w. by oral gavage, as in a previous study (Schuler and Eder (1999) Carcinogenesis 20, 1345-1350). In that study, no toxicity was reported and the highest concentrations of H-dGuo were found in the forestomach, liver, esophagus, and kidney when measured by ^{32}P-postlabeling of DNA from rats sacrificed two days after dosing. We observed moderate to severe lesions in the forestomachs of hexenal-exposed rats, with coagulative necrosis and severe edema at the high dose. Furthermore, in preliminary experiments, we were not able to quantify H-dGuo in liver DNA (n=4) of rats exposed to 500 mg hexenal/kg b.w. or forestomach DNA (n=2) of rats exposed to 200 mg hexenal/kg b.w. two days after dosing. Thus, we were not able to confirm the results of the previous report. We are continuing to analyze samples from additional time points and exposing F344 rats to repeated doses of hexenal, in order to characterize toxicity and H-dGuo formation under these conditions. This work was supported in part by grants from the NIEHS (T32ES07126, ES11746, P30ES10126), the NCI (P30CA16086, T32CA72319) and the Flavor and Extract Manufacturers Association (FEMA).

858 ORAL TREATMENT WITH 13-CIS-RETINOIC ACID (13-CIS-RA) OR ALL-TRANS-RETINOIC ACID (ALL-TRANS-RA) ALTERS SERUM LEVELS OF ALBUMIN (ALB), TRIGLYCERIDES (TRIG), TOTAL PROTEIN AND GLUCOSE OF RATS.

F. J. Cisneros[1], B. J. Gough[1], R. E. Patton[2] and S. A. Ferguson[1]. *[1]Division of Neurotox, NCTR/FDA, Jefferson, AR and [2]Charles River Laboratories, Jefferson, AR.*

13-cis-RA has been used to treat acne vulgaris since 1982. Its dermatological effect seems to occur *via* isomerization to all-trans-RA. Although the package insert suggests followup lab testing, recent reports indicate that except for teratogenicity, it is safe and effective. Thus, some suggest there is no need for routine lab followup. We evaluated the effects of 13-cis-RA and all-trans-RA on serum chemistry, leptin and adiponectin levels. Adult male and female Sprague-Dawley rats were gavaged daily for 7 days with 13-cis-RA (7.5 or 15 mg/kg), all-trans-RA (10 or 15 mg/kg) (n=24/sex/dose), or soy oil (n=16/sex) and blood collected after the last gavage. Body weight was unaffected by RA treatment. There were no RA treatment effects on levels of blood urea nitrogen, aspartate amino transferase, leptin, or adiponectin. Sex differences were noted for levels of cholesterol, creatinine, TRIG, ALB, alanine amino transferase (ALT), and total protein. Despite an RA treatment effect on ALT levels, no treated group was statistically different from controls. However, all-trans RA treatment (10 & 15 mg/kg) reduced ALB levels to ≈90% of control ($p<0.05$) and reduced total protein levels to ≈93% of control ($p<0.05$) while substantially elevating TRIG levels ≈66-99% above control ($p<0.05$). Additionally, TRIG levels of the 15 mg/kg 13-cis RA group were ≈62% higher than controls ($p<0.05$) and total protein levels were ≈5% less ($p<0.05$). Glucose levels were impacted by sex and RA treatment in that males treated with 15 mg/kg of 13-cis-RA or 10 mg/kg all-trans-RA had lower levels than same-sex controls (13-19% lower) ($p<0.05$); however, females were not affected. As expected, all-trans-RA is more potent than 13-cis-RA on a mg/kg basis. Our results suggest that in addition to recommended followups in blood lipid analysis and hepatic function, glucose levels should be monitored with special attention in males.

859 EVALUATION OF THREE SERUM BIOMARKERS OF HEPATOTOXICITY: MALATE DEHYDROGENASE, PARAOXONASE, AND PURINE NUCLEOSIDE PHOSPHORYLASE.

S. J. Schomaker, D. E. Amacher, I. M. Pruimboom-Brees and M. L. Mirsky. *Safety Sciences Groton, Pfizer Global Research and Development, Groton, CT.*

The objective of these studies was to evaluate three potential serum biomarkers of hepatotoxicity: malate dehydrogenase, paraoxonase, and purine nucleoside phosphorylase. These markers were originally identified in the Pfizer/OGS collaboration

and are targeted as those serum proteins with the greatest potential to serve as sensitive, early indicators of dose-dependent liver toxicity including steatosis, hepatocellular necrosis, or hepatobiliary injury. In the first study, male and female CD rats were dosed with acetaminophen, α-naphthylisothiocyanate, phenobarbital, or galactosamine. All of the compounds, at the specified doses, have been shown in the literature to cause increases or decreases in one or more of the proposed biomarkers. Clinical pathology and histopathology procedures were completed for correlation of the serum biomarker results to physiological toxicity. The results from the serum samples from the four rat studies paralleled the results from studies with the same compounds published in the literature. All three assays showed detectable protein levels in normal rat serum from both males and females, had a low coefficient of variation in control populations, and were easily adapted to the Cobas Fara (a clinical chemistry autoanalyzer). In a second study, male and female CD rats were dosed with acetaminophen, α-naphthylisothiocyanate, galactosamine, thioacetamide, or allyl alcohol. Clinical pathology and histopathology analyses were completed for correlation of the serum biomarker results to physiological toxicity. In general, the assays are at least as sensitive as traditional clinical chemistry hepatotoxicity assays. With allyl alcohol and α-naphthylisothiocyanate, both associated with periportal necrosis, the assays appear to be more sensitive than traditional clinical chemistry hepatotoxicity assays.

860 HEMOGLOBIN ADDUCTS OF 3, 4-EPOXY-1, 2-BUTANEDIOL IN RODENTS EXPOSED TO 3-BUTENE-1, 2-DIOL.

M. W. Powley[1], P. B. Upton[1], V. E. Walker[2] and J. A. Swenberg[1]. [1]*Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC and* [2]*Lovelace Respiratory Research Institute, Albuquerque, NM.*

1, 3-Butadiene (BD) is a rodent carcinogen and may also be a human carcinogen. BD metabolism yields several mutagenic epoxides whose contribution to carcinogenicity is not fully understood. 1, 2:3, 4-diepoxybutane and 1, 2-epoxybutene cannot completely account for the mutagenic effects of BD, suggesting that other reactive metabolites may be involved. Two potential sources arising from 3-butene-1, 2-diol (BD-diol) are 3, 4-epoxy-1, 2-butanediol (EB-diol), a known mutagen, and hydroxymethylvinyl ketone (HMVK), a compound expected to be mutagenic. Recent experiments have shown that BD-diol increases the frequency of *Hprt* mutations in splenic T-cells from rodents. Furthermore, based on a comparison of mutagenic potencies the BD-diol pathway may account for most mutagenicity in rats exposed to 625 ppm BD and contributes to the mutagenicity in mice. The objective of our current research was to measure *N*-(2, 3, 4-trihydroxybutyl)valine (THB-Val), an EB-diol derived hemoglobin adduct, to understand the involvement of this metabolite in BD-diol induced mutagenicity. To accomplish this, female B6C3F1 mice and female F344 rats were exposed to 0, 6, 18, and 36 ppm BD-diol for 4 weeks (6 hr/day x 5 days/week). Globin samples were processed and analyzed by GC-MS/MS according to a published method. Results showed that mice formed 1.01 ± 0.74, 12.3 ± 2.2, 29.2 ± 1.2, and 35.8 ± 1.6 pmol THB-Val/mg globin (mean ± SD, n≥3) at 0, 6, 18, and 36 ppm BD-diol, respectively. At the same exposure concentrations rats formed 0.37 ± 0.23, 5.8 ± 1.2, 14.75 ± 0.98, and 20.9 ± 1.3 pmol adduct/mg globin (mean ± SD, n≥3). The adduct dose-response mimicked the increase in *Hprt* mutation frequency observed in rodents following exposure to BD-diol. While the contribution to mutagenicity made by HMVK must still be determined, the results of our current study, along with earlier studies using BD or other metabolites, show that EB-diol may in part be responsible for the mutagenicity observed in rodents exposed to BD-diol or BD.

861 ACB-PCR MEASUREMENT OF *p53* MUTATION: A POTENTIAL BIOMARKER OF SKIN CANCER DEVELOPMENT.

T. L. Verkler[1], L. H. Couch[2], B. J. Miller[2], P. C. Howard[2] and B. L. Parsons[1]. [1]*Division of Genetic and Reproductive Toxicology, NCTR, USFDA, Jefferson, AR and* [2]*Division of Biochemical Toxicology, NCTR, USFDA, Jefferson, AR.*

p53 codon 270 CGT to TGT mutation is frequently detected in mouse squamous cell carcinomas (SSCs) induced by simulated solar light (SSL). Measurement of this mutation is being developed as an early biomarker for skin carcinogenesis. Allele-specific Competitive Blocker-PCR (ACB-PCR) is a sensitive, DNA-based method for the detection of rare point mutations. The method relies on selective PCR amplification of a mutation using a primer with more mismatches to wild-type than mutant DNA. ACB-PCR also uses a non-extendable blocker primer with more mismatches to mutant than wild-type DNA to reduce the signal produced from wild-type template. Mutant and wild-type PCR products were mixed in ratios corresponding to mutant fractions of 10^{-2} to 10^{-4} and compared with a no-mutant control of only wild-type PCR product. Reaction conditions were varied systematically until the assay routinely distinguished between the no-mutant control and the 10^{-4} mutant faction. Thus, the ACB-PCR assay can quantify *p53* mutation within a 10, 000-fold excess of wild-type allele. To characterize the utility of the assay, eight SSL-induced SSCs were analyzed for *p53* codon 270 CGT to TGT mutation. Genomic DNA from tumors was used to synthesize a PCR product identical to the mutant and wild-type standards. Equivalent copies of each PCR product were then analyzed in parallel with mutant fraction standards. In three independent ACB-PCR experiments, the *p53* mutant fraction in each sample was measured relative to a standard curve constructed from the mutant fraction standards. The *p53* mutation was detected as a subpopulation in each of the eight tumor DNAs analyzed. Specifically, three SSCs had *p53* mutant fractions between 10^{-4} and 10^{-3}. Five SSCs had *p53* mutant fractions between 10^{-3} and 10^{-2}. These results demonstrate that ACB-PCR can be used to quantify *p53* codon 270 CGT to TGT mutation and this mutation may be a useful biomarker for studying tumor induction and progression in response to SSL.

862 CYCLIC *N*-TERMINAL HEMOGLOBIN ADDUCT IN HUMANS, RATS AND MICE EXPOSED TO BUTADIENE.

N. I. Georgieva, G. Boysen, Y. Li and J. A. Swenberg. *Environmental Sciences and Engineering, UNC, Chapel Hill, NC.*

1, 3-Butadiene (BD) is an industrial chemical that is carcinogenic in rodents and probably humans. BD is oxidized by P450 to 1, 2:3, 4-diepoxybutane (DEB), the most mutagenic BD metabolite. DEB reacts with *N*-terminal valine in hemoglobin, forming the cyclic adduct (2, 3-dihydroxypyrrolidin-1-yl)-3-methybutanoic acid (pyrV), a potential biomarker that is specific for DEB. Sensitive isotope dilution mass spectrometric assays were developed to measure pyrV in globin from mice, rats and humans, exposed to BD or DEB. The procedure uses trypsin hydrolysis of globin, immunoaffinity (IA) purification of peptides (with antibodies specifically raised against the adducted human peptide), and quantitation of the *N*-terminal heptapeptides by capillary LC-ESI-MS/MS. The necessary internal standards (IST, D_3 labeled) for each species were synthesized and characterized by ESI-MS/MS sequencing. Quantitation was performed by LC-ESI-MS/MS in SRM mode, using both singly and doubly charged ions. This method readily detects 25 fmol of human analytical standard on column. The overall recovery was 50-90% for *in vivo* samples. Preliminary results show that the pyrV adduct was below the detection limit in 10 BD workers with exposures ranging from 0.1 to 4.2 mg/m^3 when 200 mg of globin was processed. In samples from rats exposed to 2.5 and 5 ppm DEB (4 weeks), the adduct was measured at 1.6 and 2.5 pmol/mg globin, respectively (using the human IST). In rats exposed to 1000 ppm BD (90 days) the amounts of pyrV adducts were 3-4 times lower than in the DEB exposed rats. In mice exposed to 62.5 ppm BD (4 weeks) and 1250 ppm BD (10 days) the amounts were 0.1 and 2.5 pmol/mg globin, respectively. These results provide preliminary data on the use of pyr-V as a specific and selective biomarker for internal exposure to DEB. This novel biomarker represents a useful tool to study differences in the metabolism and carcinogenicity of BD in mice, rats and humans, as well as potential gender differences and possible effects of genetic polymorphisms.

863 DETERMINING A REFERENCE VALUE FOR BLOOD CHOLINESTERASE USING US DEFENSE DEPARTMENT PERSONNEL.

S. A. McCurdy[1], J. D. Henderson[2], D. E. Arrieta[2], L. J. Lefkowitz[3], R. E. Reitstetter[4] and B. W. Wilson[2]. [1]*Epidemiology and Preventative Medicine, University of California, Davis, CA,* [2]*Environmental Toxicology, University of California, Davis, CA,* [3]*US Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD and* [4]*Clinical Investigation, Brooke Army Medical Center, San Antonio, TX.*

The use of pesticides and threats of chemical warfare establish the need for high throughput reliable standardized determinations of blood cholinesterase (ChE) as early warnings of exposure. The US Army Center for Health Promotion and Preventive Medicine (CHPPM) uses a ΔpH method based on that of Michel for the determination of red blood cell cholinesterase levels in humans. With it they monitor approximately 25, 000 DOD personnel annually. Our goal is to establish a normal range of blood ChE using ΔpH data. Blood specimens from 991 Department of Defense personnel without a potential exposure to anticholinesterase agents enrolled in a required occupational health screening program were statistically analyzed. The median age was 43 years (range 18-76). 823 specimens (82.1 %) were from men. Men were on average older than women (median age 44 vs 37 years, p<0.0001, Wilcoxon test). The mean ±SD for ΔpH was 0.75 ±0.06 units. ΔpH values were greater for men than for women (0.74 vs. 0.73, p<0.0006, Wilcoxon test). Multivariate linear regression analysis showed an association for ΔpH with age (slope +0.0008 ΔpH units for each year of age, p<0.001). There was a small, but statistically significant, reduction in ΔpH associated with test date (-0.006 ΔpH units per 100 days, p<0.001). A multiple regression model incorporating age, gender, and test date explained only 3.4% of the observed variance. The small magnitude of these effects and their minimal role in accounting for

the observed variability suggest it appropriate to ignore such factors when evaluating ΔpH data. The next step in our project is to correlate the ΔpH values with those from the more commonly used colorimetric Ellman test. Supported by DOD (DAMD17-01-1-0772), NIOSH (#CDC U07/CCU906162-06) and NIEHS (#ESO5707).

864 IDENTIFICATION OF INTER-INDIVIDUAL VARIATION IN AFLATOXIN METABOLIZING ENZYMES USING HUMAN URINARY DNA.

B. Dash, E. Afriyie-Gyawu, W. Porter, H. J. Huebner and T. D. Phillips. *Faculty of Toxicology (VAPH), Texas A&M University, College Station, TX.*

Aflatoxin B_1(AFB_1) is a factor in the etiology of hepatocellular carcinoma (HCC). Xenobiotic metabolizing enzymes (XMEs) such as mixed-function oxidases, microsomal epoxide hydrolases (mEH) and glutathione S-transferases (GSTs) are involved in the metabolism of AFB_1. It has been shown that AFB_1-albumin adduct levels may be modified by *mEH* and *GST* genotypes. Moreover, it has been hypothesized that inherited differences in DNA sequence of *mEH* and *GST* genes influence an individual's risk of HCC in response to AFB_1. In order to study genetic differences in these XMEs, a simple and non-invasive DNA extraction procedure was followed. Quantifiable amounts of DNA (9.5-573 ng ml^{-1} urine) were extracted from ≤4.5 ml of urine from AFB_1-exposed individuals and amplicons of 162 bp and 357 bp were generated for single nucleotide polymorphism (SNP) analysis of *mEH* exon 3 (*Y113H*) and exon 4 (*H139R*), respectively, by PCR-RFLP. In a single run, 49 (55%) and 71 (79%) of 89 samples were genotyped for *mEH* exon 3 and exon 4 polymorphisms, respectively. For *mEH* exon 3 SNP typed, there were 37 wild types (*113YY*), 11 heterozygotes (*113YH*) and 1 homozygote (*113HH*). All individuals SNP typed for *mEH* exon 4 were heterozygotes (*139HR*). Urinary DNA was also analyzed by multiplex-PCR to identify deletion polymorphisms in *GSTM1* and *GSTT1* genes using a fragment of the albumin gene as an internal control. Presence or absence of amplicons of 215 bp (*GSTM1*) and 450 bp (*GSTT1*) were indicative of gene polymorphisms. A total of 62 of 89 individuals were genotyped for *GSTM1* and *GSTT1* polymorphisms. Twenty-one and 48 individuals were found to be *GSTM1* and *GSTT1* null respectively, while 20 individuals were doubly null. These results suggest that exfoliated nucleated cells in urine can be a significant source of DNA and may be useful as a non-invasive method for assessing the genetic susceptibility of AFB_1 exposed populations to HCC (Supported by USAID LAG-G-00-96-90013-00, NIEHS P42-ES04917, and NIEHS Center Grant ES09106).

865 NEW APPROACH FOR MONITORING EXPOSURE TO ENVIRONMENTAL TOXIC AGENTS.

T. Berman-Shlomovich and University. Wormser. *Institute of Life Sciences, Jerusalem, Israel.*

One of the major limitations of the current methods of biological detection of exposure to hazardous environmental agents is their inability to detect long-term exposures, and exposures that occurred in the past without immediate clinical effects. In the current study we examined the potential of a new bioassay that is based on the hypothesis that serum of exposed individuals contains a toxic factor or factors produced by an affected cell or tissue in response to an exposure. In the present study PC12 cell cultures were exposed to serum samples of rats treated with the organophosphate chlorpyrifos. We observed a decrease of up to 31.8% and 40% in cell *via*bility and nerve growth factor-induced neurite outgrowth, respectively, in PC12 cells treated with serum of chlorpyrifos-exposed rats, in comparison to cells treated with serum of control rats. Maximal effect was observed for both parameters 4 weeks after exposure whereas motor activity and cholinesterase activity returned to normal levels within one week after the exposure; thereafter the rats showed no signs of toxicity, including 4 weeks post-exposure. These results demonstrate the potential of the proposed method to detect environmental exposures long after they have occurred.

866 EVALUATION OF TWO COMMERCIALLY AVAILABLE CARDIAC TROPONIN IMMUNOASSAYS FOR THE DETECTION OF DRUG-INDUCED CARDIOTOXICITY IN RATS.

C. Bozynski[1], A. Lambert[2], J. Lugo[2], J. D. VanNess[2], C. LaBare[2], J. R. Sibley[2] and D. B. Walker[2]. *[1]Faculty of Veterinary Medicine, University of Montreal, Saint Hyacinthe, QC, Canada and [2]Drug Safety, Wyeth Research, Chazy, NY.* Sponsor: F. Kirchner.

Cardiac troponin I (cTnI) and T (cTnT) are established tissue-specific biomarkers of acute myocardial injury in humans. To determine the relative value of cTnI (DPC Immulite) or cTnT (Roche Elecsys) for detection of drug-induced cardiotoxicity in rats, serum concentrations of these analytes were measured in male rats given doxorubicin using both acute and chronic dosing protocols. These levels were compared with concentrations of standard serum chemical markers of tissue injury (e.g., LDH, CPK), light microscopic findings and immunohistochemical staining of cTnI and cTnT in the myocardium. Results demonstrated good correlation between measurable serum TnT levels and doxorubicin-induced myocardial degeneration in rats. Correlations were less robust between histologically observed cardiac lesions and serum TnI levels, or the other serum markers evaluated. Immunohistochemistry of heart sections for either TnI or TnT demonstrated diffuse staining of the myocardium with attenuated, or absent staining of the degenerate myofibers indicating that cTnI and cTnT immunostaining of the myocardium can complement light microscopy in delineating foci of injury. In summary, evaluation of serum cardiac troponin T appears to be valuable for detection of some types of drug-induced cardiotoxicity in rats.

867 COMPARISON OF UNCHANGED n-HEXANE IN ALVEOLAR AIR AND 2, 5-HEXANEDIONE IN URINE FOR THE BIOLOGICAL MONITORING OF n-HEXANE EXPOSURE IN HUMAN VOLUNTEERS.

G. Hamelin[1], G. Truchon[2] and R. Tardif[1]. *[1]Occupational and Environmental Health, University of Montreal, Montreal, QC, Canada and [2]IRSST, Montreal, QC, Canada.*

Biological monitoring of exposure to n-hexane (HEX) is based on the measurement of free urinary 2, 5-hexanedione (2, 5-HD) (no hydrolysis). The current ACGIH biological exposure index (BEI) value (3.5 μmol/L) was derived from 4 field studies involving worker exposures to variable concentrations of HEX and to other solvents. This study was undertaken to characterize, for 5 consecutive days, the relationship between HEX exposure (25 and 50 ppm) and 1) 2, 5-HD urinary excretion and, 2) HEX in alveolar air. Five volunteers (3 women and 2 men) were exposed to HEX in an exposure chamber for 2 non consecutive weeks (7 hours/day). They were exposed to 50 ppm HEX, during the first week, and to 25 ppm during the second week. Unchanged HEX in alveolar air, and urinary 2, 5-HD (with or without acid or enzymatic hydrolysis) were measured. Concentrations of HEX in alveolar air were 18 ppm (25 ppm) and 37 ppm (50 ppm) which shows that 73% of inspired HEX was excreted unchanged. The urinary 2, 5-HD concentrations (mean ± standard de*via*tion) for the last 4 hours of exposure (day 5) following exposure to 50 ppm HEX were 30.4 ± 7.8 μmol/L (acid hydrolysis), 5.8 ± 1.0 μmol/L (enzymatic hydrolysis) and 6.2 ± 0.9 μmol/L (without hydrolysis). Following exposure to 25 ppm HEX, the urinary concentrations were 15.2 ± 1.9 μmol/L, 3.1 ± 0.7 μmol/L and 3.7 ± 0.5 μmol/L, respectively. The 2, 5-HD value measured in this study following exposure to 50 ppm (6.2 μmol/L), which is higher (X1.8) than the average value reported in field studies better reflects the levels of exposure to HEX alone. Inter-ethnic differences caused by genetic polymorphism, and/or metabolic interferences due to co-exposure to other solvents are likely responsible for the lower 2, 5-HD values measured in previous field studies. In conclusion, the current BEI value for HEX is most likely more protective than what has been believed up until now. (Supported by IRSST, Quebec, Canada)

868 URINARY 3-BROMOPROPIONIC ACID: AN EFFECTIVE GAS CHROMATOGRAPHIC TEST METHOD FOR QUANTIFICATION.

C. B'Hymer[1] and K. L. Cheever[2]. *[1]BHAB, NIOSH, Cincinnati, OH and [2]BHAB, NIOSH, Cincinnati, OH.*

3-Bromopropionic acid is a metabolite and possible biomarker for exposure to 1-bromopropane. 1-Bromopropane is used as an industrial solvent and exposure is a health concern for industrial workers due to its toxicity. Central neurological disorders and peripheral neuropathy has been reported in workers chronically exposed to materials composed of 1-bromopropane in the United States and animal studies have shown reproductive toxicity. Occupational exposure to this widely used industrial solvent is likely, since it is readily absorbed through the skin. A simple and effective general test method for 3-bromopropionic acid in urine samples was developed to monitor any exposed population. Urine specimens were first spiked with 3-chloropropionic acid which was used as a procedural internal standard. The samples were extracted with ethyl acetate, concentrated, and treated with N-methyl-N-[tert-butyldimethylsilyl] trifluoroacetamide (MTBSTFA) to produce the corresponding t-butyldimethylsilane derivatives of 3-bromopropionic acid and the internal standard. Quantification was by means of a gas chromatograph (GC) equipped with a mass selective detector (MSD) using a 50-m X 0.20-mm (id) HP-1 capillary column. A temperature program of 60 to 255oC was used for the gas chromatographic measurement. Ion m/z 211 was monitored for the derivative of 3-bromopropionic acid and ion m/z 165 was monitored for the internal standard. Average recovery of known 3-bromopropionic acid fortified blank urine samples was between 93-98% with relative standard de*via*tions as high as 5.7% using samples at 3-bromopropionic concentrations of 2, 10, 20 and 50 μg/ml . The limit of detection (LOD) for the developed procedure was found to be approximately 0.01 μg/ml in urine.

869 CREATININE ADJUSTED URINARY EXCRETION OF 3, 5, 6-TRICHLOROPYRIDINOL (TCP) BY CHILDREN AGED 4 TO 12 AND THEIR PARENTS.

R. I. Krieger[3, 1, 2], M. R. Oliver[3, 1], R. L. Williams[2, 3, 1] and X. Zhang[2, 3, 1]. *[1]Department of Entomology, University of California, Riverside, Riverside, CA, [2]Environmental Toxicology Graduate Program, University of California, Riverside, Riverside, CA and [3]Personal Chemical Exposure Program, University of California, Riverside, Riverside, CA.*

Complete 24-h urine samples are less often available in exposure studies than spot samples, especially for young children. Thus urinary creatinine (Cn) is useful to estimate 24-h urine volume with norms of 1.7 g/day for adult males and 1.0 g/day for adult females (ICRP, 1994). With biomarker expressed as ug /g Cn and Cn norms, daily biomarker excretion can be estimated. The relationship of daily urinary Cn excretion and age for children is not verified in exposure assessment. Cn excretion to age 12 increases at a rate of 0.08 g/year of age (Krieger, 2001). This study will clarify the relationship of Cn excretion to age and evaluate its use in pesticide exposure studies. In 2001, 42 children below 12 yrs from 13 California and 10 Missouri families and 36 California persons (>12 years of age) were recruited to participate a pesticide exposure study in which 6 consecutive 24-h urine specimens were collected. Urinary Cn was measured for all samples and TCP as a biomarker of chlorpyrifos (CP) was analyzed for the California samples. The increase of Cn excretion rate for children aged 4 to 12 was 0.075 g/year of age. The linear regression equation is urinary Cn = 0.075 × age − 0.035 (R2 = 0.71). Using this factor, urinary TCP was adjusted and the absorbed daily dosage (ADD) of CP equivalents was calculated. ADD of CP equivalents based on 24-hour urine volumes correlates with the Cn-adjusted ADD (R^2 = 0.80). The ADDs for California adults and children are 0.2 and 0.4 ug/kg-day equivalents CP ($p<0.05$), a lesser difference than often suggested. Additionally urinary Cn excretion for children aged 4 to 12 increased 0.075 g Cn/yr. This factor can be used to estimate 24-h urine volume from spot samples. This procedure may help resolve ongoing concerns about children's pesticide exposures.

870 DETERMINATION OF ETHYL GLUCURONIDE IN BIOLOGICAL MATRICES USING REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH ELECTROCHEMICAL DETECTION.

R. Kaushik[1, 2, 3], W. R. LaCourse[2], B. Levine[3, 1] and K. Squibb[1]. *[1]Toxicology, University of Maryland, Baltimore, Columbia, MD, [2]Chemistry, University of Maryland, Baltimore County, Baltimore, MD and [3]Office of the Chief Medical Examiner, Baltimore, MD.*

Currently no method exists to distinguish between ethanol levels detected in biological matrices due to alcohol consumption versus ethanol production after death as a result of decomposition. Ethyl glucuronide (EtG) is a non-volatile, water-soluble metabolite of ethanol that can serve as a biological marker of alcohol consumption. It is a highly sensitive and specific alcohol consumption marker that can be detected for up to 80 hours after alcohol elimination from the body. This has very important clinical and forensic applications. The current study involved developing a method for the detection of EtG in postmortem urine and blood samples using reversed-phase liquid chromatography with electrochemical detection. Methyl glucuronide served as the internal standard. The mobile phase consisted of 1% acetic acid/water and acetonitrile (98:2), with a 600mM sodium hydroxide post-column system attached to enable pulsed electrochemical detection (PED). This amperometric detection technique applies alternated positive and negative potential pulses at a noble metal electrode. The analyte is oxidized followed by oxidative and reductive cleaning steps. The analyte concentration is determined by measuring the electric current resulting from the molecule gaining or losing electrons. In order to separate EtG from the biological matrix a solid-phase extraction (SPE) was developed using aminopropyl columns after deproteination with hydrochloric acid. EtG was found to have a retention time of 5.3 minutes with LOQ and LOD values of 58 and 11 ug/mL respectively. The range of EtG found in postmortem samples was 3 to 700 ug/mL. Compound recovery following SPE was approximately 50%. This method showed good reproducibility and sensitivity. It is now available as a tool to clinical and forensic toxicologists for determining levels of alcohol consumption in live and deceased individuals.

871 2′-MOE ANTISENSE OLIGONUCLEOTIDES STIMULATE A PROINFLAMMATORY RESPONSE BY A TLR9-INDEPENDENT MECHANISM.

J. J. Senn, S. Burel, R. Kadri, T. Pham and S. Henry. *Toxicology, ISIS Pharmaceuticals, Carlsbad, CA.*

Oligos with a bacterial-like ″CpG″ have been shown to trigger proinflammatory responses by activating the Toll-like receptor (TLR)-9. Oligos with CpG motifs have been developed to exploit this process for use as adjuvant and cancer therapies. However, oligos intended for antisense applications (ASOs) are designed to avoid these proinflammatory responses by avoiding CpG motifs and using chemical modifications (i.e., 2′-MOE sugars and methylated cytosine residues).Although modified, ASOs are capable of eliciting a proinflammatory response in rodents at high doses. To determine if this phenomena is TLR-mediated, wild type, TLR9 and MyD88 knockout mice were treated with a CpG optimal oligo 12449, and several non-CpG ASOs. The non-CpG ASO compounds appear to have a lower potency to induce proinflammatory effects requiring a 10-fold higher dose. The inflammatory response in knockout animals to 12449 was TLR9 dependent while non-CpG ASO compounds still activated a proinflammatory response indicated by increased spleen weight and the expression of proinflammatory genes. These data implicate a TLR9-independent mechanism of action by non-CpG ASO at high concentrations. Isolated bone marrow from wild type and knockout animals treated with non-CpG ASOs responded similar to the whole animal, with production of MIP-2 and activation of MAPK pathways. We have characterized an *in vitro* system using mouse macrophage cell line RAW 264.7, that exhibit a dose-dependent increase in both ERK1/2 and p38 activation in response to treatment with ASOs, suggesting a receptor-mediated event. After 3 hr of exposure, RAW 264.7 cells release MIP-2 and MIG, and TNFα into the culture media. Also, to begin to investigate species specificity, we have examined a human macrophage cell line, U937, this cell line responds in a dose-dependent manner to ASOs treatment by activating ERK1/2 and JNK. These data suggest that there is a TLR9-independent mechanism for the stimulation of a proinflammatory response in mice to high dose non-CpG ASO treatment and that this mechanism is likely a receptor-mediated phenomenon.

872 DISTINCTIVE EFFECTS OF TOLL-LIKE RECEPTOR LIGANDS AND ETHANOL *IN VIVO, IN VITRO*, AND IN A MACROPHAGE CELL LINE: IMPLICATIONS FOR SIGNALING MECHANISMS.

Q. Zheng, R. Fan, C. Schwab and S. B. Pruett. *Cell. Biol. & Anatomy, LSU Health Sciences. Center, Shreveport, LA.*

A number of important events in innate immunity are initiated through toll-like receptors (TLRs), and the macrophage-like cell line RAW 264.7 has been extensively used to delineate signaling pathways involved in the induction of various responses. Thus, it is important to determine if the responses of these cells correspond to responses of normal macrophages (or other cell types) *in vivo* or *in vitro*. In other studies, we have determined that most of the cytokines measured in peritoneal lavage fluid are not derived from blood and were likely produced locally in the peritoneal cavity. Thus, the effects of two TLR ligands (LPS, a TLR 4 ligand and poly I:C, a TLR 3 ligand) and an inhibitor of TLR signaling (ethanol) were examined *in vivo*, with peritoneal macrophages *in vitro* and with RAW 264.7 cells *in vitro*. The results suggest that there are important differences in TLR signaling between peritoneal macrophages *in vivo* and *in vitro* compared to RAW 264.7 cells *in vitro*. In response to poly I:C, peritoneal fluid IL-10 was low (20-100 pg/ml), and IL-12 was higher (150-300 pg/ml). Ethanol given at the same time as poly I:C had no effect or increased IL-10 and decreased IL-12 concentrations. In contrast, IL-10 concentrations from peritoneal macrophages or RAW 264.7 cells in culture were much higher than IL-12 concentrations in response to poly I:C (4000-7500 pg/ml vs. <100 pg/ml). A similar pattern was noted at earlier time points in culture (4 vs. 24 hr), and a similar pattern was noted for serum concentrations of IL-10 and IL-12 in comparison with concentrations in spleen cell cultures. Ethanol did not produce the same types of effects on cytokine concentration *in vivo* as *in vitro*. These results suggest that there are fundamental differences in regulation of cytokine production *in vivo* and *in vitro* and that this should be considered in interpreting results from experiments using *in vitro* systems. This work was supported by a grant from NIAAA (ES09505).

873 PRE-ACTIVATION OF TOLL-LIKE RECEPTORS SENSITIZE MACROPHAGES TO INDUCTION OF PROINFLAMMATORY CYTOKINE GENE EXPRESSION BY DEOXYNIVALENOL AND OTHER MICROBIAL TOXINS.

J. J. Pestka[1, 2] and H. Zhou[1]. *[1]Food Science and Human Nutrition, Michigan State University, East Lansing, MI and [2]Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI.*

Stimulation of the innate immune response has the potential to increase sensitivity to xenobiotics. Asynchronous sensitization by LPS and/or other Toll-Like Receptor (TLR) agonists to induction of the proinflammatory cytokines IL-1 beta, IL-6, and TNF-alpha expression by the trichothecene deoxynivalenol (DON) and other biological toxins was investigated in primary and cloned murine macrophage models. Pretreatment of peritoneal murine macrophages or RAW 264.7 cells with the TLR-4 agonist LPS (100 ng/ml) for 16h markedly increased DON-induced IL-1 beta mRNA expression and to a lesser extent IL-6 and TNF-alpha mRNA expression. The effects of DON were dose-ldependent. In addition to LPS, similar results were

observed for other TLR agonists including zymosan (TLR2), poly [I:C] (TLR3), flagellin (TLR5), R848 (TLR 7/8) and ODN 1826 (TLR9). Furthermore, increased IL-1 beta and IL-6 mRNA expression was found in LPS-sensitized RAW 267.4 cells that were exposed to microbial toxins satratoxin G, zearalenone, ochratoxin A and Shiga toxin as compared to cells treated with LPS or the toxins alone. Taken together, the results indicate that a TLR activation sensitizes macrophages to trichothecenes and other natural toxins.

874 GALLIUM ARSENIDE EXPOSURE UPREGULATES INFLAMMATORY CYTOKINE EXPRESSION.

K. McCoy, S. Becker, T. Harrison and C. Hartmann. *Microbiology & Immunology, MCV Campus/Virginia Commonwealth University, Richmond, VA.*

The semiconductor, gallium arsenide (GaAs), is classified as an immunotoxicant and a carcinogen. We previously showed that GaAs *in vivo* induces several phenotypic changes in macrophages located at the exposure site, indicative of an activated state. To discern primary consequences, macrophage cell lines and murine peritoneal macrophages were exposed to the chemical *in vitro*. In culture, GaAs augmented invariant chain protein expression, and proteolytic activities and mRNA expression of cathepsins L and B. However, GaAs did not alter cell surface expression of major histocompatibility complex class II molecules on the macrophages, nor influence their ability to stimulate antigen-specific helper T cell s to respond to intact antigens that require processing. *In vitro* GaAs exposure did not completely mirror *in vivo* exposure, suggesting that other cells or factors may be involved. We examined the role of cytokines in GaAs-mediated macrophage activation. Blood monocytes were rapidly recruited to the exposure site, and the chemokine MCP-1 was present in the sera of exposed mice. The mRNA of seventeen chemokines and inflammatory cytokines were upregulated, while transcripts of three inhibitory cytokines diminished. Administration of latex beads caused less cytokine induction than GaAs, indicating that changes in mRNA levels could not be attributed to phagocytosis alone. Four cytokine proteins examined were mainly produced by myeloid cells. *In vivo* GaAs exposure alters cytokine gene expression which may lead to an inflammatory reaction and contribute to pathological tissue damage. This work was supported by NIEHS grant ES07199, and M. T. Harrison was supported by training grant T32 ES07087.

875 ETHANOL IS A GENERALIZED MODULATOR OF CYTOKINE PRODUCTION INDUCED THROUGH TOLL-LIKE RECEPTORS AND INHIBITS EARLY AS WELL AS LATE SIGNALING EVENTS.

C. Schwab, R. Fan, Q. Zheng, Q. Dai and S. B. Pruett. *Cell. Biol. & Anatomy, LSU Health Sciences. Center, Shreveport, LA.*

Toll-like receptors play an important role in the recognition and eventual elimination of pathogens. Once a ligand is recognized a series of signaling events begin resulting in the synthesis of proinflammatory cytokines. Previous experimentation has shown that ethanol can inhibit the ability of toll-like receptor 3 (TRL3) to induce cytokines in response to poly I:C. In this study, the effects of ethanol on cytokine production induced through TLR2, TRL3, TLR4, TLR6, TLR7, and TLR9 were examined and the mechanism by which ethanol inhibits the production of proinflammatory cytokines was examined. ELISA data indicate that serum and peritoneal cytokine levels (IL-12 and IL-6) were elevated in mice treated with a ligand alone, however, when ethanol is given in conjunction with the ligand proinflammatory cytokine production was suppressed. RNAse protection assay results indicate that in both purified and unpurified peritoneal macrophages IL-12, IL-6, IL-10, IL-15, INF-alpha, INF-beta, and INF-gamma levels are increased upon exposure to the ligand poly I:C, and this is significantly decreased, with the exception of IL-10, by ethanol. Proteins isolated from peritoneal macrophages were analyzed by Western blot. Western blot data suggest that ethanol decreases signaling early in the toll like receptor pathway. Inter/extra cellular flow cytometric data also suggest phosphorylation of p38, a kinase activated late in the signaling pathway, is decreased in macrophages exposed to ethanol. These data have implications with regard to human health, as the consumption of ethanol is prevalent, and the exposure to TLR ligands is unavoidable. This work was funded by a grant from NIAAA (AA09505).

876 METALLOTHIONEIN CAN FUNCTION AS A CHEMOTACTIC FACTOR.

X. Yin, D. A. Knecht and M. A. Lynes. *Molecular and Cell Biology, University of Connecticut, Storrs, CT.*

Mammalian cells respond to environmental stress by producing an array of stress-response proteins. One of these (metallothionein, MT), is a low molecular weight, cysteine-rich protein that can be induced by a variety of stressors, including heavy metals, organic solvents, free radicals, irradiation, and acute phase cytokines. MT sequesters toxic heavy metals, serves as a reservoir of essential divalent metal cations, regulates cellular redox status, and can modulate transcription factor activity. As a consequence, MT can regulate specific immune functions. We have noticed that the human and mouse genes which encode MT map close to the chemokines Ccl17 and Cx3Cl1. CC, CXC, and CX3C motifs that characterize chemokines are also found in the MT sequence. These observations suggested that MT might also act as a chemokine. In the experiments reported here, we show that MT can act as a chemokine for both Jurkat T cells and by cloned myeloid cells (WBC 264 9-C cells) using both the microBoyden chamber assay and the ECIS/taxis assay. This response can be blocked by two different monoclonal anti-MT antibodies (clones UC1MT and E9). Pre-incubation of Jurkat T cells with MT suppresses the response of these cells to SDF-1a, but MT does not change CXCR4 levels (the receptor for SDF-1a) on the surface of those cells. We interpret these results to suggest that MT may compete with SDF-1a and thus interfere with the SDF-1a chemotactic response. Incubation of Jurkat T cells with 2mg/ml Cholera toxin completely abrogates the chemotactic response of those cells to MT. This suggests that MT may bind to G-protein coupled receptors as it stimulates chemotaxis. These results indicate that MT that is produced in the context of exposure to environmental toxicants and released from cells may alter immune capacity in part by disturbing normal chemotactic responses. Thus, inappropriate MT synthesis may represent an important susceptibility factor in toxicant-mediated immunomodulation.

877 ENHANCED PROINFLAMMATORY CYTOKINE PRODUCTION BY ACTIVATED MICROGLIAL AND MACROPHAGE CELL LINES EXPOSED TO MANGANESE *IN VITRO*.

P. L. Crittenden and N. M. Filipov. *CEHS, Basic Sciences, Mississippi State University, Mississippi State, MS.*

Activated microglia and/or astrocytes have been proposed to play a role in the mechanism of manganese (Mn) neurotoxicity such that neurons adjacent to activated microglia could be injured by proinflammatory cytokines and reactive oxygen species elaborated from the microglia. Recently, we demonstrated that Mn greatly potentiated the LPS (lipopolysaccharide)-induced proinflammatory cytokine (IL-1β, IL-6, and TNF-α) production by the microglial cell line, N9. Because (*i*) Mn exposure may also potentiate proinflammatory cytokine production in the periphery, (*ii*) there are functional differences between brain microglia and peripheral macrophages, and (*iii*) peripheral inflammation contributes to/modulates the inflammatory response in the brain, our objective was to compare the influence of Mn on proinflammatory cytokine production by N9 microglia and J774 macrophage cell lines. Cells were exposed *in vitro* to increasing concentrations (up to 500 μM) of Mn (as $MnCl_2$) in the presence or absence of LPS (up to 1000 ng/ml). Following 24 h incubation, supernatants were collected and IL-1β, IL-6, and TNF-α concentrations were determined by ELISA. Similar to the effects already observed in N9 microglia, LPS-induced proinflammatory cytokine production was potentiated dose-dependently by Mn in the J774 macrophage cell line. This finding suggests that Mn augments proinflammatory cytokine production through a common mechanism which is now the subject of investigation. Considering that Mn exposure is not confined to the nervous system, increased inflammatory response in the periphery may be contributory to the mechanism of Mn neuro- and, possibly, systemic toxicity. (Supported by NIEHS ES11654).

878 THE EFFECTS OF INDIRUBIN ON GENE EXPRESSION PROFILES IN U937 HUMAN MONOCYTE/MACROPHAGES.

A. E. Becker and C. D. Rice. *Biological Sciences, Clemson University, Clemson, SC.*

The AhR is a ligand-activated transcription factor that alters the expression of a variety of genes relevant to toxicology and immunology. Although the AhR remains an orphan receptor, many synthetic ligands, including co-planar HAHs and TCDD, activate its specific signaling pathways. The immunotoxicity of TCDD and coplanar HAHs is fairly well characterized. However, the immunomodulatory properties of plant-based AhR ligands are just now being characterized. Indirubin (IR), a metabolite of tryptophan, can be synthesized by gut flora and is the active ingredient in an ancient Chinese remedy from Polygonum tinctgorium used to treat leukemia, used as an anti-inflammatory agent, and used to enhance detoxification processes. Despite its historical use, little is known about the effects of IR on immune function. Since TCDD and coplanar PCBs are potent immunotoxic agents, IR may have similar effects on the immune system. We treated U937 cells with IR in four different regimes representing both undifferentiated and differentiated steps in macrophage development, then monitored gene expression profiles using a macroarray for select stress and toxicity related genes. We monitored (1.) un-differentiated U937 cells treated with IR or carrier control (CC), (2.) IR or CC followed by PMA-induced differentiation to macrophages, (3.) differentiated cells

activated with LPS treated with IR or CC, and (4.) differentiated cells treated with IR or CC. Regimes 1, 2 and 3 resulted in CYP1A1 inductions of 12.7, 3.24, and 4.52 fold, respectively, supporting other findings that IR is a potent AhR ligand. COX-2 was significantly decreased in regime 2, as was iNOS in regime 3, suggesting that IR has anti-inflammatory properties. Macroarray validity was confirmed by RT-PCR on select genes. We also show that IR also induces indoleamine diooxygenase in differentiated U937 cells, suggesting an indirect of IR on immune function. Overall, our data provide evidence that IR may be a highly immunotoxic plant-based AhR ligand.

879 EFFECTS OF SELECT PARTICULATE MATTER (PM)-ASSOCIATED METALS ON MACROPHAGE (Mϕ) IRON HOMEOSTASIS.

S. P. Doherty[1], C. Prophete[1], J. Zelikoff[1], P. Maciejczyk[1], K. Salnikow[2], T. Gould[3], T. Larson[3], P. Jaques[4], J. Koenig[3], C. Sioutas[5], M. Lippmann[1] and M. Cohen[1]. *[1]Env Med., NYU, Tuxedo, NY, [2]NCI, Bethesda, MD, [3]University of Washington, Seattle, WA, [4]UCLA, Los Angeles, CA and [5]USC, Los Angeles, CA.*

Analyses of fine airborne $PM_{2.5}$ samples collected during fall 2001 indicate significant differences between the average daily PM levels in New York City (NYC), Los Angeles (LA), and Seattle (S). XRF analyses indicated that the inorganic composition of the PM varied from city to city, both in terms of absolute amounts of individual elements and their mass relationships to one another. Sulfur levels (as ng/m^3) were consistently the highest of 29 elements measured in each city's samples; however, concentrations of all other elements were very dissimilar. In NYC PM, the next most present elements were Fe, Si, Na, Ca, Al, and K. The second most prominent metal in LA and S PM was Na, followed by Si, Fe, Al, K, Ca and Cr in LA, and Fe, K and equal amounts of Cl and Si in S. We hypothesized that relative molar/mass relationships between select PM metals and Fe might govern the pulmonary immunotoxic potential of a given day's (or city's) PM by affecting alveolar Mϕ iron status, thereby altering their antibacterial/general functions. To test this, binding activity of iron response proteins (IRPs) in NR8383 rat Mϕ was assessed after exposure to select PM-associated metals (Fe, V, Mn and/or Al in the presence of apotransferrin). In all studies, relevant molar ratios of these metals, as found in a 500 μg sample of NYC PM for a specified day, were used. Results indicated that V, Mn and Al significantly changed the expression of IRP activity, although the effects were not consistently dose-dependent. Analysis of intracellular Fe status paralleled the IRP data for Mn and V, but not Al. The disruption in IRP binding activity, possibly as a result of competition for binding to the transferrin carrier, indicates that certain PM-associated metals might alter pulmonary immune competence in exposed hosts by impacting upon the Fe status of deep lung major defense cells. This work was supported by EPA/PM Center Grant R82735101.

880 EFFECTS OF PROPANIL (DCPA) ON THE NF-κB ACTIVATION PATHWAY IN MURINE MACROPHAGES.

I. Ustyugova[1], K. M. Brundage[1, 2], R. Schafer[1], C. L. Walton[1] and J. B. Barnett[1, 2]. *[1]Microbiology, Immunology and Cell Biology, West Virginia University, Morgantown, WV and [2]Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, WV.*

DCPA (3, 4-dichloropropionalide) is a post-emergent herbicide used extensively to control weeds in rice. Previous studies have demonstrated that treatment of the IC-21 macrophage cell line with DCPA resulted in changes of critical macrophage functions, such as decreases in cytokine production (TNF-α, IL-1β and IL-6). This appears to be due inhibition of the important transcription factor, NF-κB, as evidenced by reduced nuclear levels of NF-κB p65/p50 heterodimers as well as NF-κB p65/p50 DNA binding levels. To determine the cause of the decrease in nuclear levels of NF-κB, the molecules involved in the activation of NF-κB were studied. In unstimulated cells, NF-κB is bound to IκBα, IκBβ or IκBε, which conceal the nuclear localization sequence (NLS) of NF-κB required for nuclear import. Upon activation, these IκBs are phosphorylated, ubiquitinated and then degraded by 26S proteosomes to expose the NLS of NF-κB, which is then actively translocated into the nucleus. Inhibition of IκB phosphorylation would prevent ubiquitination and subsequent proteosomal degradation of IκB that would result in decreased nuclear levels of NF-κB. Therefore, LPS-induced phosphorylation and degradation of IκBα in the cytosol was studied in DCPA treated cells by Western Blot. No differences in phosphorylation or degradation of IκBα were seen in DCPA-treated versus vehicle-treated cells, however, the kinetics of IκBβ degradation was delayed in DCPA-treated cells versus vehicle-treated cells. Moreover, the hypophosphorylated form of IκBβ is less pronounced in DCPA treated cells. These data provide evidence that DCPA affects the NF-κB activation pathway by changing the phosphorylation state of IκBβ, thereby interfering with its degradation and preventing NF-κB shuttling to the nucleus. Supported by NIEHS grant ES11311.

881 SMAD3 MEDIATES SUPPRESSION OF T CELL RECEPTOR-INDUCED BUT NOT IL-2-INDUCED T CELL PROLIFERATION BY TGF-β1.

S. C. MCKARNS[1], N. E. Kaminski[2] and R. H. Schwartz[1]. *[1]Laboratory of Cellular and Molecular Immunology, NIAID/NIH, Bethesda, MD and [2]Deptartment of Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

TGF-β1 plays a critical role in regulating immune homeostasis. It is well documented that TGF-β1 can impair T cell proliferation and IL-2 production; however, the mechanisms of suppression remain elusive. TGF-β1 signals through a transmembrane serine/threonine kinase receptor complex to activate Smad-dependent and -independent intracellular signaling cascades. We have previously shown that Smad3 is critical for suppression of IL-2 production by TGF-β1. Utilizing Smad3-deficient mice and CFSE labeling, we now demonstrate that β-CD3 β-CD28-stimulated $Smad3^{-/-}$ splenic T cells and thymocytes are partially resistant to growth inhibition by TGF-β1. However, in striking contrast, TGF-β1 suppresses IL-2-induced proliferation indepdendent of Smad3. Our data implicate critical roles for TCR-induced Smad3-dependent and IL-2-induced, Smad3-independent signaling pathways in the regulation of T cell homeostasis by TGF-β1. These differential immune suppressive properties of TGF-β1 may allow for selective regulation of T cell expansion during an immune response.

882 INHIBITION OF INTERLEUKIN-2 (IL-2) SECRETION BY ANANDAMIDE IS MEDIATED BY A CYCLOOXYGENASE (COX) METABOLITE.

C. E. Rockwell and N. E. Kaminski. *Pharmacology & Toxicology, Michigan State University, East Lansing, MI.*

The putative endogenous cannabinoid, arachidonoyl ethanolamine, which is commonly known as anandamide (AEA), is an arachidonic acid derivative that has been shown through radioligand binding studies to bind to the cannabinoid receptors, CB1 and CB2. Anandamide mimics many of the immunomodulatory effects of the plant-derived cannabinoids, including inhibition of cytokine production. Specific to the present studies, AEA inhibits IL-2 secretion that has been induced by the phorbol ester, phorbol-12-myristate-13-acetate (PMA), and the calcium ionophore, ionomycin. The objective of the present studies was to determine the mechanism for this effect and the role of the CB1 and CB2 receptors. The CB1 and CB2 antagonists, SR141716A and SR144528, when used in combination, did not antagonize the inhibition of IL-2 secretion by AEA. Furthermore, neither AM404, the inhibitor of the putative AEA membrane transporter (AMT), nor methyl arachidonoyl fluorophosphonate (MAFP), the inhibitor of fatty acid amidohydrolase (FAAH), attenuated the effects of AEA upon IL-2 secretion. Interestingly, arachidonic acid caused a similar concentration-dependent inhibition of IL-2 secretion (IC_{50}=12.3μM) as structurally-related AEA (IC_{50}=14.1μM). The nonselective COX inhibitor, flurbiprofen, reversed inhibition of IL-2 mediated by AEA as well as by arachidonic acid. The COX-2 specific inhibitor, SC58125, had no effect upon the inhibitory activity of either AEA or arachidonic acid on IL-2 secretion. These data suggest that inhibition of IL-2 by AEA is independent of CB1/CB2 and the AMT/FAAH system. These data also suggest that inhibition of IL-2 is mediated by a COX-1 metabolite of arachidonic acid rather than AEA itself and that the enzyme responsible for the hydrolysis of AEA is not FAAH. (Supported by NIH grants DA12740 and DA15276).

883 ROLE OF EXTRACELLULAR SIGNAL-REGULATED PROTEIN KINASE 1/2 (ERK1/2) IN THE INHIBITION OF T-CELL FUNCTION BY ALKENYLBENZENES.

S. Yea[1, 2], H. Jeong[1], C. Kim[1], Y. Park[1], S. Lee[2], J. Shin[2] and C. Yun[3]. *[1]Department of Biochemistry, Inje University, Pusan, South Korea, [2]Pharmacogenomics Research Center, Inje University, Pusan, South Korea and [3]Department of Genetic Engineering, Pai-Chai University, Taejon, South Korea.* Sponsor: H. Kim.

Human may consume alkenylbenzenes as natural components of certain spices, essential oils or vegetables. However, the potential of alkenylbenzenes for immune modulation and their mechanism have not known. In the present study, we investigate the effect of several naturally occurring alkenylbenzenes on T lymphocytes that play an important role in immune response through the action of cytokines they elaborate. Anethole, eugenol, isoeugenol and myristicin produced inhibition of concanavalin A-induced lymphoproliferation in B6C3F1 mouse splenocytes. In light of the fact that IL-2 is responsible for the clonal expansion of T-cells, effect of the alkenylbenzenes on IL-2 expression was determined. PMA plus ionomycin (Io) induced IL-2 mRNA expression and protein secretion in EL4.IL-2 T-cells, which were inhibited by anethole, eugenol, isoeugenol and myristicin as determined by real-time RT-PCR and ELISA, respectively. Electrophoretic mobility shift assay was performed to evaluate the effect of the alkenylbenzenes on binding activity of transcription factors for IL-2 gene expression, and the alkenylbenzenes decreased the

NF-AT and AP-1 binding activity in PMA/Io-stimulated EL4.IL-2 cells. Western blot analysis showed that the alkenylbenzenes inhibited the phosphorylation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2). These results suggest that the alkenylbenzenes have the potential for immune modulation, which is due to the inhibition of IL-2 expression and is mediated, at least in part, through the blocking of ERK1/2 signaling.

884 SODIUM ARSENITE INHIBITS ERK1/2 PHOSPHORYLATION IN MICE LYMPHOCYTES STIMULATED WITH PHYTOHEMAGGLUTININ.

P. Conde-Moo[1], L. C. Acosta-Saavedra[1], M. E. Cebrian[1] and E. S. Calderon-Aranda[1, 2]. *[1]Seccion Externa de Toxicologia, Cinvestav, Mexico, DF, Mexico and [2]Department of Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.*

Arsenic immunotoxic effects include inhibition of the proliferative responses elicited by mitogens such as phytohemagglutinin (PHA) in humans and animal lymphocytes, although the molecular mechanism remains to be defined. Previous studies have shown that arsenite induces activation of mitogen-activated protein kinase (MAPK) in non-stimulated lymphocytes, but the effect on PHA-stimulated lymphocytes has not been evaluated. The aim of this study was to establish the possible role of ERK on the inhibition of PHA-induced proliferation of mice T lymphocytes, produced by arsenite. For this purpose, T lymphocytes from female C57BL/6 mice spleen were used. The effects of arsenite on proliferative response of T lymphocytes induced by PHA (10 ug/ml) were measured by incorporation of [^{3}H]-Thymidine and the phosphorylation of ERK1/2 was determined by immunoblotting using an anti-phosphorylated-ERK antibody. The simultaneous treatment of PHA-stimulated lymphocytes with 10 uM arsenite had an inhibitory effect (80%) on PHA-induced proliferation. The phosphorylation of ERK normally elicited by PHA, was inhibited by arsenite at the concentration of 10 uM, but not by 1.0 uM. These results suggest that the MAPK family members, such as ERK, could be involved in the inhibitory effect of sodium arsenite on T-cell proliferation which probably explains in part, immunosupression. Whether this effect is mediated by other MAPK member remains to be determined. This work was partially supported by the Mexican Council for Science and Technology (Conacyt 34508-M) to ES Calderon-Aranda.

885 PRETREATMENT WITH CIGARETTE SMOKE EXTRACT DECREASES CELL DEATH IN PMA-TREATED NEUTROPHILS.

H. C. O'Neill[2] and K. A. Stringer[1, 2]. *[1]Department of Pharmaceutical Practice, UCHSC, Denver, CO and [2]Department of Pharmaceutical Sciences, UCHSC, Denver, CO.*

Neutrophil (PMN) accumulation in the lung is implicated in the pathogenesis of inflammatory pulmonary diseases such as COPD and emphysema. Cigarette smoke (CS) recruits PMNs into the lungs and may alter PMN function. Timely PMN apoptosis is necessary for resolution of inflammation. We have previously determined that pre-treatment of normal, human PMNs with Camel CS extract (CSE) reduces phorbol 12-myristate 13-acetate (PMA)-induced reactive oxygen species (ROS) production. Because of the important relationship between PMN ROS production and lifespan, we investigated the ability of CSE to influence PMN survival. Peripheral neutrophils were isolated from normal healthy volunteers and treated with PMA, Camel CSE, or CSE and PMA and incubated for 0, 4, 8, 12, or 24 hr. Pretreatment with CSE slowed PMA-induced cleavage of caspase 3 as measure by Western immunoblotting. However, morphological fluoresent microscopy studies (Hoeschst 33258 dye) showed PMA-induced necrosis rather than apoptosis which was prevented by pre-treatment with CSE. There was no difference between CSE alone and untreated control cells either morphologically or as measured by caspase 3 immunoblotting. These data suggest that CSE is capable of modulating PMA-induced necrosis in human PMNs. Whether this translates to apoptotic mediated events and its relationship to PMN ROS production are currently under investigation. If CSE interferes with activator-induced apoptosis, it may limit resolution of inflammation which could contribute to the pathogenesis of inflammatory lung diseases such as COPD and emphysema. Research described in this article was supported by Philip Morris USA Inc.

886 GW7845, A PPARγ AGONIST, INDUCES MAP KINASE-DEPENDENT APOPTOSIS IN PRO/PRE-B CELLS.

D. Liu[1], J. Emberley[2], D. H. Sherr[1] and J. J. Schlezinger[1]. *[1]Environmental Health, Boston University School of Public Health, Boston, MA and [2]Microbiology, Boston University, Boston, MA.*

Peroxisome proliferator-activated receptor γ (PPARγ) is expressed highly in the immune system and influences multiple aspects of immune function. Previously we have shown that activation of PPARγ by the high affinity agonist GW7845 results in apoptosis in pro/pre-B lymphocytes. PPARγ agonists are known to influence MAP kinase cascades, therefore we investigated the activation of these casades in pro/pre- B cells and their role in apoptosis induction. Treatment of a non-transformed murine pro/pre-B cell line (BU-11) with GW7845 (40 μM) strongly induced the phosphorylation of p38 MAPK, while the phosphorylation of p44/42 MAPK and JNK was induced minimally. This is in contrast to the pattern of phosphorylation seen following treatment with hydrogen peroxide (200 μM), which strongly induced the phosphorylation of all of the kinases. Phosphorylation of p38 MAPK was accompanied by induction of its kinase activity as evidenced by a significant increase in the phosphorylation of its substrate ATF-2 by p38 MAPK immunoprecipitated from GW7845-treated BU-11. No change in AP-1 DNA-binding was observed following treatment with GW7845, suggesting that the small increase in JNK phosphorylation did not lead to significant induction of its kinase activity. Pre-treatment with the p38 MAPK inhibitor SB202190 (30 μM), but not JNK (SP600125) or p44/42 (PD98059) inhibitors, prevented GW7845-induced B cell apoptosis. Activation of PPARγ appears to initiate apoptotic signaling in B cells in early maturation stages, potentially through the activation of a p38 MAPK cascade.

887 AN ENVIRONMENTAL PPARγ AGONIST, MONO-(2-ETHYLHEXYL) PHTHALATE, INDUCES PRO/PRE-B CELL TOXICITY: INTERACTIONS WITH 9-*CIS*-RETINOIC ACID AND 15-DEOXY-$\Delta^{12, 14}$-PROSTAGLANDIN J_2.

J. J. Schlezinger[1], G. Howard[1], C. H. Hurst[2], T. Webster[1], D. J. Waxman[2] and D. H. Sherr[1]. *[1]Environmental Health, Boston University School of Public Health, Boston, MA and [2]Biology, Boston University, Boston, MA.*

The common industrial and commercial use of phthalate esters has resulted in significant human exposure to these bioactive compounds. The facts that phthalate ester metabolites are peroxisome proliferator-activated receptor (PPAR) agonists and that PPARγ agonists induce lymphocyte apoptosis suggest that phthalate esters may be immunotoxicants. Here, we examined the effects of a metabolite of one such environmental phthalate, mono-(2-ethylhexyl) phthalate (MEHP), on developing B cells. MEHP inhibited thymidine incorporation by primary bone marrow pro/pre-B cells and by a non-transformed pro/pre-B cell line (BU-11). Co-treatment with a retinoid X receptor α (RXRα) ligand, 9-*cis*-retinoic acid, decreased thymidine incorporation synergistically, a characteristic feature of PPARγ-mediated apoptosis. These results were similar to those obtained with the natural PPARγ ligand 15-deoxy-$\Delta^{12, 14}$-prostaglandin J_2 (15d-PGJ_2). At moderate doses of MEHP (150-200 μM), the inhibition of thymidine incorporation resulted primarily from induction of pro/pre-B cell apoptosis, while at low doses (50-100 μM), the inhibition reflected growth arrest in the absence of apoptosis. Co-treatment of bone marrow B cells with 15d-PGJ_2 and MEHP significantly enhanced the inhibition of thymidine incorporation seen with MEHP alone, potentially mimicking exposures in the bone marrow microenvironment where prostaglandin concentrations are high. MEHP and 15d-PGJ_2 activated PPARγ and induced a significant increase in NF-κB. These data demonstrate that an environmental phthalate ester can inhibit proliferation of and induce apoptosis in developing bone marrow B cells *via* PPARγ activation. The activation of NF-κB suggests a signaling pathway through which these are effected. Finally, the data suggest that bone marrow B cells are particularly susceptible to phthalate toxicity.

888 INDUCTION OF COMPETING PRO-APOPTOTIC AND PRO-SURVIVAL SIGNALING PATHWAYS IN THE MACROPHAGE BY THE TRICHOTHECENE DEOXYNIVALENOL.

H. Zhou[1] and J. J. Pestka[1, 2, 3]. *[1]Food Science and Human Nutrition, Michigan State University, East Lansing, MI, [2]Department of Microbiology and Molecular Genetics, Michigan state University, East Lansing, MI and [3]Institute for Environmental Toxicology, Michigan state University, East Lansing, MI.*

Deoxynivalenol (DON) and other trichothecene mycotoxins cause immune suppression by inducing apoptosis in leukocytes. The capacity of DON to activate pro-apoptotic and pro-survival signaling pathways was assessed in the murine RAW 264.7 macrophage model. Induction of the p53-driven Bax/Mitochondira/Caspase-9 pathway was first investigated. DON at 250 ng/ml readily induced the phosphorylation and the binding activity of p53 as determined by Western analysis and electrophoretic mobility shift assay, respectively. DON exposure also resulted in translocation of Bax, altered mitochondrial membrane potential and release of cytochrome C. PFT-α (Pifithrin-α), an inhibitor of p53, reduced DON-induced phosphorylation of p53 as well as the p53 binding activity. Moreover, PFT-α decreased DON-induced caspase-3 activity and subsequent apoptosis in a dose dependent manner. Interestingly, DON concurrently activated an anti-apoptotic pathway as evidenced by induction of Akt and p90Rsk phospho-

rylation and, subsequently, Bad . Taken together, the results demonstrate the DON competing apoptotic (p53/Bax/Mitochondrial/Caspase-9) and survival (Akt/p90Rsk/Bad) pathways in the macrophage.(Supported by NIH Grant ES-03358 and USDA-ARS Grant 59-070-060).

889 AhR LIGANDS INHIBIT PROLIFERATION OF ACTIVATED HUMAN B CELLS.

L. L. Allan[1], H. Ryu[2], J. K. Emberley[1], J. J. Schlezinger[2] and D. H. Sherr[2]. *[1]Microbiology, Boston University School of Medicine, Boston, MA and [2]Environmental Health, Boston University School of Medicine, Boston, MA.*

Polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic hydrocarbons (HAHs), ubiquitous environmental contaminants, are immunosuppressive AhR ligands. Previous studies suggested that several types of activated cells, including lymphoid tumors, have high AhR levels. Here, the consequences of this putative up-regulation, with regard to normal human B cell function and susceptibility to AhR ligands, were studied. *In vitro* activation of primary human B lymphocytes with either CpG (a TLR-9 ligand) or CD40 ligand, stimuli that model B cell activation during innate and adaptive immune responses respectively, dramatically up-regulated AhR mRNA and protein levels. Furthermore, constitutive AhR activation was suggested by its nuclear translocation and by an increase in CYP1A1 mRNA levels. Treatment of activated B cells with B[a]P or TCDD further augmented CYP1A1 expression, suggesting that these cells with high AhR levels would remain or become more sensitive to environmental AhR agonists. To test this hypothesis, the consequences of PAH or HAH exposure on the proliferation of CD40L and CpG-activated B cells were assessed. B[a]P (10-5 to 10-7 M) significantly reduced the proliferation of CD40L-activated B cells while B[e]P, a B[a]P congener and weak AhR agonist, had no effect. Conversely, TCDD (10-8 to 10-10 M) greatly inhibited proliferation of human B cells activated with CpG. Collectively, these data demonstrate that activated human B cells express high AhR levels, making them extremely sensitive targets of AhR-mediated PAH/HAH immunotoxicity.

890 HEMATOTOXIC EFFECTS OF HEPTACHLOR ON B LYMPHOPOIESIS.

S. V. Dodson, D. A. Piktel, J. B. Barnett and K. S. Landreth. *Microbiology, Immunology, and Cell Biology, West Virginia University, Morgantown, WV.*

Hematotoxicity has been documented in adults exposed to high levels of organochlorine pesticides. We previously evaluated effects of prenatal heptachlor exposure on development of embryonic hematopoiesis and observed that embryonic exposure consistently resulted in altered numbers of fetal liver lymphoid progenitor cells. Prenatal heptachlor exposure did not affect development of myeloid progenitor cells. To further investigate mechanisms of lymphoid hematotoxicity, we evaluated direct effects of heptachlor on lymphoid cell lines at different stages of maturation. Following exposure, 70Z/3 pre-B cells withdrew from cell cycle and expressed immunoglobulin kappa chains, indicative of maturation in these cells. Nuclear factor-kappa B (NF-κB), a transcription factor known to promote kappa light chain transcription, was increased in nuclei of pre-B cells exposed to heptachlor. Exposure of the pro B cell line, C1.92, to heptachlor resulted in failure to proliferate and onset of apoptosis. We determined that NF-κB is decreased in nuclei of pro-B cells following exposure to heptachlor. Pro-B cells require stromal cell contact and cytokines produced by stromal cells for normal development. Pro-B cells cultured with stromal cells were more resistant to onset of apoptosis following heptachlor exposure than pro-B cells treated in the absence of stroma. In order to determine whether hematotoxicity was limited to direct effects on developing lymphoid cells, we exposed stromal cells to heptachlor. Heptachlor pretreatment of stroma resulted in decreased stem cell factor mRNA, VCAM-1 protein, and pro-B cell proliferation. Taken together, these data suggest that heptachlor directly alters lymphoid cell development by interrupting expansion of pro-B cells and causing premature differentiation of pre-B cells through alterations of NF-κB. Additionally, heptachlor exposure affects hematopoiesis through alteration in stromal cell function. Supported by NIEHS ES010953

891 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN ALTERS THE REGULATION OF PAIRED BOX GENE 5 (*PAX5*) IN B CELLS.

D. Shnaider[1, 3], B. S. Yoo[5, 1], D. R. Boverhof[2, 3], R. B. Crawford[1], T. R. Zacharewski[2, 3, 4] and N. E. Kaminski[1, 3, 4]. *[1]Pharmacology & Toxicology, Michigan State University, East Lansing, MI, [2]Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, [3]Institute of Environmental Toxicology, Michigan State University, East Lansing, MI, [4]National Food Safety & Toxicology Center, Michigan State University, East Lansing, MI and [5]Biology, Kyonggi University, Paldal-gu, Suwon-Si, South Korea.*

The environmental contaminant, 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), produces a suppression of the primary immunoglobulin-M (IgM) antibody response. The suppression of IgM production by TCDD can occur through direct interactions with the B cell, is aryl hydrocarbon receptor-dependent and results from interference with the differentiation of B cells to plasma cell stage. Pax5, also known as B cell-lineage-specific activator protein, is a crucial repressor of B cell differentiation. The objective of the present investigation was to characterize the effects of TCDD on the regulation of Pax5 and its downstream targets: immunoglobulin heavy chain (IgH), light chain (Igk), *J chain* and X box protein-1 (XBP-1). Lipopolysaccharide (LPS)-activation of CH12.LX cells induced a time-dependent decrease in cellular Pax5 mRNA, protein and Pax5 DNA binding activity during a 72 hour culture period, which was almost completely blocked in the presence of TCDD. Concordant with sustained *Pax5* expression, *IgH*, *Igk, J chain* and XBP-1 mRNA levels were strongly suppressed in LPS-activated CH12.LX cells treated with TCDD. Collectively, these studies suggest that the suppression of the primary IgM antibody response by TCDD is intimately related to altered Pax5 cellular levels and function. (NIH Grant ES02520)

892 TCDD-INDUCED MODULATION OF THE 3′α ENHANCER.

C. Sulentic[1], W. Zhang[2], Y. Na[3] and N. Kaminski[2]. *[1]Pharmacology & Toxicology, Wright State University, Dayton, OH, [2]Pharmacology & Toxicology, Michigan State University, East Lansing, MI and [3]Korea Advanced Institute of Science and Technology, Daejon, South Korea.*

Transcriptional regulation of the immunoglobulin heavy chain gene involves several regulatory elements including the 3'a enhancer, which is composed of four distinct regulatory domains. DNA binding sites for several transcription factors, including B cell-specific activator protein, NF-κB and Oct have been identified within the 3'a enhancer domains and are believed to be important in regulating 3'a enhancer activity. We have identified an additional DNA binding motif, the dioxin responsive element (DRE) which can contribute to 3'a enhancer regulation. 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), a known disruptor of B cell differentiation (i.e., decreased plasma cell formation, inhibition of mu heavy chain expression and suppression of IgM secretion), induces binding of the aryl hydrocarbon receptor (AhR) nuclear complex to DREs. TCDD also induces AhR binding to the hs4 domain of the 3'a enhancer. Interestingly, TCDD enhances LPS-induced activation of the hs4 domain but profoundly inhibits LPS-induced activation of the complete 3′α enhancer. Furthermore, site-directed mutational analysis demonstrated that a DRE and κB element in the hs4 domain is modulated by TCDD in LPS-activated B cells. We propose that the AhR is a novel transcriptional regulator of the 3′α enhancer, which can mediate, at least in part, the effects of TCDD on the 3′α enhancer and its domains, putatively contributing to a marked suppression of IgM production (Supported by NIH ES02520 and NIEHS 1F32ES05914).

893 DEVELOPMENTAL LEAD EXPOSURE IMPACTS ON SPATIAL LEARNING AND MEMORY: CHOLINERGIC AND GLUTAMATERGIC INVOLVEMENT.

E. D. Levin[1, 2], K. Sato[2], J. H. Freedman[2], J. Skene[3] and G. Harry[4]. *[1]Psychiatry, Duke University Med. Ctr, Durham, NC, [2]Nicholas School of Earth and Environmental Sciences, Duke University, Durham, NC, [3]Neurobiology, Duke University Med. Ctr, Durham, NC and [4]NIEHS, Research Triangle Park, NC.*

Developmental lead exposure has been shown to cause persisting neurobehavioral effects in various species including humans, non-human primates, and rats. To provide data concerning a mouse model for lead effects, CD1 mice (dams with their litters) received lead acetate (0.2 percent in drinking water) upon parturition of pups until weaning at day 21. No lead-induced changes in body weight or developmental landmarks were evident. One male and one female from each litter were trained as adults for 18 sessions on the 8-arm radial maze. During the initial block of 3 sessions a paradoxical lead-related improvement in choice accuracy was noted. For the rest of the training period no lead-induced effects were seen with choice accuracy. Response latency during acquisition training was affected by lead with a significant decrease in lead-exposed females, but not males. After acquisition, the mice received amnestic pharmacological challenges with scopolamine, a muscarinic cholinergic antagonist and dizocilpine, an NMDA glutamate antagonist. Scopolamine challenge uncovered a significant choice accuracy impairment in the lead-exposed female offspring relative to controls. There was also evidence for complex lead-induced alterations in response to the amnestic effects of dizocilpine challenge. This mouse model can facilitate genetic analysis to determine the molecular mechanisms of persisting lead-induced neural damage. Preliminary cDNA array studies have suggested specific effects on a very limited number of genes. An early induction of the transthyrethrin gene suggests either an early maturation of the choroid plexus or a disruption in the thyroid hormone status with developmental lead exposure. The mouse model provides the opportunity to determine the relationship between lead effects on genetic expression and neurobehavioral function. (Supported by Duke University and NIEHS.)

894 NEUROGENESIS IN THE RAT DENTATE GYRUS IS DECREASED BY CHRONIC DEVELOPMENTAL LEAD EXPOSURE.

T. Verina[1], C. A. Rohde[2] and T. R. Guilarte[1]. *[1]Environmental Health Sciences, Johns Hopkins University, Baltimore, MD and [2]Biostatistics, Johns Hopkins University, Baltimore, MD.*

Developmental Pb2+ exposure impairs spatial learning and synaptic plasticity in the hippocampus of young adult rats [Nihei et al., Neurosci. 99:233, 2000]. We have previously reported preliminary evidence of decreased neurogenesis in the dentate gyrus (DG) of Pb2+ exposed rats [Verina et al., Toxicology. Sciences., 66(S1) 129, 2002]. DG neurogenesis is important for some forms of hippocampal-dependent learning and memory [Shors et al., Nature 410: 372, 2001]. Changes in hippocampal neurogenesis may serve as a novel cellular mechanism of cognitive deficits caused by Pb2+. To further investigate this question in a larger cohort of animals, we counted newly generated cells labeled with the thymidine analog bromodeoxyuridine (BrdU) using stereology. The phenotype of BrdU-labeled cells was assessed by using doublecortin (DCX), a marker of immature neurons, or the glial marker GFAP. Pb2+-exposed or control rats were injected daily with BrdU (100mg/kg) from postnatal days (PN) 45 to 49 and sacrificed by transcardial perfusion at PN50. Systematically selected sections from the dorsal aspects of the DG were processed for either BrdU immunohistochemistry or triple-immunofluorescence with BrdU, DCX, and GFAP. BrdU-positive cells were analyzed using a Poisson regression model with count as the response variable, area as an offset, and group (control & lead) as a factor. We measured a 13% decrease ($p < 0.001$) in BrdU-labeled cells in the granular layer and subgranular zone of the DG from Pb2+-exposed rats relative to controls (1673 vs 1915 total cells, respectively). Approximately 78% of the BrdU cells were colocalized with the immature neuronal maker DXC, 2% with the astrocytic marker GFAP, and 18% did not label with either marker. No treatment effects on the percent distribution of cellular phenotypes were found. These findings indicate that chronic exposure to Pb2+ during development reduces neurogenesis in the rat DG and may form the basis for learning impairments associated with Pb2+ exposure [supported by NIEHS ES06189 to TRG].

895 CAMKII ACTIVITY, PROTEIN AND GENE EXPRESSION IN THE HIPPOCAMPUS OF LEAD-EXPOSED RATS.

C. D. Toscano, J. R. Moss and T. R. Guilarte. *Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.*

The NMDA receptor (NMDAR) is a ligand-gated calcium channel essential for learning and memory. We have shown previously that developmental Pb2+ exposure alters the expression of NMDAR subunits in the rat hippocampus (Toscano et al, Dev. Brain Res. 139(2):217, 2002), a brain region essential for some forms of learning and memory. The calcium-calmodulin dependent kinase II (CAMKII) is directly activated by NMDAR-mediated calcium signaling in the post-synaptic membrane. Further, CAMKII is an important kinase in the propagation of calcium-sensitive signaling essential for learning and memory. We hypothesized that developmental Pb2+ exposure alters NMDAR-mediated calcium signaling and affects the activity of key kinases such as CAMKII. Control and Pb2+-exposed rats were used at 50 days of age, a time point at which deficits in spatial learning, synaptic plasticity, and NMDAR subunit expression are observed (Nihei et al, Neuroscience 99:233-42, 2000). Hippocampal CAMKII activity was measured in the presence of activators. Kinetic analysis showed that Pb2+-exposed rats exhibit a 55% decrease in Vmax [Control =192.9 ± 92.1 pmol/μg/min vs. Pb2+= 88.1 ± 31.2 pmol/μg /min; $p<0.02$] with no change in Km [Control = 34.2 ± 17.2 μM vs. Pb2+= 20.5 ± 7.0 μM; $p>0.05$] of hippocampal CAMKII activity. The decrease in Vmax cannot be explained by a direct effect of Pb2+ on CAMKII since incubation of hippocampal homogenates with Pb2+ [10^{-8} to 10^{-5}M] did not significantly affect CAMKII activity. In addition, the decrease in Vmax is not due to an alteration in the gene or protein expression of CAMKII as demonstrated by in situ hybridization and immunoblot analysis. While the nature of the Vmax decrease is unknown, it is possible that an alteration in the expression of CAMKII isoforms or posttranslational modifications occurs with Pb2+ exposure. A decrease in CAMKII activity could alter its ability to effectively phosphorylate substrates in an activity-dependent manner affecting neuronal function, synaptic plasticity, learning and memory [Supported by NIEHS ES06189 to TRG]

896 THE INFLUENCE OF LEAD ON THE EXPRESSION OF OCT-2 AND THE REGULATION OF ITS TARGET GENES.

S. A. Bakheet and N. H. Zawia. *Biomedical Sciences, University of Rhode Island, Kingston, RI.*

The nervous system is the principal target for a number of environmental metals, which are well known for their neurological and behavioral effects in humans. Lead (Pb) exposure is highly neurotoxic, particularly to the developing central nervous system. Exposure to lead may alter developmental gene expression and brain development through selective effects on the transcriptional machinery. In this study macroarray analysis was used in an attempt to identify transcription factors that are involved in the response to Pb-exposure. Rat pups were exposed to Pb-acetate (0.2 %) from birth to weaning *via* lactating dams. On various postnatal days the hippocampi of control and Pb-exposed pups were examined by macroarray analysis for changes in the developmental profiles of approximately 30 transcription factors, belonging to known transcription factor families. A number of transcription factors appear to be altered following lead exposure and have distinctive temporal patterns of expression. The most prominent changes were exhibited by the Sp and Oct families. The results of macroarray screening were validated by using alternative methods (RT-PCR). Also the functional properties of Oct transcription factor were tested by using EMSA. We found that Oct-2 mRNA expression and DNA-binding follow a similar pattern after lead exposure. The expression of the nitric oxide synthase (NOS) gene was examined to test whether Pb-induced changes in Oct-2 DNA-binding translate into alterations in the expression of its target genes. We found that NOS mRNA levels and Oct-2 DNA-binding were inversely related consistent with the predominant inhibitory effects of Oct-2 on NOS expression in neuronal cells. In the future, we plan to examine other target genes regulated by Oct-2 transcription factor along with the signaling pathways involved following lead exposure in order to determine the role of Oct-2 transcription factor in lead neurotoxicity.

897 DEVELOPMENTAL EXPOSURE TO LEAD AND RESPOSIVNESS OF THE APP GENE IN THE SENESCENT BRAIN.

N. Benitez, N. H. Zawia and M. Basha. *Biomedical Sciences, University of Rhode Island, Kingston, RI.*

Although AD is a disease of the elderly, environmental influences that impact the onset and severity of neurodegeneration may occur much earlier in life. A potential mechanism through which Pb could promote neurodegeneration is by interfering with programmatic regulation of the APP gene established during early development. The regulatory region of the APP gene contains elements recognized by the transcription factor Spl, which is essential for the activation of the APP gene. An induction in the activity of Spl would consequentially increase the expression of the APP gene, subsequently increasing the supply of APP substrates which could be processed to generate higher levels of AP associated with the neuropathological symptoms of AD. The DNA-binding and transcriptional activity of Spl was found to be induced following neonatal exposure of rats to Pb. Long-term analysis of Spl DNA-binding of the same progeny of rats exposed to Pb during the postnatal stage revealed that Spl activity is maintained at resting levels for more than a year but exhibits a rise in activity 20 months later, when compared to unexposed controls. Furthermore the expression of the APP gene follows a pattern that is similar to that of Sp1 DNA-binding and Sp1 MNRA expression, while the levels of a house keeping gene are not changed. These delayed elevations of both Spl DNA-binding and APP expression are exacerbated when postnatally-exposed animals are challenged by renewed Pb-exposure during their old age. Interestingly, none of these effects are observed if exposure occurred only during old age, strongly suggesting that Pb interferes with gene imprinting which is established during development and which is critical for the responsiveness of the gene regulatory apparatus in the adult.

898 INTERACTIVE EFFECTS OF CHRONIC POSTWEANING PB EXPOSURE AND ENVIRONMENTAL STRESS.

D. A. Cory-Slechta[1], M. B. Virgolini[1] and D. Weston[2]. *[1]Env Comm. Med., EOHSI, Piscataway, NJ and [2]University of Rochester, Rochester, NY.*

Lower socioeconomic status (SES) children in the US also sustain the highest blood lead (Pb) levels. Low SES itself is a known risk factor for adverse health outcomes and behavioral dysfunctions, a phenomenon attributed to higher levels of stress and prolonged cortisol elevation. Pb exposure and elevated cortisol are associated with similar behavioral alterations and target mesolimbic dopamine (DA) systems. The hypothesis that Pb interacts with environmental stress was examined here in male rats subjected to chronic postweaning Pb exposure (0, 50 or 150 ppm in drinking water) with or without environmental stress (groups: control no-stress (C-NS), control stress (C-S), Pb50 no-stress (50-NS), Pb50 stress (50-S), Pb150 no-stress (150-NS) & Pb150 stress (150-S)). As in our past studies, Pb increased response rates on a fixed interval (FI) schedule of food reward by 38% and 58% at 50 and 150 ppm, respectively. Functionality of the HPA axis was measured *via* blood corticosterone (CORT) levels. Pb itself decreased basal CORT levels at 50 ppm (31%) with less pronounced effects (18%) at 150 ppm. Introduction into a novel environment increased ambulatory and horizontal activity only in the 150-S group and suppressed FI response rates only in the Pb groups, even though equivalent increases in CORT were observed across all groups. Brief exposure to a cold environment increased CORT levels only in the 50-S and 150-S groups but did not differ-

entially influence FI performance. Interactions of Pb and stress cannot be easily ascribed to changes in brain monoamine alterations since changes in levels of DA and serotonin and metabolites in striatum and frontal cortex were primarily due to Pb alone, and no changes were found in nucleus accumbens. These results indicate that Pb exposure can modulate responses to stress situations and stress can unmask effects of Pb. These findings raise questions about stress-related mechanisms of Pb effects as well as the adequacy of risk assessments based on the study of Pb in isolation. Supported by ES05017, ES01247.

899 LONG-LASTING BEHAVIORAL AND NEUROCHEMICAL CONSEQUENCES OF MATERNAL LEAD (PB) EXPOSURE AND STRESS IN FEMALE OFFSPRING.

M. B. Virgolini and D. A. Cory-Slechta. *EOHSI, UMDNJ and Rutgers University, Piscataway, NJ.*

Elevated Pb body burden is a particular problem for low socioeconomic status children in the US, populations also considered to sustain the highest levels of environmental stress. Since both Pb and stress target the same brain sites and behavioral functions, their potential interactions were examined here. Dams exposed to 150 ppm Pb in drinking water during gestation and lactation were subjected to restraint stress 3 times per day on gestational days 16-17 yielding 4 groups: control no-stress (C-NS), control stress (C-S), Pb no-stress (Pb-NS) and Pb stress (Pb-S). In assessments of fixed-interval (FI) schedule controlled behavior of adult female offspring, stress alone (C-S) increased overall response rates and decreased pause time, while Pb exposure alone (Pb-NS) had no effect. In contrast, Pb-S significantly decreased response rates. To assess impacts on the HPA axis, blood basal and stress-induced corticosterone (CORT) levels were measured. Prenatal Pb exposure alone permanently increased CORT levels (Pb-NS and Pb-S groups). Restraint stress resulted in maximal CORT levels in all groups and no differences in subsequent FI performance. However, a brief exposure to a cold environment increased CORT levels above baseline in all groups except the Pb-S group. Finally, monoamines and metabolites were determined to evaluate long-lasting effects of the combined Pb/stress exposure in mesolimbic and nigrostriatal terminals. In nucleus accumbens, Pb-induced changes in levels of dopamine (DA) and 5-HT and metabolites were not altered by stress. However, in striatum, multiple interactions between Pb and stress were found in which generally Pb increased but Pb-S decreased neurotransmitter levels. A significant elevation in frontal cortex DA levels was observed only in the Pb-S group. Since Pb and stress can modulate each other's effects, the study of Pb as a risk factor in isolation may be unrepresentative of its actual adverse effects. ES05017 and ES01247.

900 EFFECTS OF EARLY LEAD EXPOSURE ON DOPAMINERGIC AND GLUTAMATERGIC NEURONAL MARKERS IN THE AGING RAT BRAIN.

J. L. McGlothan, T. Verina, C. D. Toscano and T. R. Guilarte. *Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.*

Childhood lead (Pb2+) intoxication is a major public health problem with thousands of children exposed each year in the US. It is well recognized that childhood Pb2+ exposure results in long-term changes in brain chemistry leading to deficits in cognitive function. Although these changes have been known to persist for years, little is known about the effects of Pb2+ exposure during early life on the aging brain. We examined the effects of chronic Pb2+ exposure on the dopaminergic and glutamatergic neuronal systems in the aging rat. Rats exposed to Pb2+ from gestation until 235 days of age were euthanized for neurochemical assessment at 1.3 years of age. Analysis of tyrosine hydroxylase (TH) immunoreactive neurons in the substantia nigra (SN) indicate a significant decrease (22.8%; $p < 0.05$) in Pb2+-exposed animals relative to controls. We also measured the levels of dopamine transporters (DAT) in the corpus striatum and SN using 125I-RTI quantitative autoradiography. Consistent with the loss of TH-positive neurons in the SN of Pb2+-exposed rats, we found a 41.4% decrease ($p < 0.01$) in the levels of DAT in the SN. On the other hand, there was a 19.4% ($p < 0.05$) increase in the levels of DAT in the striatum of Pb2+-exposed rats. We also measured the expression of NMDA receptor subunit genes in the hippocampus of the same animals. We found no significant differences in the NR1 or NR2A mRNA levels in the hippocampus of Pb2+-exposed rats. However, there was a significant decrease in NR2B subunit mRNA in the CA1 (12.6%; $p < 0.05$) and CA4 (13.1%; $p < 0.05$) subfields of the pyramidal cell layer and in the granule cell layer of the dentate gyrus (13.6%) in Pb2+-exposed rats. In summary, these studies provide evidence that early exposure to Pb2+ produces changes in dopaminergic and glutamatergic neuronal markers in the aging brain. These molecular and cellular changes may be associated with deficits in neurological function in the aging animal [Supported by NIEHS ES06189 to TRG]

901 PRENATAL EXPOSURE TO METHYLMERCURY ALTERS NEUROBEHAVIOR AND BRAIN MONOAMINE OXIDASE ACTIVITY IN SPRAGUE-DAWLEY RAT OFFSPRING.

C. J. Stamler[1], P. Beyrouty[3], K. M. Loua[1] and L. Chan[2]. *[1]Dietetics and Human Nutrition, McGill University, Montreal, QC, Canada, [2]Centre for Indigenous Peoples' Nutrition and Environment, McGill University, Montreal, QC, Canada and [3]Natural Resource Sciences, McGill University, Montreal, QC, Canada.*

Epidemiological data have shown that prenatal chronic low dose exposure to methylmercury (MeHg) is a risk factor for neurobehavior alterations in infants. Monoamine oxidase (MAO) regulates serotonin and dopamine levels in the developing brain and has recently been shown to play a critical role in neurodevelopment. The objective of this study was to evaluate the effects of gestational MeHg exposure on neurobehavior and brain MAO activity in different brain regions in rat offspring. Adult female rats were treated by gavage with saline or MeHg (0.5 or 1.0 mg/kg/d) for four weeks prior to mating and throughout pregnancy. Tests to assess cognitive, sensory and motor function were performed on the offspring between postnatal day (PND) 20 and 38. Auditory startle tests performed on offspring (PND 23) revealed a marked augmentation of the startle reflex amplitude at both dose levels. In addition, there was a gender difference in responses. Male offspring in the 1.0 mg/kg/d exposure group displayed a shorter response time for the hot plate test, an increased state of arousal and memory deficits as assessed by the passive avoidance tasks. Female offspring in the 0.5 mg/kg/d exposure group showed an increase in conditioning latency time. The offspring exhibited no significant alterations in motor activity, swim performance, Cincinnati water maze test or rotorod test. MAO activity in the offspring (PND 41) was significantly ($p<0.01$) decreased in the cerebral cortex of the 0.5 and 1.0 mg/kg/d exposure groups (22% and 19%, respectively) and in the striatum of the 1.0 mg/kg/d exposure group (24%) when compared to controls. Our data demonstrates a low-level gestational exposure to MeHg results in altered neurobehavioral function and a disruption in brain MAO activity in rat offspring. These neurochemical changes may be one of the underlying mechanisms of MeHg neurotoxicity.

902 EFFECTS OF EARLY POSTNATAL METHYLMERCURY ADMINISTRATION ON EPH RECEPTOR EXPRESSION AND SPATIAL LEARNING IN THE MOUSE.

A. Halladay[1, 2], D. T. Wilson[1], R. Zhou[3], G. C. Wagner[4, 1] and K. R. Reuhl[1, 2]. *[1]Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ, [2]Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ, [3]Department of Chemical Biology, Rutgers University, Piscataway, NJ and [4]Department of Psychology, Rutgers University, Piscataway, NJ.*

The appropriate spatio-temporal expression of the Eph family of tyrosine kinase receptors and their ligands is critical for neural migration and establishment of proper topographic projections in the central nervous system. Dysregulation of Eph tyrosine kinase receptor expression results in cognitive and motor impairments, as well as misguided axonal projections. Previous studies demonstrated that gestational and lactational administration of methylmercury (MeHg) produced an increase in the expression of Ephrin-A5, an Eph ligand involved in hippocampal development. In order to examine these effects of early postnatal MeHg exposure on Eph receptor expression as well as habituation and hippocampal-dependent spatial learning, mice were treated with either PBS or MeHg on alternate postnatal days 3-15 and allowed to explore an open field and trained on the water maze on days 24 and 25. Compared to PBS treated mice, MeHg treatment resulted in increased exploration following dishabituation in an open field. PBS-treated mice learned the water maze task after several trials, while MeHg treatment produced a significant, persistent deficit in escape latency throughout training. Using a RNase Protection Assay to examine Eph receptor mRNA levels in the hippocampus, an increase in EphA4 and A5 RNA was detected following repeated spatial navigation training trials, an effect attenuated by MeHg pretreatment. These results suggest that induction of Eph receptor expression may participate in morphological changes that underlie learning events in the hippocampus; and may partially underlie MeHg-induced cognitive deficits in rodent models. (Supported by NIH ES05022, NIH/EPA ES011256 and NIH ES11279)

903 ENVIRONMENTAL MERCURY EXPOSURE AND COGNITIVE FUNCTION IN OLDER ADULTS.

M. E. Weil[1], J. Bressler[1], T. Glass[1], P. Parsons[2], J. Hidalgo[3] and B. Schwartz[1]. *[1]Johns Hopkins University, Baltimore, MD, [2]New York State Department of Health, Albany, NY and [3]Autonomous University of Barcelona, Bellaterra, Spain.*

The neurotoxicology of low-level mercury (Hg) exposure has not been adequately studied in the general adult population. We evaluated associations of blood Hg with neurobehavioral test scores, and effect modification by factors that could influence the toxicology of Hg. Toxicokinetic differences might be due to differential

binding of Hg; metallothionein (MT) and hemoglobin (Hb) bind Hg avidly and both exhibit inter-individual variability. Toxicodynamic differences could be mediated by apolipoprotein (ApoE) genotype; previous studies have reported that the ApoE ε-4 allele may make the nervous system more susceptible to lead. We performed cross-sectional analysis of data from a longitudinal study of cognitive decline in 1, 140 50-70 year old Baltimore residents. A total of 474 subjects were randomly selected for a sub-study examining neurobehavioral test scores (NBT), blood Hg (cold-vapor atomic absorption spectrometry), fish consumption (Block 98.2), blood MT (radio-immunoassay) and Hb (cyanmethemoglobin). The sub-study consisted of 31% men, 54% whites and 39% blacks. The median Hg level was 2.1 μg/L (range: 0-16 μg/L). Linear regression was used to model NBT adjusting for age, gender, race, education and technician. Results indicate that higher Hg was associated with worse performance on Rey Complex Figure Delayed Recall (visual memory; $p = 0.04$) and Rey Auditory Verbal Learning Test Delayed Recall (verbal memory; $p = 0.08$). In contrast, higher Hg was associated with better performance on Purdue Pegboard (manual dexterity; $p < 0.01$), Finger-Tapping tests (manual dexterity; $p < 0.02$) and Category Fluency Score (language; $p = 0.04$). MT, Hb and ApoE genotype were not associated with NBT; nor did these factors modify the relation between Hg and test scores. Analysis of fish consumption data is ongoing. Overall the data suggest that blood Hg, a biomarker of relatively recent dose, was associated with poorer performance on memory tests and improved performance on tests of eye-hand coordination.

904 ASSESSMENT OF POSTNATAL EXPOSURE TO THIMEROSAL AND METHYL MERCURY USING A MORRIS WATER MAZE PROCEDURE IN B6C3F1 MICE.

J. Smythe, J. EuDaly, W. C. Griffin, D. E. Keil and M. Peden-Adams. *Medical University of South Carolina, Charleston, SC.*

The National Institute of Mental Health has stated that autism affects approximately 1 in 1000 children with an ever-increasing rate of diagnosis; however, the cause(s) of autism is unknown. Current attention has lead to the examination of thimerosal (TH) containing vaccines administered to infants. While the toxicity of TH has not been previously examined, mercury and other mercury containing compounds are known to affect the developing nervous system. Thus, we performed comparative studies to assess spatial learning and memory after postnatal exposure to either methylmercury (MeHg) or TH in male B6C3F1 using the Morris Water Maze (MWM). The MWM procedure required mice to learn the location of an escape platform hidden just below the surface in a pool of opaque water using spatially arranged visual cues. The mice were given 4 daily trials of 90 seconds each for 9 days. The first experiment compared adult male B6C3F1 mice with the parental strains of adult male C3H and C57 mice. The B6C3F1 mice readily acquired the maze problem as indicated by significantly greater declines in escape latency and steeper slopes than the parental strains. In the second experiment, male pups were exposed to either MeHg (0, 10, or 50 ug/kg) or TH (0, 20, 100, or 200 ug/kg) on postnatal days 7, 10, and 12 and examined in the MWM procedure at 8 weeks of age. No treatment effect was observed due to exposure to MeHg or TH. Although no treatment effects were observed after postnatal exposure to MeHg or TH, this study demonstrated that B6C3F1 mice readily learn under the conditions of the MWM procedure.

905 AGE-DEPENDENT SENSITIVITY OF STRIATAL MITOCHONDRIA TO MANGANESE-INDUCED CYTOTOXICITY.

J. Li[1, 2], R. Geng[1], J. Chu[1] and W. Zheng[2]. *[1]School of Public Health & Family Medicine, Capital University of Medical Sciences, Beijing, China and [2]School of Health Sciences, Purdue University, West Lafayette, IN.*

The mitochondrion is a known target for manganese (Mn) toxicity. However, the question as to whether the mitochondria in different ages may have the differential sensitivity to Mn insults remained unclear. The purpose of this study was to test the hypothesis that the mitochondria of the aged animals were more vulnerable to Mn cytotoxicity than those from the young ones. The mitochondrial fractions were collected from the striatal area of Wistar rats aged either 4 months old (the young group) or 18 months old (the aged group). Mitochondrial preparations were incubated with Mn at various concentrations at 37°C for 10 min. The functions of mitochondria were examined for the activities of Complex-I/III and Complex-II, mitochondrial membrane potential (MP), and mitochondrial superoxide dismutase (SOD) activity. The results showed that following Mn exposure (50, 100, 200, and 400 uM as $MnCl_2$), there was a dose-dependent inhibition of Complex-I/III in the young group (14, 24, 36, and 45% of inhibition) as well as in the aged group (15, 28, 41, and 49% of inhibition). In comparison to the control group, Mn exposure caused 57% inhibition of Complex-II in the young, whereas it caused 66% inhibition in the aged group. The age factor did not seem to affect the sensitivity of mitochondrial MP to Mn exposure, as determined by laser scanning confocal microscopy; a Mn dose-dependent decrease (50-400 uM) in the mitochondrial MP was observed in both age groups with 15-49% decrease in the aged group vs. 14-51% in the young group. Mn treatment at 20 uM, however, significantly inhibited SOD activity, and the effect was more profound in the aged group (93% inhibition) than in the young group (69% inhibition) ($p<0.05$, $n=15$). These data suggest that the age plays a significant role in the sensitivity of brain mitochondria to Mn-induced cytotoxicity. (Supported in part by PRC-National Natural Science Foundation #3000140, PRC-Beijing Science Committee # 9558102800, and USA-NIH/NIEHS ES08146)

906 IRON DEFICIENCY (ID) INCREASES MANGANESE (MN) ACCUMULATION IN THE DEVELOPING RAT BRAIN.

S. J. Garcia[1], T. Syversen[2], E. Steinnes[3], K. Gellein[3] and M. Aschner[1]. *[1]Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC, [2]Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway and [3]Chemistry, Norwegian University of Science and Technology, Trondheim, Norway.*

ID is a prevalent nutritional disorder, affecting ~2 billion people, particularly pregnant and lactating women and children. Since iron (Fe) and Mn share similar transport mechanisms, ID enhances Mn accumulation even in the absence of excess dietary Mn. While Mn neurotoxicity in adults results in neurological disturbances, little is known regarding Mn toxicity during brain development. Moreover, few studies have considered the interactions of ID and Mn throughout early brain development, a time when the brain is most vulnerable to change. Pregnant Sprague-Dawley rats were fed either control (35 mg Fe/kg, 10 mg Mn/kg diet) or low Fe (3 mg Fe/kg, 10 mg Mn/kg diet) semi-purified diets (AIN-93-G) from gestational day (GD) 7 until weaning at postnatal day (PN) 21. Neonates were cross-fostered from control dams to control or low Fe dams on PN4 and were exposed to the diets *via* maternal milk until PN21. To evaluate the relationship between systemic Fe status and Mn accumulation within the brain, we assessed hematological parameters and metal levels, including Mn, by inductively coupled plasma mass spectrometry (ICP-MS). Measurement of hematological parameters confirmed ID in dams and pups fed a low Fe diet. Hemoglobin was reduced in the Fe-deficient dams throughout lactation and in pups at PN21. Furthermore, Fe-deficient rats had decreased plasma Fe and increased plasma transferrin and total iron binding capacity. In Fe-deficient pups, Fe, chromium (Cr), and molybdenum (Mo) brain levels decreased while Mn and copper (Cu) levels increased. Surprisingly, no regional differences were detected in the striatum, hippocampus, midbrain or cerebellum. These data demonstrate that ID increases brain Mn during early development and that ID is a risk factor for Mn as well as Cr, Mo, and Cu alterations in the brain. Supported by NIEHS 07331 (MA) and 12768 (SJG)

907 THE EFFECTS OF TRIBUTYLTIN (TBT) ON NEUROTRANSMITTERS AND NMDA RECEPTORS IN THE BRAINS OF ICR MOUSE OFFSPRING.

M. Tsunoda[1], N. Konno[2] and Y. Sugita-Konishi[3]. *[1]Public Health, Fukushima Medical University, Fukushima, Japan, [2]Koriyama Women's University and College, Koriyama, Japan and [3]Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan.*

Tributyltin (TBT) compounds have been widely used as biocides and antifouling agents. TBT compounds have been detected in marine waters, fish and shellfish. The central nervous system is one of the target organs of TBT. TBT-induced modulations of neurotransmitters and binding to NMDA receptors in the brains of adult mice have been reported. However, little is known about the developmental neurotoxicity of TBT. In this study, we evaluated the neurotoxic effects of TBT on the offspring in ICR mice. Pregnant ICR mice were exposed to TBT chloride at concentrations of 0, 15, and 50 ppm in water and 125 ppm in diet. Exposure was continued until the time offspring were weaned (3 weeks). Male offspring were sacrificed at 1, 2 and 3 weeks after birth. Brain samples were dissected into the cerebrum, cerebellum, medulla oblongata, midbrain, corpus striatum and hypothalamus. The concentrations of norepinephrine, dopamine, dihydoxyphenylacetic acid(DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindolacetic acid (5-HIAA) were determined in different brain regions by HPLC. The binding of [3H]MK-801, which is the NMDA non-competitive antagonist, was also investigated in membrane preparations from the cerebrum. Since all offspring from the 125 ppm group died immediately after birth, comparisons were made among living groups only. Decreases in mean body weight were observed in TBT-exposed groups most notably at the first week. A significant increase in TBT-treated groups compared to the control was observed for HVA in the cerebrum and 5-HT in the medulla oblongata at the third week. The significant decrease in the binding of [3H]MK-801 was observed in TBT-treated groups at the third week. For neuro-

transmitters and binding to NMDA receptors in the brain of offspring, the effects of TBT were observed at the third week at the relatively lower levels than those observed in previous studies for adult mice.

908 BEHAVIORAL CONSEQUENCES OF PERINATAL MONOMETHYLTIN EXPOSURE IN RATS.

K. D. Ehman[2], K. L. McDaniel[1], P. M. Phillips[1] and V. C. Moser[1]. *[1]NTD/NHEERL, USEPA, Research Triangle Park, NC and [2]Curriculum in Toxicology, UNC/USEPA, Research Triangle Park, NC.*

Organotins, such as monomethyltin (MMT), are widely used as heat stabilizers in PVC and CPVC piping, which results in their presence in drinking water supplies. Concern for neurotoxicity produced by organotin exposure during development has been raised by published findings of a deficit on a runway learning task in rat pups perinatally exposed to either MMT or trimethyltin. The objective of the first study was to replicate the earlier publication and further define the dose-response characteristics of MMT. Female Sprague-Dawley rats were exposed *via* drinking water to MMT (0, 10, 50, 245 ppm) before mating and throughout gestation and lactation (until weaning at postnatal day, or PND, 21). Behavioral assessments included: the runway test (PND 11) in which the rat pups learned to negotiate a runway for dry suckling reward; motor activity habituation (PNDs 13, 17, and 21); and learning in the Morris water maze (as adults). Contrary to the earlier finding, there were no effects on any measure of growth, development, or cognitive function following MMT exposure. These findings were confirmed in a subsequent study in which the developmental effects of 500 ppm MMT in drinking water were explored in pregnant rats exposed from gestational day 7 until weaning. The behavioral assessments included the runway task (PND 11) and motor activity habituation (PND 17). In this second study, MMT-exposed females consumed significantly less water than the controls throughout both gestation and lactation, although neither dam nor pup weights were affected. As in the first experiment, MMT-exposure did not alter pup runway performance or motor activity habituation. These results indicate that perinatal exposure to MMT, even at concentrations which decrease fluid intake, does not result in significant neurobehavioral or cognitive deficits. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

909 COMBINED EFFECTS OF FLUTAMIDE AND β-ESTRADIOL 3-BENZOATE ON ADULT MOUSE TESTES.

R. Anahara[1], Y. Toyama[2] and C. Mori[1, 3]. *[1]Bioenviron Med., Grad Sch Med., Chiba University, Chiba, Japan, [2]Anat Devel Biol, Grad Sch Med., Chiba University, Chiba, Japan and [3]CREST, JST, Kawaguchi, Japan.*

Many reports on endocrine disrupting chemicals (EDCs) were investigated using single compounds. Little information is available about the combined effect of hormone active compounds. The present study investigated the combined-effect of β-estradiol 3-benzoate (E2B) and flutamide (Flu) on mouse testes by electron-microscopic observation. Both E2B and Flu were subcutaneously and simultaneously administrated to adult ICR mice (12-week-old) for 5 days. The combination of the doses was 20μg/kg/day (histological LOEL) of E2B and any one of 0.01, 0.1, 1 or 10μg/kg/day of Flu. Single treatments of Flu were also examined. Testes (n=5) were fixed on the 6th day for electron microscopy. The number of abnormal spermatids in the treated-mice and normal-mice were counted on semi-thin sections. Deformation of acrosomes and/or nuclei of spermatids and complete or partial deletion of the ectoplasmic specialization between the Sertoli cell and spermatids were observed. There were no differences in ultrastructure between the combined-treatment and single-treatments of Flu. Disarrangements of seminiferous cycles were observed in combined-treatment by light microscopy, but these changes were not seen in single-treatments. Moreover, the percentages of abnormal spermatids were increased at histological NOEL in combined-treatments. The effects of combined treatment on testes of spermatids have tendencies with no linear-correlation in accordance with dose. There were no differences in the observed histological change between the single-treatment and combined-treatment, even though in the combined & treatment of E2B and the highest-dose of Flu (10μg/kg/day). In this study, the dose of histological NOEL of Flu was decreased in the presence of exogenous estrogen, and also xenoestrogenic actions were observed in the combined-treatments than in single-treatments. In conclusion, the combined treatments of both estrogen (histological LOEL) and low-dose-Flu on mouse testes induced synergic effect and strengthened the effect of Flu.

910 VALIDATION OF A METHOD OF INTRAPROSTATIC ADMINISTRATION OF TEST MATERIAL TO THE MOUSE.

S. Grainger and G. Hale. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: D. Everett.

The dorsal and lateral prostate glands of the mouse are composed of branching ducts and glands surrounded by loose connective tissue and adipose tissue situated around the dorsal and lateral aspects of the proximal urethra. A method was developed to deliver test material (in this case, a viral gene vector) to the dorsal prostate of the mouse. The method and its validation for use are described. Mice are anaesthetised with isoflurane in oxygen. Antibiotics and analgesics are administered and the animal placed on a heated pad. Using aseptic surgical technique the urinary bladder is exposed at laparotomy. The ventral prostate is visualised by gentle traction on the bladder and, if necessary, section of the middle ligament of the bladder and blunt dissection of fatty tissue around the bladder neck. Using the ventral prostate as a guide, test material (50 μl) is deposited in the area of the dorsal prostate by transurethral injection using a 300 μl insulin syringe with a 30 gauge needle. Surgical repair is routine. For validation purposes the administration procedure was carried out on ten male CD-1 mice freshly killed by exposure to a rising concentration of carbon dioxide in a chamber. 50 μl of indian ink was injected into the area of the dorsal prostate. Immediately after injection the bladder, proximal urethra, prostate, seminal vesicles and coagulating glands were removed in one piece and fixed in 10% neutral buffered formalin prior to processing to paraffin wax blocks, sectioning and staining with haematoxylin and eosin. During organ removal and histological processing care was taken to maintain anatomical relationships as closely as possible. The distribution and density of indian ink particles was assessed in the histological sections and correlated with observations made at the time of injection. When the administration had been thought satisfactory, there was a clear concentration of indian ink particles in the loose connective and adipose tissues surrounding the lobules of the dorsal and lateral prostate and the base of the anterior prostate (coagulating glands).

911 EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO 17 ALPHA-ETHYNYLESTRADIOL ON SPERM QUALITY AND EGG FERTILIZING ABILITY OF MALE OFFSPRING IN MICE.

J. HAN[1, 3], P. M. Saama[1], T. R. Zacharewski[2, 3, 4] and K. Chou[1, 3, 4]. *[1]Animal Science, Michigan State University, East Lansing, MI, [2]Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI, [3]Institute for Environmental Toxicology, Michigan State University, East Lansing, MI and [4]National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI.*

Previously, we reported decreases in sperm production and fertilizing ability *in utero* and lactational exposure to high doses (1 and 10 ug/kg of maternal BW) of diethylstilbestrol (DES) and increases in fertilizing ability after low dose exposure. The objective of this study was to examin the effect of 17 alpha-ethynylestradiol (EE2), the major estrogenic component of oral contraceptives, on sperm production and fertilizing ability in male offspring. Pregnant C57BL/6 mice (F-0), bred with DBA/2 male, were gavaged with 0, 0.1, 1, or 10 ug EE2/kg of BW per day from gestational day 12 to postnatal day (PND) 20. The male offspring (F-1), at 15-17 or 45-48 weeks of age, were examined for sperm quality by using sperm motion analysis and *in vitro* fertilization (IVF) assay. The anogenital distance (AGD) of the male offspring in the 1 and 10 ug dose groups was shorter than that of the control animals on PND21. Sperm concentration from 45-48 week-old male offspring was lower in the 10 ug dose group than that in the control ($P<.05$), but not at 15-17 weeks of age. However, when equal sperm concentrations were used in IVF assay, no treatment effect on fertilization in either age group was observed. Results from this study suggest that exposure to high doses of EE2 during gestation could decrease sperm production, body weight, and AGD, while there is no apparent effect on sperm fertilizing ability. Similar to the observation in our DES study, low dose exposure, 0.1 ug/kg of maternal body weight, had no detrimental effect on sperm production of offspring. The median dose tested, 1 ug/kg, is about 1.5 to 2 times of the dosage used in oral contraceptives. (Acknowledgement: EPA, R827-420-01-0, and Michigan Agricultural Experiment Station)

912 DISRUPTION OF MALE REPRODUCTIVE DEVELOPMENT IN THE RAT BY A SINGLE DOSE OF THE ANTIANDROGEN, FLUTAMIDE, ADMINISTERED ON DIFFERENT GESTATIONAL DAYS.

P. M. Foster and M. W. Harris. *DHHS, NIH, NIEHS, Research Triangle Park, NC.*

Previous studies have indicated that the antiandrogen, flutamide, can produce a suite of reproductive malformations in the rat when administered daily on gestation days (GD) 12-21. The objective of this study was to investigate the time dependence for the induction of these malformations to establish a robust animal model for future studies of gene expression related to specific malformations. Groups of timed-pregnant Sprague-Dawley rats (GD 0 = day of mating) were administered flutamide as a single gavage dose (50 mg/kg) on GD 16, 17, 18, or 19 with 10 dams per group. Control animals (5 dams / time group) were administered corn oil vehicle (1ml/kg). Dams were allowed to litter and their adult male offspring were killed at postnatal day (PND) 100±10. Anogenital distance was measured at PND 1 and 100. Areolae were scored at PND 13 and permanent nipples evaluated at PND 100. No reproductive tract malformations were found in control male offspring. In

the treated groups, malformations were noted following exposure at every GD, although the incidence of specific malformations varied by GD. At GD 16, the highest incidence was noted for permanent nipples (46% pups, 60% litters), epispadias (12% pups, 30% litters), and missing epididymal components (5% pups, 20% litters). The highest incidences for hypospadias (58% pups, 80% litters), vaginal pouch (49% pups, 70% litters), cleft prepuce (29% pups, 60% litters) and missing prostate lobes (12% pups, 60% litters) were noted at GD 17. At GD 18 the highest incidence of malformations noted were epispadias (5% pups, 30% litters), reduced prostate size (32% pups, 90% litters) and abnormal kidneys (3% pups, 30% litters) and bladders (7% pups, 30% litters), while on GD 19 70% of the litters had animals with abnormal seminal vesicles. Thus, a single gestational exposure of flutamide induced numerous reproductive tract malformations consistent with previously reports following multiple exposures, with the timing of the exposure producing marked tissue selectivity in the response noted in adult offspring.

913 EFFECTS OF SULFASALAZINE ON SPERM ACROSOME REACTION AND GENE EXPRESSION IN THE REPRODUCTIVE ORGANS.

T. Fukushima[1, 2], M. Kato[1], T. Adachi[3], Y. Hamada[1], M. Horimoto[1], M. Komiyama[2], C. Mori[2] and I. Horii[1]. *[1]PGRD Nagoya Lab., Pfizr.Inc., Taketoyo, Aichi, Japan, [2]Department of Bioenvironmental Mdedicine, Graduate School of Medicine, Chiba university, Inohana, Chiba, Japan and [3]Center for Research and Development of Bioresources, Reserch Institute for Advanced Sciences and Technology, Osaka Prefecture University, Sakai, Osaka, Japan.* Sponsor: M. Kurata.

Sulfasalazine (SASP) has been reported to depress the fertility in men. This study was undertaken to investigate the mechanism by which SASP affects fertility. After we confirmed the effect of SASP on the fertility of rats administered the drug at the dose of 600 mg/kg/day for 28 days, we investigated its effects on sperm motion and acrosome reaction using FITC-concanavalin A lectin stain and on gene expression using cDNA microarray and real-time RT-PCR in the reproductive organs. We observed a decrease in sperm velocity and a depression of acrosome reaction in the SASP treated group. In the testes, acrosome membrane related genes (CD59) expression was slightly altered in the SASP treated group. There were, however, no changes in the expression of the genes related to spermatogenesis. In the epididymides, the expression of CD59 and other acrosome membrane related genes, membrane cofactor protein (MCP) and decay accelerating factor (DAF), were decreased in the SASP treated group. It is known that CD59, MCP and DAF have biological functions related to sperm motion and that MCP is involved in the acrosome reaction or binding of the sperm with the egg. From these findings, it was elucidated that the effects of SASP on fertility were mediated by a depression of sperm velocity and acrosome reaction. These findings correlated with the decreased expression of CD59, MCP and DAF gene in the epididymides.

914 METHYL TERTIARY-BUTYL ETHER INDUCES ALTERATIONS IN MOUSE TESTIS WEIGHT, TESTOSTERONE PRODUCTION AND MORPHOLOGY.

L. Almeida and E. Hall. *Biology, Rhode Island College, Providence, RI.*

Methyl tertiary-butyl ether (MTBE) is the most common fuel oxygenate used today. Oxygenating fuel serves to induce a more complete combustion reaction, thus reducing CO and hydrocarbon emissions. Because of the high water solubility and widespread use of MTBE, there is a high risk of exposure through groundwater that has been contaminated with MTBE oxygenated fuel from leaking, underground storage tanks and pipelines. In this study, mice were exposed to doses of 80, 800, and 8000 ppb MTBE in drinking water for 28 days. Results demonstrated a significant, dose-dependent increase in mean combined testis weight, mean seminal vesicle weight, mean seminiferous tubule diameter, and incidence of abnormal tubules. Serum testosterone was substantially decreased by exposure to 800 and 8000 ppb of MTBE. These results indicate that MTBE may act *via* a disruption in endocrine-mediated Leydig cell testosterone production. Supported by NIH RR16457 from the BRIN Program of the NCRR and a grant from the Rhode Island College Faculty Research Committee.

915 *IN VIVO* EXPOSURE OF YOUNG ADULT RATS TO METHOXYCHLOR (M) REDUCES SERUM TESTOSTERONE (T) LEVELS, BASAL LEYDIG CELL (LC) T FORMATION, LC CYTOCHROME P450 CHOLESTEROL SIDE-CHAIN CLEAVAGE (P450SCC) ACTIVITY AND SERUM DEHYDROEPIANDROSTERONE (DHEA) LEVELS.

E. P. Murono and R. C. Derk. *Pathology and Physiology Research Branch, CDC/NIOSH, Morgantown, WV.* Sponsor: V. Castranova.

M is a pesticide developed as a replacement for dichlorodiphenyltrichloroethane (DDT). Although its metabolite, 2, 2-bis(p-hydroxyphenyl)-1, 1, 1-trichloroethane (HPTE), is thought to be the active compound, both M and HPTE have been reported to exhibit weak estrogenic and/or antiandrogenic activities and thereby produce adverse reproductive effects in rodents. In the current studies young adult male rats (at least 11 animals per each treatment group) were gavaged once daily between days 54-60 days of age with 0, 5, 40 or 200 mg M/kg body weight in corn oil. Animals were sacrificed ~24 h after the last exposure to assess the effects of M on LC steroidogenic competence. Serum T levels declined from 4.40 ± 0.52 ng/ml (control) to 1.82 ± 0.27 ng/ml at the 200 mg/kg dose. Similarly, both wet and expressed seminal vesicle weights declined to 44 and 60% of control, respectively, at the highest exposure dose. However, serum LH and FSH levels were unaffected by M. In addition, testicular LC isolated from exposed animals produced less T under basal conditions following a 4 h incubation period (2.20 ± 0.13 ng T/4 h/10^5 cells in LC from 200 mg/kg exposed animals vs 4.50 ± 0.31 ng/4 h/10^5 cells in control cells). Also, P450scc activity of isolated LC declined from 20.44 ± 0.92 ng side-chain/h/10^5 cells (control) to 16.10 ± 1.29 and 10.15 ± 0.98 ng/h/10^5 cells in LC from animals exposed to 40 and 200 mg/kg M, respectively. Although serum corticosterone levels were unaffected by M, serum DHEA levels declined from 0.11 ± 0.01 ng/ml (control) to 0.05 ± 0.01 ng/ml in 200 mg/kg exposed animals. Whether this decline in DHEA represents adrenal or LC androgen remains to be determined. These studies suggest that *in vivo* exposure of M to young adult male rats directly reduces LC T formation and that this decline is due to inhibition of LC P450scc activity.

916 DI(*N*-BUTYL) PHTHALATE RAPIDLY REPRESSES STEROIDOGENESIS IN THE FETAL TESTIS AND INTERFERES WITH ADRENAL STEROIDOGENESIS THROUGH AN ALTERNATIVE MECHANISM.

C. Thompson, S. M. Ross, S. Heinze and K. W. Gaido. *CIIT Centers for Health Research, Reserach Triangle Park, NC.*

The phthalate ester di(*n*-butyl) phthalate (DBP) produces antiandrogenic effects on reproductive development in male rats by interfering with testosterone production. We have shown that several genes involved in steroidogenesis are downregulated in the fetal testis following *in utero* exposure to DBP. Expression of these genes returns to control levels within 48 hours of DBP withdrawal. Our aim was to determine how rapidly testosterone synthesis is affected following exposure to DBP. Also, since several genes repressed by DBP are expressed in other steroidogenic tissues, we evaluated the effects of DBP exposure on steroidogenesis in the fetal adrenal. Pregnant Sprague-Dawley rats were dosed *via* oral gavage with 500 mg/kg/day DBP or corn oil from gestational day (gd) 12 to 19. The start of DBP dosing was shifted from gd 12 one day later in gestation for each treatment group, so that the final group was dosed only on gd 19. On gd 19, testes were removed for testosterone, RNA, and protein isolation. Testosterone production was significantly repressed in all groups exposed to DBP. The mean testosterone concentration in the fetuses dosed only on gd 19 was 44% of control. Mean testosterone concentration of all other dose groups was 13% of control. mRNA and protein levels for StAR, SR-B1, $P450_{SCC}$, and CYP17 were correspondingly repressed at all time points. In the adrenal gland, corticosterone content was decreased 42% in male fetuses and 48% in female fetuses following DBP treatment from gd 12-19, although only the decrease observed in the females was statistically significant. mRNA expression of SR-B1, StAR, and $P450_{SCC}$ were not significantly altered in the adrenal following DBP treatment. Our results show that DBP rapidly leads to transcriptional repression in the fetal testis resulting in diminished testosterone production. Diminution of steroid concentrations in the fetal adrenal occurs through a mechanism distinct from the transcriptional repression caused by DBP in the fetal testis.

917 THE EFFECTS OF NEONATAL EXPOSURE TO DIETHYLSTILBESTROL AND 17β-ESTRADIOL IN MOUSE EPIDIDYMIS.

K. Yamazaki[1], Y. Ono[1], T. Adachi[2], H. Fukata[3], K. Kojima[4], K. Chiba[4], C. Mori[1, 5] and M. Komiyama[1, 6]. *[1]Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Chiba, Japan, [2]Center for Research and Development of Bioresources, Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai, Osaka, Japan, [3]Department of Bioenvironmental Medicine (SRL), Graduate School of Medicine, Chiba University, Chiba, Chiba, Japan, [4]Laboratory of Biochemical Pharmacology and Toxicology, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Chiba, Japan, [5]Core Research for Evolutional Science of Technology (CREST), Japan Science and Technology Corporation (JST), Kawaguchi, Saitama, Japan and [6]Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Chiba, Japan.*

Recently, several studies have reported that semen quality has decreased. Endocrine disruptors (EDs) have been thought to be one of the possible causes. Epididymis is an important organ for maturation of spermatozoa. Perinatal exposure to EDs has induced morphological changes in epididymis. However, there is little information about the effects of EDs on epididymal gene expression. In this study, we examined

morphological changes and alteration of gene expression using *in house* cDNA microarray in epididymis after neonatal exposure to diethylstilbestrol (DES) or 17β-estradiol (E_2). Male newborn ICR mice were treated with DES or E_2 (5 μg/mouse/day) for 5 successive days, and their epididymides were dissected at 2, 4, and 8 weeks of age. Morphological observation revealed that the height of epithelial cells was lower than control, and unusual supranuclear vesicles were observed in the initial segment of epididymal duct at 2, 4 and 8 weeks of age in both groups. In microarray analysis, the expression of 340 and 196 genes was altered at 4 and 8 weeks of age in DES group, respectively, as compared with control. In E_2 group, 5350 and 985 genes were different in expression from control at 4 and 8 weeks of age, respectively. These results suggest that neonatal exposure to exogenous estrogen alters the expression of several genes and induces the morphological changes in epididymis.

918 IMPACT OF METHODS OF EUTHANASIA ON SPERM MOTILITY OF OLDER ADULT SPRAGUE-DAWLEY RATS.

S. L. Lohrke[1], S. A. Stutler[1], E. W. Johnson[1], J. E. Miller[1], K. Carnes[2], R. A. Hess[2], D. L. Schaeffer[2] and D. P. Arfsten[1]. [1]*Naval Health Research Center Toxicology Detachment, Wright-Patterson AFB, OH and* [2]*College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL.*

There are several agents and methods that are approved by the American Veterinary Medical Association for administering euthanasia. Most involve administering an overdose of inhaled or injectable anesthetic. It is known that the administration of drugs or chemicals can have an impact on sperm motility and *via*bility. NHRC scientists carried out studies to compare the effects of euthanasia on sperm motility in the interest of determining whether reported absolute sperm motility/*via*bility were comparable across studies in situations where the methods or agent of euthanasia were different. In this study, the impacts of different euthanasia methods on sperm motility and *via*bility were determined in 22 week-old Sprague-Dawley rats. Agents tested in this study were CO_2, halothane, isoflurane, enflurane, sevoflurane sodium pentabarbitol, and Buthansia D solution. Sperm motility and *via*bility were compared with those of animals euthanized by rapid decapitation (negative controls) or treated with α — chlorohydrin (positive controls) prior to rapid decapitation. Sperm motility and *via*bility was measured using a Hamilton Thorne sperm analyzer (HTM-IVOS Version 12). Preliminary results suggest that there does not appear to be significant differences in sperm motility or *via*bility between animals euthanized rapid decapitation versus those euthanized by CO_2 asphixiation or an overdose of sodium pentobarbital. Work is continuing on determining the effects (if any) of halothane, isoflurane, enflurane, and sevoflurane on sperm motility and *via*bility as compared with controls.

919 *IN VITRO* EXPOSURE TO 8-MOP DAMAGES MALE REPRODUCTIVE CELLS.

M. M. Diawara and J. Carsella. *Biology, Colorado State University - Pueblo, Pueblo, CO.*

The psoralens occur naturally in many plants. Synthetic forms of 8-methoxypsoralen (8-MOP) have been widely used to treat skin disorders. Studies have shown that Wistar rats have low birth rates when both male and female are dosed with 8-MOP or when only female in a mated pair is exposed. Reduced sperm counts, reduced litter size, decreased weight of individual pups and birth defects were also exhibited when only the male is dosed. This was accompanied by other teratogenic effects such as missing limbs and discolored fetuses in offspring of naive females mated to dosed males, suggesting adverse effect on the sperm in dosed males. Therefore, the current study was initiated to see if 8-MOP exposure would damage the DNA of rat spermatozoa. A modified comet assay protocol was used to test spermatozoa DNA for 8-MOP-induced damage. Spermatozoa samples were collected from the testes of mature Wistar rats and reacted with varying concentrations of 8-MOP (0, 2, 4, 6, 8, 10 mg/ml Acetone solution). Some of the treated samples were exposed to long-range UVA radiation and some were not. Samples were then digested with proteinase K in 1% SDS and TES buffer for 60 minutes. The cells were then placed in a low melting point gel and lysed. DNA damage in the samples was scored. Images were gathered using a fluorescence microscope and analyzed by computer. The moments (ratio of nucleus to tail for each gamete) of comets were statistically analyzed. Treated, irradiated sperm samples sustained significantly more damage than untreated, irradiated samples. The treated, non-irradiated sperm samples also sustained significantly more damage than the untreated, non-irradiated samples. The damage in these sperm samples occurred in a dose-dependant manner in both irradiated and non-irradiated groups. These results, which are consistent with previous findings, provide evidence that the linear furanocoumarins could compromise the reproductive function in a naive female by indirect effect on the sperm in the seminal fluid. (Supported by NIGMS grant 2S06 GM008197-20 MPRC-B)

920 FUNCTIONAL EXPRESSION OF PPAR GAMMA IN SERTOLI CELLS.

Y. Ye and J. H. Richburg. *College of Pharmacy, The University of Texas at Austin, Austin, TX.*

Recently, mono-2-ethylhexyl phthalate (MEHP) has been shown to activate the PPAR gamma receptor in granulosa cells and is likely responsible for the MEHP-induced suppression estradiol production by the ovary. Since the Sertoli cell is the primary target cell of MEHP in the testis, we hypothesized similar mechanisms of action may contribute to MEHP-induced testicular injury in the testis. To test this hypothesis, we examined the expression of PPAR gamma receptor in primary Sertoli cell cultures and the mouse TM4 Sertoli cell line. We also utilized 15-deoxy-Delta12, 14-PGJ2 (15d-PGJ2), an endogenous ligand for PPARgamma, to examine the functional consequences of PPAR gamma receptor activation in TM4 cells. Using immunohistochemical staining, a nuclear localization of PPAR gamma receptor was detected in both primary Sertoli cells and TM4 cells. Staining was not observed in germ cells of primary Sertoli-germ cell co-cultures. Incubation of TM4 cells with 15d-PGJ2 (10 μM) for 24 hours significantly inhibited the proliferation of TM4 cells (a decreased of 64 percent). However, the growth inhibition effect of 15d-PGJ2 was not reversed by GW9662, a specific PPAR gamma antagonist. 15d-PGJ2 (10 μM) was able to rapidly (1h) increase the phosphorylation of the kinase ERK1/2 by 127 percent in TM4 cells. In contrast, MEHP (200 μM) caused only marginal ERK1/2 increases (27 percent) that were maximal at 30 minutes. These novel findings indicate that Sertoli cells express PPAR gamma receptor and that 15d-PGJ2 is capable of triggering an ERK1/2 response. These observations provide for a foundation for our future investigations on the role of PPAR gamma in Sertoli cells and its possible modulation by MEHP. (Supported, in part by grants from NIH/NIEHS ES09145 & ES07784).

921 CRITICAL WINDOWS OF VULNERABILITY FOR EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON PRENATAL PROSTATE DEVELOPMENT IN MICE.

S. M. Hicks, T. Lin and R. E. Peterson. *School of Pharmacy, University of Wisconsin, Madison, WI.*

A single maternal dose of TCDD on gestation day (GD) 13 impairs prostate development in C57BL/6J mice. The effect is most severe in ventral prostate where TCDD causes complete agenesis by inhibiting formation of epithelial buds. TCDD does not appear to inhibit early bud formation in dorsolateral and anterior prostate but alters number and patterning of buds leading to smaller adult organs. A time course study was performed to define critical windows of vulnerability to TCDD for the various prostate lobes. Pregnant mice were dosed orally with 0, 5, or 25 μg/kg TCDD on GD 13, 14, 15, 16, or 17. Urogenital sinus (UGS) from male fetuses was collected on GD 18 and evaluated for epithelial bud formation by scanning electron microscopy. In all cases, the 25 μg/kg dose slightly enhanced the effects seen in male fetuses from dams exposed to 5 μg/kg TCDD on the same GD, which are detailed below. Ventral budding was completely inhibited in fetuses exposed on or before GD 15 but was normal in those exposed on GD 17. There was some ventral budding in fetuses exposed on GD 16, but buds were laterally dislocated. In fetuses exposed on or before GD 14, lateral buds moved up to the anterior-dorsal surface and dorsal buds crowded along the dorsal-posterior ridge. There was moderate recovery of dorsolateral bud position if TCDD exposure occurred on GD 15 or 16 and full recovery if exposure occurred on GD 17. Anterior buds appeared normal in all samples. However, the buds were proportionally larger in males exposed later in gestation, a trend also apparent in dorsolateral buds. In summary, UGS regions that develop into the lobes of the adult mouse prostate are vulnerable to disruption by TCDD at different times during gestation. The anterior region is susceptible beginning on or before GD 13 to changes affecting bud growth. Maximum vulnerability of the dorsolateral regions begins on GD 14, though some effect remains in mice exposed later in gestation. Ventral budding can be completely inhibited by exposure as late as GD15. (NIH grant ES 01332)

922 PROTEOME ANALYSIS OF THE EFFECTS OF *IN UTERO* 2, 3, 7, 8- TETRACHLORODIBENZO-*P*-DIOXIN EXPOSURE ON MALE C57BL/6 MOUSE UROGENITAL SINUS.

R. Karjanlahti[1, 2], University. Simanainen[1, 2], M. Tuomainen[2], S. Ryhanen[2], T. Lin[3], J. Tuomisto[1, 2], S. Karenlampi[2], R. E. Peterson[3] and M. Viluksela[1]. [1]*National Public Health Institute, Kuopio, 70701, Finland,* [2]*University of Kuopio, Kuopio, 70211, Finland and* [3] *University of Wisconsin, Madison, WI.*

The earliest visible stage of prostate development is bud formation from the urogenital sinus (UGS) epithelium. This early stage of prostate development is vulnerable to environmental disturbances. *In utero* 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin

(TCDD) exposure on gestational day (GD) 13 disrupts prostate development in a lobe-specific manner by preventing ventral bud formation, reducing dorsolateral bud number and delaying dorsolateral and anterior bud formation in male mice. This effect of TCDD is aryl hydrocarbon receptor (AHR) dependent. In UGS gene expression analysis mRNA levels were altered on GD 16 after a single maternal dose of 5 μg TCDD/kg on GD 13. Using proteomics as a tool we analyzed the effect of a single maternal dose of TCDD on the pattern of UGS protein expression. Pregnant C57BL/6 mice were administered po. 5 μg TCDD/kg or vehicle on GD 13 and the UGS from male fetuses were collected on GD 16. Five to eight UGS per sample were pooled and lysed directly into rehydration solution containing protease free Dnase/Rnase. For each gel, 150 μg of protein was first applied to a 24 cm, pH 4-7 IPG strip (Amersham Biosciences) using Ettan™ IPGphor™ Isoelectric Focusing System and the second dimension was run by Hoefer™ Dalt vertical system according to manufacturer's instructions. The gels were stained with SYPRO™ Ruby fluorescence dye and imaged on an FLA3000 reader. In total about 700 proteins were analyzed with PDQuest (7.0.1, Bio-Rad) and statistical analysis with SPSS (version 11.5). The expression of 65 proteins were significantly altered. Of those, 24 were up-regulated after TCDD exposure. Determining the identity of the altered proteins will provide greater insight into mechanism by which TCDD disrupts prostate development. (Supported by the Academy of Finland, Grant 53307/2001, and Graduate School of Environmental Health).

923 EXAMINING THE POSSIBILITY THAT CHRONIC AZATHIOPRINE TREATMENT POTENTIATES GERMLINE TRANSMISSION OF *HPRT* MUTATIONS IN C57BL/6 MICE.

S. Bendre[1, 2], J. G. Shaddock[2], V. N. Dobrovolsky[2] and R. H. Heflich[2, 1]. [1]*Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR and* [2]*NCTR, Jefferson, AR.*

Azathioprine (Aza), a pro-drug of 6-mercaptopurine, is used in human medicine to prevent transplant rejection and for the treatment of autoimmune diseases. Patients undergoing chronic Aza therapy can have extremely large increases in *Hprt* lymphocyte mutant frequency (MF) (up to 10 x 10^{-2}) that may be caused by selection and amplification of pre-existing *Hprt* mutant lymphocytes. We have developed a mouse model to test the hypothesis that *in vivo* selection by Aza increases germ cell, as well as somatic cell *Hprt* MFs. 55 male C57BL/6 mice were treated with 10 mg/kg Aza for 23 weeks; 10 control mice were treated with the vehicle. Each of the treated animals then was bred with 3 females, and each of the controls with 1 female for a total period of 8 weeks. The resulting litters were sexed, weighed and counted, and the female offspring were sacrificed at 28 days of age to measure splenic lymphocyte *Hprt* MFs. Since the *Hprt* gene is on the X-chromosome, female mice with a germline *Hprt* mutation should have lymphocyte *Hprt* MFs between 1 and 5 x 10^{-1}. The results indicate that 335 of the female offspring have *Hprt* MFs of 0-6 x 10^{-6} (the normal range for mice this age), but 5 of the offspring have slightly elevated lymphocyte *Hprt* MFs of 15-30 x 10^{-6}. Since one of these mice was fathered by a control male, these relatively high MFs may be part of the normal variation in lymphocyte *Hprt* MFs for mice this age. The mutants from these mice are being analyzed for alterations in the *Hprt* gene to determine if they were due to clonal expansion of single mutations or are independent events. The males treated with Aza were also evaluated for *Hprt* mutants, and 12 had lymphocyte MFs of 0.01-25 x 10^{-2}, indicating that the Aza treatment successfully selected somatic cell mutants. Although data continue to be collected on additional offspring of Aza-treated mice, the present results indicate that Aza therapy does not promote high levels of germline *Hprt* mutation transmission.

924 TRANSPLACENTAL EXPOSURE TO 17-α-ETHYNYL ESTRADIOL INDUCES A SPECIFIC GENE EXPRESSION PROFILE IN THE DEVELOPING MALE RAT REPRODUCTIVE SYSTEM.

J. M. Naciff, K. A. Hess, S. M. Torontali, J. P. Tiesman, G. J. Overmann, G. J. Carr and G. P. Daston. *Central Product Safety, Procter & Gamble Company, Cincinnati, OH.*

Exposure of developing fetal rodents to estrogenic compounds at relatively high doses induces abnormalities in the male reproductive system and causes a predisposition to abnormal function during adulthood. We hypothesize these latent developmental effects are preceded by immediate changes in the fetal gene expression. Thus, an approach to address the potential impact on the physiology of the male reproductive system to estrogenic exposure is the evaluation of the gene expression changes elicited in this system. In this study, high-density oligonucleotide arrays were used to determine the transcriptional program produced by transplacental exposure to 17 α-ethynyl estradiol (EE), in the developing rat testis and epididymis on gestation day (GD) 20, following EE exposure from GD11 to GD20. At the highest dose (10 μg EE/kg/day) less than 1% of the detected mRNAs (83 genes; out of 8740) showed a statistically significant change in expression levels, without inducing detectable morphological changes. Dose-dependent analysis of the transcript profile revealed the expression of a set of 56 genes significantly modified in the fetal testis by transplacental exposure to EE. Comparing the effect of EE exposure on the testis versus the uterus, EE induces changes in the expression of 19 transcripts exclusively in the developing testis, suggesting these transcripts represent male-specific estrogenic activity. The expression of several proteins was affected in a similar manner by EE as the gene expression profile. For example, steroidogenic acute regulatory protein (StAR) was significantly decreased in developing testes. Thus, our data demonstrate that transplacental exposure to a potent ER agonist changes the gene expression profile of estrogen-sensitive tissues of the male, and that these transcripts could be valuable markers of estrogen exposure. These studies have lead to the further investigation of additional compounds and the potential effects at very low doses.

925 MORPHOLOGY OF THE FETAL RAT TESTIS PRESERVED IN DIFFERENT FIXATIVES.

P. C. Howroyd[2, 1], R. Hoyle-Thacker[1], O. Lyght[1], D. Williams[1] and E. Kleymenova[1]. [1]*CIIT Centers for Health Research, Research Triangle Park, NC and* [2]*Experimental Pathology Laboratories, Inc., Research Triangle Park, NC.* Sponsor: E. Gross.

Developmental toxicity studies play a significant part in risk assessment. Histopathological examination of the fetal and neonate testes is important for the detection of developmental effects. The rat is the preferred species for such studies. This study was conducted to compare the morphology of H&E or immunostained fetal rat testes after fixation in modified Davidson fluid (mDF), Bouin fluid or 10% neutral buffered formalin (NBF). Fetal testes with epididymides, fixed in situ, were removed from the abdomen, embedded in paraffin, and 5 micron sections cut. Sections were stained with H&E, for apoptotic cells by the TUNEL technique, or immunostained for BrdU. In 10% NBF-fixed testes, chromatin and nucleoli were not easily discernable in the nuclei of cells above the basal layer of the seminiferous epithelium. In Bouin-fixed testes, the clarity of nuclear detail especially in suprabasal cells was better than with 10% NBF, as nucleoli were visible as basophilic structures. In mDF-fixed testes, nuclear detail was even clearer in supra-basal cells with some gonocyte nucleoli clearly visible as eosinophilic structures, in contrast to cells in the basal layer and interstitium, in which nucleoli were basophilic. In 10% NBF-fixed testes, there was only mild shrinkage artifact affecting cells in the seminiferous epithelium. In BrdU-stained slides, clarity of nuclear counter-staining was better in Bouin or mDF-fixed testes than with 10% NBF, although the numbers of positive cells and the intensity of staining were similar regardless of the fixative used. In TUNEL-stained slides, nuclear detail was clearer with mDF compared to 10% NBF fixation. Bouin fixation gave far worse visualization of nuclear detail and TUNEL-stained nuclei, and high background staining. We concluded that fixation in mDF resulted in better nuclear detail in seminiferous epithelium of rat fetuses compared to fixation in 10% NBF or Bouin fluid. Use of Bouin fluid did not allow satisfactory detection of fluorescent TUNEL staining

926 DEVELOPMENTAL EXPOSURE TO DI-N-BUTYL PHTHALATE AFFECTS CORD ORGANIZATION AND SERTOLI CELL-GONOCYTE INTERACTIONS IN THE FETAL RAT TESTIS.

E. Kleymenova, E. Dei Rossi, K. Liu, C. Swanson, L. Pluta and K. Gaido. *CIIT Centers for Health Research, Research Triangle Park, NC.*

Impaired spermatogenesis in adults has been associated with abnormal development of Sertoli and Leydig cells during fetal life. We used rats *in utero* exposed to di-n-butyl phthalate (DBP) to gain insights into fetal abnormalities underlying impaired spermatogenesis. In earlier studies, exposure to 500 mg/kg/day DBP from gestation day (GD) 12 to 21 caused multinucleated gonocytes, Leydig cell hyperplasia, and incomplete spermatogenesis. We hypothesized that this outcome is linked to abnormal cell proliferation and apoptosis in the DBP-exposed fetal testis. Cell proliferation was assessed on tissue slides by histological methods and BrdU incorporation, and apoptosis was measured by TUNEL assay. Cadherins that may play a role in Sertoli cell-gonocyte interactions were analyzed by immunostaining. In rats exposed to 500 mg/kg/day from GD 12 to the termination day, the total number of cells and the number of tubular cells were significantly (two-way ANOVA) decreased starting on GD 17. BrdU incorporation and TUNEL assays suggest that this decrease is caused by altered proliferation of Sertoli and peritubular cells but not apoptosis. A pilot study revealed that at GD 19 this decrease appears to be dose-dependent in the range from 0.1 to 500 mg/kg/day but NOAEL was not determined due to small sample size. As early as GD 17, treated testes had fewer cords that were enlarged and less convoluted. Beginning on GD 20, Sertoli cells in treated testes exhibited hypertrophy. Multinucleated gonocytes, first detected at GD 19, appeared to be arrested in telophase/cytokinesis I phase of meosis.

Immunostaining for P- and N-cadherins suggested changes in Sertoli cell - gonocyte interactions in the DBP-treated testis. Our data indicate that *in utero* exposure to a high dose of DBP affects cords formation and development of Sertoli cells that may alter meiosis in gonocytes. These data support the hypothesis linking impaired spermatogenesis in the adults with failure of fetal Sertoli cells to proliferate and mature.

927 MODULATION OF HOST DEFENSES BY AIR POLLUTANTS.

M. D. Reed[1] and I. Gilmour[2]. *[1]Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM and [2]Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, USEPA, Research Triangle Park, NC.*

Epidemiological evidence links air pollutants with increases in morbidity and mortality in susceptible populations. Among the health effects of long term and episodic air pollution, parameters associated with respiratory infections in susceptible populations, especially small children and the elderly, may be of primary importance. The mechanistic work detailing the modulation of infection by air pollutants is a developing and exciting field of research that brings together cutting edge biology and mechanistic toxicology. For example, the role of toll-like receptors (tlr) in response to infection and airborne particulates has been recognized. This symposium will address the epidemiological evidence suggesting that ambient air pollutants may modulate the occurrence and/or severity of respiratory infection. Subsequently, the current state of experimental models of host defense mechanisms and pathogenesis will be presented in relation to both bacterial and viral respiratory infection as modulated by individual pollutants and pollutant mixtures. Speakers will detail susceptibility to, and lung clearance of gram positive and negative bacteria, and viruses such as RSV. Focus will be placed on infection models modulated by ambient particulates, source emissions (coal fly ash and diesel exhaust), and metals. Target Audience: This Symposia encompasses the fields of microbial pathogenesis, immunology, air pollution, mixtures and mechanistic toxicology.

928 MECHANISMS OF HOST DEFENSE.

I. Gilmour and M. K. Selgrade. *Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, USEPA, Research Triangle Park, NC.*

The respiratory tract is maintained in a remarkably sterile condition by the combined anti-microbial activity of the mucociliary, phagocytic and immune systems. Epidemiological studies of smokers and others exposed to high levels of air pollutants have demonstrated that these individuals suffer from more frequent and severe respiratory infections. Experiments in animals have also shown that a wide range of airborne pollutants including cigarette smoke, oxidant gases, acid aerosols, metals, organic compounds, and combustion products can interfere with the normal defense processes of the lung to enhance susceptibility to respiratory infection. The mechanisms for these effects include decreases in mucociliary clearance and macrophage phagocytosis, as well as reduced specific immune responses such as antibody formation, and natural killer (NK) and T cell function. Although there is some evidence that similar effects occur in humans, the link to ambient air pollutants are not as strong because of incomplete individual exposure histories. Animal studies provide dosimetric information, which can be used to predict the relative health risk of simple and complex exposures, and also lend insight into the mechanisms of air pollution toxicity. This presentation will describe host defense processes in the respiratory tract and illustrate how these may be reduced by air pollutant exposure resulting in increased severity of lung disease. These data can then be applied to risk assessment through *in vitro* and *in vivo* comparisons of immune function in animals and humans. This abstract does not reflect EPA policy.

929 EPIDEMIOLOGY OF AMBIENT AIR POLLUTION AND PULMONARY INFECTION.

A. Pope. *Department of Economics, Brigham Young University, Provo, UT.* Sponsor: M. Reed.

Substantial epidemiological evidence suggests that air pollution common to many urban and industrial environments is a risk factor contributing to the exacerbation or development of pulmonary infection in susceptible populations including children and the elderly. Associations observed include an increased prevalence of respiratory symptoms consistent with chronic and acute pulmonary infection, and increased hospitalizations for bronchitis and pneumonia. These findings suggest that inhaling air pollutants may modulate host defenses and immunity. This presentation will review the current state of epidemiological associations of air pollutants, especially those of ambient air, with risk of pulmonary infection.

930 INCREASED LUNG PATHOGENESIS TO RESPIRATORY VIRAL INFECTION BY DIESEL ENGINE COMBUSTION COMPONENTS.

K. S. Harrod[1], J. A. Berger[1], J. D. McDonald[2] and M. D. Reed[2]. *[1]Asthma and Pulmonary Immunology Program, Lovelace Respiratory Research Institute, Albuquerque, NM and [2]Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM.*

Epidemiology studies clearly indicate a relationship between poor air quality and childhood illness, particularly respiratory infections. However, experimental evidence is lacking, leaving a poor understanding of the mechanisms by which air pollutants alter susceptibility to pulmonary infections and disease. Using an inhalation exposure system of diesel engine emissions (DEE), altered susceptibility to a common childhood pathogen, respiratory syncytial virus (RSV), was examined in an *in vivo* experimental model. At concentrations reflecting ambient exposures, RSV persistence was increased in the respiratory tract following exposure to inhaled DEE, and increased lung viral burdens were concordant with increased lung pathogenesis and disease. Following exposure to DEE, distinct lung epithelial cell populations exhibited altered remodeling and/or injury to RSV infection. Evidence shows that lung epithelial-specific mechanisms of host defense or immunomodulation were altered by prior exposure to DEE. Furthermore, adaptive and innate immune mechanisms were exacerbated by DEE following infection, suggesting a greater reliance on immune mechanisms for clearance of RSV from infected lungs. Collectively, these studies suggest a role for air pollutants from engine combustion sources in the increased susceptibility to respiratory infection and provide an experimental model for elucidation of mechanisms by which these pollutants contribute to respiratory disease in childhood.

931 EFFECT OF WORKPLACE PARTICULATES ON THE SUSCEPTIBILITY TO BACTERIAL INFECTION AND THE SUPPRESSION OF LUNG DEFENSE RESPONSES IN RATS.

J. M. Antonini. *Health Effects Laboratory Division, National Institutes of Occupational Safety and Health, Morgantown, WV.*

Inhalation of increased levels of air pollutants generated in the workplace and environment may augment pulmonary infection and increase morbidity and mortality. The effect of exposure to different workplace particulates on lung defense responses was investigated using a rat bacterial infectivity model. Male Sprague-Dawley rats were pretreated by intratracheal instillation with 1.0 mg/100 g body wt of residual oil fly ash (ROFA), stainless steel welding fumes particulate matter (SS), or silica (Si) prior to bacterial inoculation with 5×10^3 or 5×10^5 Listeria monocytogenes. Particle-induced effects on pulmonary clearance of the bacteria, lung macrophage function (phagocytosis, oxidant production, and bacterial killing), and the secretion of lung cytokines, important in immune responses, were assessed. Pretreatment with ROFA and SS significantly slowed the lung clearance of bacteria, increased animal morbidity, suppressed macrophage function, and altered IL-2, IL-6, and IL-10 production after infection. The suppression observed for ROFA was due to soluble metals, whereas a combination of soluble and insoluble metals was responsible for the effect seen with SS. Conversely, Si pretreatment enhanced lung bacterial clearance and significantly upregulated non-specific lung defense responses (macrophage phagocytosis, oxidant production, and neutrophil activation) despite the presence of significant lung inflammation and fibrosis. In conclusion, inhalation of different workplace particulates may induce varied lung defense responses to infection. Chronic exposure to metal particles in the workplace may alter macrophage function and increase the susceptibility to lung infection in exposed workers.

932 EXACERBATION OF PULMONARY PNEUMONIA BY INHALED AMBIENT PARTICULATE MATTER AND ASSOCIATED METALS.

J. T. Zelikoff, Y. Li, K. Schermerhorn, M. D. Cohen and R. B. Schlesinger. *Nelson Institute of Environmental, NYU School of Medicine, Tuxedo, NY.*

Epidemiological studies demonstrate that pneumonia contributes to the increased morbidity among individuals following exposure to ambient particulate matter (PM). This suggests that inhaled PM can act as an immunosuppressant. A 5 hr exposure of Streptococcus pneumoniae-infected rats to concentrated PM 2.5 μm from N.Y City air exacerbates infection and depresses antimicrobial pulmonary defense mechanisms. Infected rats exposed to PM 48 hr following bacterial instillation have increased: pulmonary bacterial burdens; bacterial-associated lung lesions; and, bacteremia compared to sham. Exposure to CAPS also decreased lavageable neutrophils, cytokines, and bronchus associated lung tissue. To identity the possible constituents responsible for the worsening pneumonia, studies were undertaken to

correlate the physiochemical attributes of CAPS with *in vivo* immunotoxicity. Using a similar model, rats were exposed by inhalation to Fe, Cu, Mg, Ni, or Zn. Uninfected rats were assessed for pulmonary cytokine levels, markers of cell damage, and oxyradical production, or histopathology; effects on systemic immunity were determined by examining blood cell profiles and splenic lymphoproliferation. Results showed that while Mn or Cu had no effect on local or systemic immunity, Fe, Zn, and Ni either reduced or delayed pulmonary bacterial clearance in infected rats. Metal interractions were observed in infected animals exposed to metals in combination. Collectively, these results indicate that PM can worsen the outcome of an ongoing pulmonary infection and that associated Fe, and possibly Ni and/or Zn, play a role in this effect. EPA Center Grant No. R827351.

933 ROLE OF TOLL-LIKE RECEPTORS (TLRS) IN RESPONSES TO AIR POLLUTANTS AND INFECTIONS.

S. R. Kleeberger. *Environmental Genetics Group, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

The innate immune response is the first line of defense against infectious diseases. Unlike the adaptive immune system (i.e., T-cell receptor and B-cell antibody receptor), cells of the innate immune system such as macrophages and dendritic cells discriminate between 'self' and infectious 'non-self' *via* constitutive receptors that identify pattern ligands synthesized exclusively by pathogens (e.g., lipopolysaccharide, bacterial lipoproteins BLP, bacterial DNA), so called pattern recognition factors (PRFs). TLRs are emerging as key regulators of host responses to injury and infection. Recent studies with mice have shown that immune cell activation by the PRFs of Gram-negative bacteria requires a pattern recognition receptor, TLR4. TLR4 is known as a lipopolysaccharide (LPS) receptor in mice, and altered TLR4 function by the mutation in C3H/HeJ mice is believed to confer resistance to LPS in this strain. Moreover, studies with Tlr4-deficient mice (Tlr4null) strongly supported the role of murine TLR4 in LPS responsiveness. The mutation of Tlr4 limits the pulmonary hyperpermeability and inflammation induced by exposure to particulate matter and ozone in mice. In addition, decreased innate immune responses (i.e., natural killer cell trafficking, IL-12 expression) were observed in the lungs of Tlr4-deficient mice after respiratory syncytial virus infection. Considerable progress has been made to define the TLR signaling pathways, and it is becoming increasingly clear that multiple ligands bind each of the TLRs. A more clear understanding of the interactions between inhaled respiratory pollutants/pathogens and TLRs should enhance strategies to protect individuals against these agents.

934 THE PRESENT AND FUTURE OF TOXICOGENOMICS IN PRECLINICAL DRUG DEVELOPMENT.

J. K. Leighton[2] and K. L. Kolaja[1]. [1]*Toxicology, Iconix Pharmaceuticals, Mountain View, CA and* [2]*Food and Drug Administration, Laurel, MD.*

Toxicogenomics, the genome scale analyses of chemically induced changes in complex populations of mRNA to understand toxicity, has the potential to dramatically improve predictive, mechanistic and descriptive insights into drug development candidates prior to human exposures. Currently, toxicogenomics and microarray research are used selectively in drug development, primarily to facilitate the generation of proto-type compound databases and/or mechanistic and assay development research on compounds dropped from development consideration. However, as regulatory guidance begins to crystallize regarding toxicogenomics, the opportunity to use transcription profiling to improve and broaden understanding of efficacy and safety of development candidate(s) early in the testing paradigm could become more and more common. This session will discuss the current implementation of toxicogenomics in pharmaceutical companies and delve into the not-so-distant future for this technology. The first speaker will outline some critical steps needed to allow the true value of genome wide expression analysis to be realized. In order to improve human health using transcription profiling, a number of technical, biological, and regulatory/procedural issues will need to be resolved. The remaining speakers will discuss focused hypothesis-driven research projects highlighting the strategic advantage(s) inherent to toxicogenomics.

935 TECHNICAL, PROCEDURAL, AND BIOLOGICAL ISSUES IN THE INCORPORATION OF TOXICOGENOMICS INTO THE REGULATORY PROCESS.

J. K. Leighton. *USFDA/CDER, Rockville, MD.* Sponsor: F. Sistare.

Critical steps are needed to allow the true value of genome-wide expression data from nonclinical stages of pharmaceutical investigations to be realized. In order to improve human health using transcription profiling data from early phases of pharmaceutical development that are destined for regulatory review groups, a number of technical, biological, and regulatory/procedural issues need to be resolved. FDA has been working to address these issues and to enable the application of this technology by pharmaceutical developers in a way that maximizes confidence in the data, maximizes mutual biological understanding of compound actions, and promotes evolution of this new technology, while minimizing risks to adverse regulatory actions. Sets of gene expression data that have been submitted to FDA will be described and the regulatory value and limitations of these data, technical and biological lessons learned, and the procedural pathways forward that are being taken will be discussed.

936 GENE EXPRESSION PROFILING IN PHARMACEUTICAL TOXICOLOGY RESEARCH.

R. G. Ulrich. *Molecular Profiling, Rosetta Inpharmatics-Merck Research Laboratories, Kirkland, WA.* Sponsor: K. Kolaja.

Toxicogenomics, defined as the study of the structure and output of the genome as it relates and responds to adverse xenobiotic exposure, is impacting the way new drugs are discovered. Molecular profiling of cell or tissue gene expressional changes using DNA microarrays, when coupled with appropriate bioinformatics, generates compound-specific transcriptional fingerprints that reflect molecular phenotypes. Importantly, these fingerprints includes both on-target and off-target responses and thus illuminates pathways positively affected for a new drug candidate based on interactions with the intended target and adverse effects due to untoward downstream responses from the therapeutic target or to off-target interactions. Using unsupervised hierarchical clustering algorithms it has been found that compounds with similar putative toxic mechanisms form clusters based on similar effects on gene expression. Using a database of such gene expressional profiles, called a response compendium, it is possible to classify the effects of new or unknown compounds even in the absence of pathology. Thus, relative adverse effects can be identified using either unsupervised or supervised clustering approaches, and specific predictions can be made using classification approaches. Toxicogenomics is also used to describe potential biological consequences of exposure in great detail. Where sufficient annotations exist, discrete gene analysis can help identify specific affected metabolic pathways (both on- and off-target) and identify putative toxic mechanisms. While not currently used for risk assessment, molecular profiling approaches are quickly becoming part of the toxicology paradigm.

937 IDENTIFICATION AND EVALUATION OF GENOMIC BIOMARKERS OF TOXICITY.

F. M. Goodsaid. *Genetic and Molecular Toxicology, Schering-Plough Research Institute, Lafayette, NJ.* Sponsor: W. Choy.

The application of genomic biomarkers of toxicity in drug discovery and development requires several levels of evaluation. These levels include cross-species evaluation, evaluation across multiple toxic compounds, in silico evaluation in toxicogenomic databases, and pilot evaluation in discovery. We have developed quantitative RT-PCR assays for biomarker candidates previously published for rat hepatotoxicity and nephrotoxicity and tested them in non-rodent species. We have evaluated the sensitivity and specificity of these candidates in tissues from a rat tissue bank at Iconix Pharmaceuticals based on studies using a variety of toxic compounds, and we have also queried their databases for an in silico evaluation of these candidate biomarkers of toxicity. Through this process, we have assessed the performance of KIM1/HAVcr-1 and clusterin as predictive genomic biomarkers of nephrotoxicity and investigated the transfer of these tests across different platforms with the throughput required for drug discovery applications. This assessment has shown that induction of either the clusterin or KIM1/HAVcr-1 genes in rat kidney is a genomic indicator of nephrotoxicity for a test compound. We conclude that these genes are good candidates for further evaluation in drug discovery.

938 IMPLEMENTATION OF STRATEGY TOWARD IMPACTING PRECLINICAL DEVELOPMENT WITH GENOMICS APPROACHES.

C. Afshari. *Amgen Inc., Thousand Oaks, CA.*

Studies in toxicology are conducted in order to facilitate identification of safety thresholds and potential health risks associated with new drug candidates in development. The rapid development and evolution of genomic, proteomic, and metabonomic based technologies has led to the development of the new field of toxicogenomics that will allow application of these technologies to improve the efficiency of safety and risk assessments by facilitating better understanding of the mechanisms by which compound induced injury occurs. The initial phase of devel-

opment of this program at Amgen is focused on using global gene expression technologies to explore mechanisms of toxicity and reveal early, sensitive candidate biomarkers of toxicity with a focus on liver and kidney toxicity. We are working to use these findings to develop new screens that will contribute early in the drug development process and to integrate these data to reveal mechanistic information about the underlying biology of the development of potential toxicities. This talk will highlight ongoing work being conducted at Amgen as well as work that is being conducted in collaboration with the International Life Sciences (ILSI) HESI-Genomics/Nephrotoxicity Subcommittee.

939 APPLICATION OF TOXICOGENOMICS TO PHARMACEUTICAL DRUG DISCOVERY AND DEVELOPMENT.

Z. Jayyosi. *eSafety-Drug Safety Evaluation, Aventis Inc., Bridgewater, NJ.*

Kinase inhibitors are an important class of compounds being developed as pharmaceuticals for the treatment of diseases, such as asthma and cancer. Because of the numerous intracellular kinases, specificity of a selected kinase inhibitor is crucial for its pharmacological action, as well as, its potential toxicity. Within a class of kinase inhibitors, toxicities may be similar, but not likely identical. Toxicogenomics was used to evaluate alterations in gene expression in response to compound treatment to differentiate the mechanism(s) of toxicity from the mechanism(s) of action, and to determine the overall utility of toxicogenomics in drug discovery and development. Spleen kinase (syk) plays a central role in mast cell activation. Therefore, syk inhibitors may be useful in the treatment of inflammatory diseases. This presentation will review the exploratory toxicogenomic studies conducted in rats to evaluate the potential toxicity of a syk inhibitor. In a 14-day study in rats, target organ toxicities were observed in liver, kidney, nasal epithelium and immune-related organs, including spleen and lymph nodes. The expression profiles of approximately 8, 800 genes in the target organs were analyzed and evaluated against the biochemical toxicology, histopathology and clinical pathology data. Interpretation of gene expression data revealed a distinct separation between the pharmacological and toxicological effects of the subject syk inhibitor. This study provided a number of valuable clues as to the mechanism of toxicity of this compound in the liver, kidney and spleen including oxidative stress, effects on energy metabolism and potential for immune suppression. Overall, gene expression analysis was found to be a valuable tool for the elucidation of mechanisms of toxicities when complemented with other safety assessment tools, including histopathology and clinical pathology.

940 USE OF A LARGE CHEMOGENOMIC DATABASE TO FACILITATE DRUG DEVELOPMENT.

K. L. Kolaja. *Toxicology, Iconix Pharmaceuticals, Mountain View, CA.*

Chemogenomics can broadly be considered the study of pharmacology and toxicology using genomic analysis. The key is to combine the benefits of the study an organism as a whole with genome-scale approach such as microarray analysis of gene expression and broad molecular pharmacology profiling. The knowledge gained from screening a wide range of drugs, chemicals, and toxicants across a broad spectrum of assays has helped uncover previously uncharacterized activities, both good and bad. This reference database, combined with a set of statistical predictive tools, can be used to help characterize drug candidates very early in the development process and optimize the selection process. This presentation will present examples of findings generated from this database, with a particular focus on the potential mechanisms of hepatotoxicity. One key example will discuss how a contextual reference dataset reveal a potential explaination for a multi-organ model of hepatotoxicity of non-steroidal anti-inflammatories (NSAIDS).

941 INTRODUCTION TO THE SYMPOSIUM ON "TISSUE AND SPECIES DIFFERENCES IN REGULATION OF CYTOCHROME P450S".

X. Ding[1, 2]. [1]*Wadsworth Center, New York State Department of Health, Albany, NY and* [2]*School of Public Health, SUNY at Albany, Albany, NY.*

Tissue differences in the expression and regulation of various xenobiotic-metabolizing cytochrome P450 genes are critical determinants of organ-selective chemical toxicity, and species differences in P450 expression and regulation will impact risk assessment. Although tremendous progress has been made in recent years on the identification and characterization of biotransformation enzymes involved in metabolic activation, we still know very little about the expression and regulation of these enzymes in various extrahepatic target tissues, in either humans or laboratory animals. The goal of this symposium is to provide a timely forum for the dissemination of recent progress in studying P450 regulation in several important extrahepatic organs, including the brain, the lung, the skin, and the nasal mucosa. The individual research topics to be discussed will be diverse, involving different tissues, different P450 genes, different modes of regulation, and different approaches. However, a common theme of tissue- and species differences will be emphasized, and the approaches used will likely benefit studies on gene regulation in other tissues.

942 TISSUE DIFFERENCES IN THE REGULATION OF THE CYP2A GENES.

X. Ding[1, 2]. [1]*Wadsworth Center, New York State Department of Health, Albany, NY and* [2]*School of Public Health, SUNY at Albany, Albany, NY.*

The CYP2A genes play important roles in the metabolic activation of many procarcinogens and toxicants. CYP2A3, and its mouse and human orthologs (CYP2A5 and CYP2A13, respectively), are expressed preferentially in the olfactory mucosa (OM). We previously found that a nuclear factor 1 (NFI)-related regulatory element (NPTA element) in the proximal promoter region of the CYP2A3 gene is critical for transcriptional activation *in vitro*. Recently, we used DNA-affinity purification to isolate the NPTA-binding proteins from rat OM. Mass spectral analysis showed that isoforms corresponding to each of the four NFI genes are present in the purified NPTA-binding fraction, with NFI-A being the most abundant. Further studies led to the identification of a novel NFI-A isoform (NFI-A-short), which has a truncated transactivation/repression domain. Transient transfection assays showed that NFI-A2, an OM-specific NFI, transactivated the CYP2A3 promoter, whereas NFI-A-short offset the activation. These data suggest that NFI may play important roles in the tissue-specific expression of the CYP2A3 gene. In another study, we examined tissue differences in the control of CYP2A5 expression by circadian rhythm in mice, which has been previously demonstrated in mouse liver. We found that, the circadian variation in CYP2A5 mRNA level occurred in the kidney, as well as in the liver, but not in the OM. Additionally, we and others have recently discovered a global induction of microsomal P450s, including CYP2A5, in the liver of mice with liver-specific deletion of the NADPH-cytochrome P450 reductase (Cpr) gene. This finding revealed novel regulatory pathways of CYP expression associated with altered cellular homeostasis. We are characterizing another mouse model, which exhibits down-regulation of the Cpr gene in all organs, to determine whether the loss-of-CPR associated P450 induction also occurs in the OM and other extrahepatic tissues. (Supported in part by NIH grant ES07462)

943 OLFACTORY MUCOSAL METABOLIC ENZYMES: MODULATION OF TOXIC ENDPOINTS BASED ON DISTRIBUTION, INDUCTION, AND AGE-AND SPECIES VARIABLES.

M. Genter. *Department of Environmental Health and Center for Environmental Genetics, University of Cincinnati, Cincinnati, OH.*

The olfactory mucosa (OM) is a common site of toxicant induced damage by inhaled and systemically-administered chemicals, often as a result of target tissue bioactivation. Alachlor (ALA), 2, 6-dimethylaniline (DMA), and naphthalene (NP) cause OM tumors in the rat, likely as a result of local bioactivation by cytochromes P450 (CYPs) 2A3 and 2f2. Because OM enzymes are clearly involved in the bioactivation of ALA and/or its intermediate metabolites, we have been studying a number of possible ALA bioactivation pathways, including N-hydroxylation of the intermediate metabolite 2, 6-diethylaniline, acetylation, and DNA adduct formation, to clarify its mechanism of action. Mice and rats display different carcinogenic outcomes following chronic administration of ALA or NP. The species-related differences in response to these compounds may be due to differential pharmacokinetics or to differences in ratios of bioactivating:detoxification enzymes. OM metabolic enzymes can be induced, though generally not to the same degree as hepatic enzymes. For example, lidocaine metabolism was increased in OM microsomes from TCDD-treated rats, coincident with induction of CYPs 1A1/2, 2B1, 2C11 and microsomal epoxide hydrolase. Similarly, inhibition of Phase 1 and Phase 2 OM enzymes is associated with modulation of the toxic effects of compounds, e.g. vinyl acetate and dichlobenil. OM enzymes, including CYP2A13, superoxide dismutases, flavin containing monooxygenases, and glutathione-S-transferases, vary in expression with age, suggesting differing potential for toxicity depending on the age of the exposed individual. Given that the mechanism underlying the development of chemically-induced nasal cancers in humans (e.g. by wood dust or nickel) is poorly understood, the role of concomitant environmental exposures that could modify the effects of these compounds needs further investigation. Supported by NIH grant ES08799.

944 GENE REGULATION BY THE AHR IN HUMAN KERATINOCYTES.

H. I. Swanson, S. S. Ray, E. M. Hoagland, E. Thompson, D. Pupula and Z. M. Georgia. *Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, KY.*

While the mechanisms governing gene regulation by the AHR have been well characterized in hepatocytes, those that exist in epithelial cells are largely unexplored. Our recent findings indicate that as primary human keratinocytes undergo senescence/differentiation, both the expression of the AHR and its ability to transactivate genes such as CYP1A1 dramatically increases. In addition, the increase in intracellular calcium levels that typically accompany keratinocyte differentiation is sufficient to increase both TCDD-induced gene activation and DNA binding of the AHR/ARNT heterodimer. Using microarray analyses, we have identified novel TCDD responsive genes. In the differentiating keratinocytes, a number of gene products are upregulated by TCDD. This includes the previously identified TCDD target genes, CYP1B1, CYP1A1 and CYP1A2. In addition, several upregulated genes are those involved in retinoid signaling, including NADP-dependent retinol dehydrogenase, RXRB and RAR beta. Genes that have been identified to be downregulated by TCDD in an AHR dependent manner include tumor suppressors such as p16, p19 and p53. Alterations of these gene pathways may be a critical factor in the ability of TCDD and the AHR to alter epithelial cell fate.

945 NICOTINE INCREASES CYP2B IN THE BRAIN BUT NOT IN THE LIVER: CONTRASTING THE REGULATION IN RODENTS, NON-HUMAN PRIMATES AND PEOPLE.

R. F. Tyndale, A. Lee and S. Miksys. *Department of Pharmacology, Center for Addiction and Mental Health, University of Toronto, Toronto, ON, Canada.* Sponsor: X. Ding.

Approximately 25% of adult Americans smoke daily. Nicotine is consumed not only by smokers, but also by passive smokers and ex-smokers on nicotine replacement therapies (NRTs), and NRTs are in clinical trials for the treatment of a number of neurological diseases. The metabolism of drugs and toxins can be substantially altered by enzyme induction by constituents of tobacco smoke such as nicotine. We have found that in rat, low, behaviorally relevant doses of nicotine given for 7 days (s.c.) increase CYP2B in the brains but not in the livers. The induction by nicotine in the brain is specific for both cell-type and brain region. The elevation in brain CYP2B protein, determined by western blotting and immunocytochemistry, is dose-dependently related to increased CYP2B1 mRNA. These data suggest that the regulation of CNS CYP2B by nicotine involves transcriptional mechanisms. In human brain, we have found brain region- and cell type-specific distributions of CYP2B6. We also have found elevated levels of CYP2B6 in the brains from smokers, compared to non-smokers. The regional and cellular localization of brain CYP2B6 in smokers differs from the regional and cellular localization of nicotine-induced CYP2B in the rat brain. To investigate this further, we treated non-human primates (African Green Monkeys) with nicotine and are currently investigating the presence and induction by nicotine of CYP2B in liver and brain. As predicted from rat and human data, there is no elevation of CYP2B in monkey liver. Data derived from the monkey brain should help us to differentiate species effects (rodent versus primate) from inducer effects (nicotine versus cigarette smoking), and allow us to validate the African Green Monkey as a model for human smokers. Funded by CIHR MT 14173 and by a Canadian Research Chair in Pharmacogenetics to RFT.

946 MECHANISMS THAT CONTROL THE SELECTIVE EXPRESSION OF CYP2F1, CYP4B1, AND CYP3A5 IN HUMAN LUNG.

G. S. Yost[1] and R. N. Hines[2]. *[1]Pharmacology and Toxicology, University of Utah, Salt Lake City, UT and [2]Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI.*

The toxicity of many acute pneumotoxicants and carcinogens to lung tissues requires bioactivation by the CYP2F1, CYP4B1, or CYP3A5 enzymes. In many cases, toxicant-specificity to lung tissues is highly related to the selective expression of these P450 genes in specific cells in the lung. However, the mechanisms responsible for the selective expression of these genes has not been elucidated. In our work we have characterized the structures of the *CYP2F1, CYP4B1, CYP3A4, and CYP3A5* genes, and have identified several novel regulatory elements in the proximal promoter regions of these genes. Specific promoter elements were identified within the first 200 bp of the *CYP2F1, CYP4B1, and CYP3A4/5* genes. These elements were shown by functional reporter gene activation to regulate transcription in human lung cells, and a novel nuclear transcription factor was identified, that formed protein/DNA transcriptional complexes with the *CYP2F1* gene at the -152 to -182 bp motif [*J. Biol. Chem.* **278**, 15473-15483 (2003)]. Four putative Sp1/3 binding sites were shown to coordinately regulate basal *CYP2F1* transcription in A549 lung cells. Other proteins, belonging to the Sp1/3 (*CYP4B1*) or E-box families of transcriptional factors (*CYP3A4/5*), from human lung tissues or lung cells, formed specific binding complexes with the regulatory motifs of these other genes. Ten additional transcriptional factors were excluded (by competitive EMSA analyses) as candidates for the tissue-selective regulation of *CYP4B1* expression. Inclusion of a double E-box "silencer" element (-127 to -70) from the *CYP3A4* promoter region into the *CYP3A5* promoter dramatically decreased reporter gene activation of *CYP3A5* in A549 lung cells. Therefore, the results strongly support the hypothesis that promoter elements in these lung-selective genes control organ-specific transcription by recruiting transcription factors that are selectively expressed in lung cells. (Supported by the NIH grants: HL13645 and HL60143)

947 SYSTEMIC DRUG ALLERGY:FREQUENCY, CHALLENGES, MECHANISMS AND NEED FOR PREDICTIVE MODELS.

J. Dean[2] and R. Pieters[1]. *[1]Immunotoxicology, IRAS/Utrecht University, Utrecht, Netherlands and [2]Sanofi-Synthelabo, Inc., Malvern, Philadelphia, PA.*

A number of drugs induce hypersensitivity responses that may result in systemic allergy and/or autoimmune-like phenomena in susceptible individuals. These reactions can be very serious and even life-threatening. The susceptibility for immune-mediated adverse drug reactions (ADRs) is dependent on a complex interplay of inherent idiosyncratic factors combined with poorly defined environmental factors. Due to the complexity of the mechanisms underlying these ADRs and the lack of predictive models, the potential for these reactions can be missed in routine toxicology testing and often remains undetected until the drug is already on the market. Therefore, a better understanding of the mechanisms of ADRs as well as the development and validation of predictive tests represent important issues for the pharmaceutical industry and for governmental agencies. Rat (in particular Brown Norway strain) as well as certain mouse models are in use to study mechanisms of ADRs by specific drugs, but these models are not applicable as general screening models. For screening for hazard identification two assays have been evaluated, the popliteal lymph node assay (PLNA) and a recently investigated modification of the Local Lymph Node Assay. These assays detect direct immunostimulatory effects of chemicals following subcutaneous administration, and PLNA data collected over the past 15-20 years correlated well with documented ADRs in man. As many drugs are administered by the oral route, an additional assay using the oral route of exposure is desirable. Recent efforts combining relevant routes of exposures with simple read-out systems such as the local lymph node assays have shown promise.

948 MECHANISMS OF ADVERSE EFFECTS OF LOW MOLECULAR WEIGHT CHEMICALS.

J. Uetrecht. *Faculty of Pharmacy and Medicine, University of Toronto, Toronto, ON, Canada.*

Idiosyncratic drug reactions are a major clinical problem as well as a major impediment to drug development. The characteristics of these reactions strongly suggest that most idiosyncratic reactions are immune-mediated, and there is also a large amount of circumstantial evidence that reactive metabolites are involved. The hypothesis that has been used to link these two concepts is the Hapten Hypothesis in which reactive metabolites bind to proteins and make them foreign. A more recent hypothesis, the Danger Hypothesis, proposes that it is cell damage, presumably caused by reactive metabolites, rather than foreignness that leads to an immune response. The P-I Hypothesis questions the need for reactive metabolites and proposes that a reversible pharmacological interaction between drug and the MHC II / T cell receptor complex is sufficient to initiate an immune response. A new animal model, i.e. nevirapine-induced skin rash in the rat, will be presented which can be used to test these hypotheses. The incidence of the rash varies with rat strain: 0% in Lewis rats, 20% in Sprague-Dawley rats and 100% in Brown Norway rats. The rash only affects female animals of the strains tested. Tolerance can be induced by treatment with a lower dose for 2 weeks. The onset takes 2-3 weeks on initial exposure but the ears turn red within 24 hours on reexposure in a sensitized animal. The rash is immune-mediated because this sensitization can be transferred to naive rats with spleen cells. It could also be prevented by the immunosuppressant tacrolimus. The characteristics of this rash are very similar to the rash that occurs in some humans who are treated with the drug. In order to design safer drugs we need a much better understanding of the mechanisms of idiosyncratic drug reactions, and such animal models, although exceedingly rare, represent a very powerful tool for mechanistic studies. This work was supported by grants form the Canadian Institutes of Health Research and Boehringer Ingelheim.

949 POPLITEAL LYMPH NODE ASSAY AND RECENT MECHANISTIC STUDIES.

R. Pieters. *Immunotoxicology, IRAS/Utrecht University, Utrecht, Netherlands.*
Sponsor: J. Dean.

Generally applicable animal models to predict drug hypersensitivity based on systemic or clinical effects do not exist, although some chemicals induce adverse immune-mediated effects in some animal strains. This unpredictibility is thought to be due to a complex interplay of predisposing idiosyncratic and environmental factors. Because drug hypersensitivity reactions often mimick graft-versus-host (GVH) reactions the popliteal lymph node assay (PLNA), originally used to measure GVH activity, was introduced to study chemical induced hypersensitivity. To date, around 140 different chemicals (drugs and homologues thereof as well as other compounds) have been tested in various isolated PLNA studies. When the lack of metabolism in the PLNA is taken into account the outcomes of this simple screening test correlated well with documented adverse effects in man. Modifications of the simple primary PLNA by including immunologically more relevant parameters than cell proliferation have allowed mechanism-based and therefore better assessment of sensitizing potential of chemicals. Recent studies, using KO-mice and specific blocking monoclonals have shown that costimulatory molecules (CD40L and CD86) are crucial for induction of PLNA responses by Th-2 sensitizing model-drugs like D-penicillamine and DPH studies. The response to the Th-1 stimulating model compound streptozotocin however, particularly involves CD80, and is more or less independent of CD40L. This indicates that immunosensitization by drugs follows general immunological patterns, but also that expression of costimulation may be used as an alternative read-out parameter. In all, the PLNA appears not only suitable to screen for the sensitizing potential of series of drugs (including structural homologues) but also to study fundamental aspects of drug-induced hypersensitivity.

950 MODIFICATION OF THE LOCAL LYMPH NODE ASSAY TO EVALUATE THE POTENTIAL FOR ADVERSE IMMUNOLOGICALLY-MEDIATED DRUG REACTIONS.

B. J. Meade[1] and J. L. Weaver[2]. *[1]NIOSH, Morgantown, WV and [2]CDER, USFDA, Laurel, MD.*

There is no generally accepted preclinical method to predict the potential for drug allergy to systemically administered drugs. A collaborative research project, involving members of industry, government and academia, was initiated to evaluate an assay which uses the basic protocol of the Local Lymph Node Assay (LLNA, an assay accepted by regulatory agencies for hazard identification of skin sensitizers) modified by administration of test article by subcutaneous injections between the ears, rather than the prescribed topical exposure. Test compounds were selected from marketed drugs known to cause human drug allergy. Selection of active drugs was based on reports of adverse events found in at least two independent data sources including FDA postmarketing data, drug labeling, clinical trial data, and publications. The following drugs associated with adverse reactions were tested by at least three laboratories: streptozotocin, hydralazine, sulfamethoxazole, diphenhydramine, procainamide, clonidine, ofloxacin, chlorpromazine, nevirapine, abacavir, lamotrigine, and zomepirac. Clinically negative drugs tested were phenobarbital and metformin. The pure compound was used if available; otherwise the marketed drug formulation was used. Range finding studies in mice were performed to allow selection of the highest dose that did not produce systemic toxicity. Both clinically negative drugs tested negative in the assay. Six of the 12 clinically positive drugs were positive in the modified LLNA (mLLNA). For a limited set of drugs, a concurrent Popliteal Lymph Node Assay was performed and the results were comparable to those obtained in the mLLNA. Evaluation of the lymph node cell phenotypes by flow cytometry showed population changes in general agreement with the mLLNA data. These results suggest that the mLLNA holds promise as a screen for systemic hypersensitivity inducing drugs and the use of concurrent flow cytometry may provide insight into the underlying mechanisms.

951 THE USE OF THE PLNA IN RELATION TO ORAL ROUTE OF EXPOSURE TO LMWCS.

B. W. Gutting. *Chemical Biological Systems Technology Division, Naval Surface Warfare Center, Dahlgren Division, Dahlgren, VA.*

This work examined diclofenac (an NSAID) and oxazolone (a contact sensitizer) in the Direct- and RA-PLNAs in naive mice and mice that had been orally pretreated with the respective LMWC. Both caused T cell-mediated PLN reactions in naive mice. Oxazolone increased the percent of CD4+ PLN T cells and decreased the percent of CD8+ PLN T cells, with a significant increase in CD4+ T cells having an activated phenotype (highCD44, lowCD62L). With diclofenac the PLN reaction was significantly attenuated in T cell-deficient mice, while wild-type mice showed an accumulation of CD4+ and CD8+ T cells with an activated phenotype. When co-injected with TNP-OVA, diclofenac also selectively induced IgG1 AFCs, IgE AFCs, and CD4+IL-4+INFg- T cells. Oral pretreatment with either compound appeared to affect the respective PLN reactions. Oral pretreatment with oxazolone (3 doses) rendered mice hypo-responsive to a footpad injection with oxazolone and the suppression could be adoptively transferred using CD8+ splenocytes. Oral pretreatment with diclofenac yielded a mixed effect on a subsequent diclofenac-induced PLN reaction. For example, mice gavaged a single time had augmented and accelerated diclofenac-induced PLN reactions both in the direct PLNA and when co-injected with TNP-Ficoll (increases of IgM, IgG1 and IgG3 AFCs). In contrast, 3 oral doses with diclofenac rendered mice hypo-responsive following footpad injection by an as of yet unidentified mechanism. These observations suggest the PLNA may be well suited to study the systemic immunomodulating effects of chemicals following oral priming and support further examination of the assays.

952 SCIENCE IN THE LEGISLATIVE PROCESS: A CONGRESSIONAL AND SCIENTIFIC VIEW.

W. J. Brock[1] and K. Olden[2]. *[1]ENVIRON Health Sciences Institute, Arlington, VA and [2]National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

A primary goal for the Society of Toxicology is to promote the use of sound toxicological science in regulatory and legislative practices and policies. To achieve this goal, the SOT focuses significant activity to support science-related functions in regulatory agencies and legislative bodies with the objective of achieving better working relationships with policy makers. In this session, representatives from Congress and experienced SOT members will explore those practices that have worked best in developing and promoting good science policy. Topics to be included in this session will be a Congressional view on the "rights" and the "wrongs" of working with Congressional committees or individual representatives. It is anticipated that a speaker from both the Senate and House of Representatives will provide special insights for the SOT membership on developing relationships with Congressional members and their staff. Also, SOT members with significant experience with presentations before Congress or have worked closely with Congressional members will present on the practices that work best for the scientific community. A general discussion between speakers will address the importance of strategy and effective communication between policy makers and scientists during those times when scientific topics are being addressed by Congress. A key outcome from this roundtable discussion will be to provide SOT members with greater perspective of how the process of legislation works and how scientists can be a part of that process.

953 THE ROLE OF ESTROGEN IN THE MAINTENANCE OF AH RECEPTOR EXPRESSION IN MCF-7 BREAST CANCER CELLS.

D. C. Spink, B. H. Katz and B. C. Spink. *New York State Department of Health, Wadsworth Center, Albany, NY.*

Numerous studies indicate cross-talk between estrogen receptor-alpha- and Ah receptor(AhR)-mediated signaling in breast and endometrial cells. In this study we examined the effects of long-term estrogen deprivation and estrogen re-exposure on Ah responsiveness in MCF-7 human breast cancer cells. Estrogen-deprived MCF-7 cells were obtained by long-term culture in medium prepared with 5% bovine calf serum, which contains minimal estrogen. These estrogen-deprived cells showed depressed levels of AhR mRNA and protein and reduced responsiveness for induction of CYP1A1 and CYP1B1 by dioxin. The reduced Ah-responsiveness was reversed by culture for four passages in medium supplemented with 1 nM estradiol (E2). Expression from a transiently transfected CYP1B1-promoter-luciferase reporter construct was reduced in the long-term estrogen-deprived cells, but was restored by co-transfection with an AhR expression construct, indicating that AhR expression was limiting in the estrogen-deprived cells. To determine whether the reduced AhR expression in estrogen-deprived cells involved alterations in transcription of the AhR, we investigated AhR promoter activity using a vector that contained 5.8 kb of the human AhR gene promoter cloned into the luciferase reporter vector, pGL3-Basic. A deletion construct containing 1.9 kb of the AhR proximal promoter was also used. Use of either promoter-reporter construct showed significantly lower AhR promoter activity in long-term estrogen-deprived MCF-7 cells compared with cells maintained in estrogen-containing medium. When estrogen- deprived cells were cultured for four passages in medium containing 1 nM E2, AhR promoter activity was restored, implicating E2 in the regulation of AhR expression. These studies indicate that the continued presence of estrogen is required to maintain high levels of AhR expression and inducibility of CYP1A1 and CYP1B1 in MCF-7 cells and that the reduced expression of AhR in long-term estrogen-deprived MCF-7 cells involves altered transcriptional regulation of the AhR. (Supported by NIH grant CA81243.)

954 A PROPOSED ROLE FOR THE ARYLHYDROCARBON RECEPTOR (AHR) IN NON-PHOTIC FEEDBACK TO THE MASTER CIRCADIAN CLOCK.

L. T. Frame[1], W. Li[1], J. D. Miller[2] and R. L. Dickerson[1]. [1]*Pharmacology and Neuroscience, Texas Tech Health Sciences Center, Lubbock, TX and* [2]*Department of Cell and Neurobiology, Keck School of Medicine of USC, Los Angeles, CA.*

The arylhydrocarbon receptor (AhR) is a ligand-activated transcription factor and PAS-domain protein, best known for its role in mediating the toxicity of dioxin. Despite a wealth of knowledge about the molecular biology of the AhR, its physiological role remains unknown. We propose a novel role for the AhR in non-photic feedback to circadian clocks. In this scheme, stimulation of the AhR pathway opposes behavior associated with the expression of clock-associated PAS-domain proteins in the suprachiasmatic nucleus (SCN) of the hypothalamus. According to this model, many natural AhR agonists are produced as a consequence of specific interactions with the environment (i.e. behavior). Under normal conditions, these chemical signals transiently stimulate the AhR pathway, and play a role in daily resetting of the circadian clock. More significant changes to the circadian program may occur with stress, when production of other relevant chemical signals may enhance stimulation of the receptor. In the extreme, persistent activation of the AhR pathway (i.e. TCDD) leads to total failure of circadian homeostasis. This hypothesis evolved from recent data in our C57B6 mouse model, which showed 1) TCDD-associated inhibition of the clock-associated protein, mPER, within pacemaker cells of the SCN; 2) low-dose TCDD phase-response curves similar to other non-photic stimuli, such as serotonin and neuropeptide Y; 3) behavioral splitting and arrhythmicity as a consequence of high-dose TCDD; and 4) behavior in AhR knockout animals that appears consistent with a non-functioning negative feedback pathway to the master circadian clock. This model provides a framework for understanding the role of the AhR in both regulation and dysregulation of the circadian system and sets the premise for mechanistic studies. It also puts PAS-domain proteins at every level of circadian control, with the possibility of cross-talk from the molecular to behavioral level.

955 LIGHT-INDUCED SIGNALING VIA THE ARYL HYDROCARBON RECEPTOR (AHR).

A. Rannug[1], M. Oberg[1], L. Bergander[2], H. Hakansson[1] and University. Rannug[2]. [1]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden and* [2]*Cellular and Genetic Toxicology, Stockholm University, Stockholm, Sweden.*

The AHR is a ligand-activated transcription factor that is implicated in the mechanism of dioxin induced toxicity. The physiological roles of the AHR as well as the possible endogenous ligand(s) are still mostly unknown. We have introduced a new concept in dioxin research, suggesting that the AHR has a physiological function as a sensor of chemical signals for light. UV irradiation of the essential amino acid tryptophan gives rise to substances of the formylindolo[3, 2-b]carbazole type. These bind to the AHR with very high affinity and induce the AHR battery of genes. In accordance with hormone signaling, the presence of the photoproducts seems to be strictly regulated and the target cells degrade them *via* the upregulated biotransformation enzymes to terminate the signal. The aim of this project was to study and quantify the impact of light, in combination with different medium components, on the CYP1A1 activity in rat hepatoma cells. In addition, we aimed at identifying the active photoproduct(s). Cell culture media with or without tryptophan (16 mg/l) were exposed to visible light (60 W light bulb) for 24 hours and then fractionated to enable identification of the formed photoproduct(s). The light exposed medium was tested for CYP1A1 inducing activity. Several non-polar substances were formed in tryptophan containing medium and for the first time, one of the inducers was identified and found to be identical to the previously identified compound 6-formylindolo[3, 2-b]carbazol. This finding ultimately explains many reports in the literature, describing 1) increased CYP1A1 gene expression after change of medium; 2) high constitutive CYP1A1 mRNA levels and low responsiveness to induction by dioxin in cells that lack CYP1A1 enzyme activity; and 3) the resetting of circadian rhythms that has been observed in cells in culture after medium change. Also, the results clearly show that formation of AHR ligands from tryptophan, should be considered in mechanistic studies performed with cells grown in culture.

956 AH RECEPTOR-REGULATED CHROMATIN REMODELING AND TRANSCRIPTIONAL ELONGATION: *Sequential recruitment of transcription factors and differential phosphorylation of c-terminal domain of rna polymerase ii at cyp1a1 promoter.*

Y. Tian and S. Ke. *Vet. Physiology and Pharmacology, Texas A&M University, College Station, TX.*

The expression of cytochrome P450 1A1 gene (*cyp1a1*) is regulated by the aryl hydrocarbon receptor (AhR), which is a ligand-activated transcription factor that mediates most toxic responses induced by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). In the nucleus, ligand-activated AhR binds to the xenobiotic response elements, initiating chromatin remodeling and recruitment of coregulators, leading to the formation of preinitiation complex followed by elongation. Here, we report that ligand-activated AhR plays an active role in chromatin remodeling/histone modifications as well as control of transcriptional elongation. Using chromatin immunoprecipitation assay, we found that, in Hepa1c1c7 cells, TCDD treatment resulted in recruitment of histone methyltransferase (CARM1) which methylates argine 17 of histone H3 at *cyp1a1* promoter. The recruitment of CARM1 is likely through direct ligand-dependent association between AhR and CARM1 as determined by the co-immunoprecipitation assay using Hepa1c1c7 cells as well as by the GST-pulldown assay. Furthermore, we found that AhR actively regulates *cyp1a1* transcriptional elongation. We found that AhR recruits the positive transcriptional elongation factor (P-TEFb) and RNA polymerase II (RNA P II) to *cyp1a1* promoter with concomitant phosphorylation of RNA P II carboxyl domain (CTD). Inhibition of P-TEFb kinase activity by 5, 6-dichloro-1-β-D-ribofuranosyl-benzimidazole (DRB) suppressed CTD phosphorylation (especially serine 2 phosphorylaiton) and abolished processive elongation without disrupting the assembly of the preinitiation complex at *cyp1a1* promoter, suggesting that the formation of the preinitiation complex and elongation are interconnected but distinct transcriptional processes. We showed that ligand-activated AhR associated with P-TEFb through the C-terminus of cyclin T1, suggesting that AhR recruit the P-TEFb to cyp1a1 promoter whereupon its kinase subunit phosphorylates the RNA P II CTD.

957 COREGULATOR DYNAMICS OF AHR-MEDIATED TRANSCRIPTIONAL ACTIVATION: RECRUITMENT OF ERα TO TCDD RESPONSIVE PROMOTERS.

J. Matthews[1], B. Wihlen[1] and J. Gustafsson[2, 1]. [1]*Biosciences, Karolinska Institutet, Huddinge, Sweden and* [2]*Medical Nutrition, Karolinska Institutet, Huddinge, Sweden.*
Sponsor: T. Zacharewski.

Using the chromatin immunoprecipitation (ChIP) assay we studied the TCDD-mediated recruitment of the AhR and ARNT heterodimeric complex (AHRC) and several coregulators to the prototypic AhR target gene CYP1A1 in MCF-7 human breast cancer cells. AhR and ARNT displayed time-dependent recruitment to the CYP1A1 promoter, reaching a peak at 75 min and maintaining promoter occupancy for the remainder of the 165 min time course. Recruitment of AhR and ARNT was closely followed by AIB1/SRC3 and TIF2/SRC2, which preceded the accumulation of CBP and p300. The arrival of HATs coincided with that of the TRAP/SMCC/mediator complex, which was followed by histone H3 acetylation and the phosphorylation of RNAPII. Mutual recruitment of the AHRC to a DRE rich region approximately 1000 bp upstream from the promoter and the tata-box region suggests the formation of a larger transcriptionally active complex that bridges the proximal and distal promoter regions. No recruitment of AHRC was observed to a region within the CYP1A1 coding sequence, demonstrating the specific recruitment of AHRC to the promoter region. Interestingly, ERα displayed a TCDD and time-dependent recruitment to the CYP1A1 promoter region. Cotreatment experiments with TCDD and estradiol showed a significant increase in the recruitment of ERα to CYP1A1. Transient transfection experiments HuH7 liver and MDA-MB231 breast cancer cell lines confirmed previously reported ERα enhancement of AHRC gene activity. In contrast to other reports, TCDD did not induce the recruitment of ERα to the estrogen responsive pS2 promoter, and cotreatment experiments demonstrated a time-dependent reduction in the recruitment ERα to estrogen responsive promoters. Collectively, these data suggest that ERα acts as a coregulator of AhR signaling cascades and the recruitment of ERα by AHRC represents a further mechanism of AhR-ERα cross-talk.

958 AGONIST AND CHEMOPREVENTATIVE LIGANDS INDUCE DIFFERENTIAL TRANSCRIPTIONAL COFACTOR RECRUITMENT BY ARYL HYDROCARBON RECEPTOR.

E. Hestermann[1, 2, 3] and M. Brown[2, 3]. [1]*Biology Department, Furman University, Greenville, SC,* [2]*Medical Oncology, Dana Farber Cancer Institute, Boston, MA and* [3]*Harvard Medical School, Boston, MA.*

Aryl hydrocarbon receptor (AHR) is a transcription factor whose activity is regulated by environmental agents, including several carcinogenic agonists. We measured recruitment of AHR and associated proteins to the human cytochrome P4501A1 gene promoter *in vivo* by chromatin immunoprecipitation. Upon treatment with the agonist beta-naphthoflavone (BNF), AHR is rapidly associated with the promoter, and recruits the three members of the p160 family of coactivators as well as the p300 histone acetyltransferase, leading to recruitment of RNA polymerase II (Pol II) and induction of gene transcription. AHR, coactivators, and Pol II cycle on and off the promoter, with a period of ~60 minutes. In contrast, the chemopreventative AHR ligand 3, 3'-diindolylmethane (DIM) promotes AHR nu-

clear translocation and p160 coactivator recruitment, but, remarkably, fails to recruit Pol II or cause histone acetylation. This novel mechanism of receptor antagonism may account for the antitumor properties of chemopreventative compounds targeting the AHR.

959 PROTEIN KINASE C (PKC)-ELICITED PHOSPHORYLATION OF THE ARYL HYDROCARBON RECEPTOR (AHR) IS INHIBITED BY MUTATION OF AHR TYROSINE 9.

G. D. Minsavage[1], G. S. Bedi[2] and T. A. Gasiewicz[1]. *[1]Toxicology Training Program, Department of Environmental Medicine, University of Rochester, Rochester, NY and [2]Biochemistry and Biophysics Center for Oral Biology, University of Rochester, Rochester, NY.*

The aryl hydrocarbon receptor (AhR) is a ligand-activated member of the bHLH-PAS transcription factor family which includes PER, AhR nuclear translocator (ARNT or Hypoxia Inducible Factor 1-beta), SIM, and HIF 1-alpha. Xenobiotics, such as TCDD, bind to the AhR and initiate pleiotropic responses. It has been reported that formation of the AhR/ARNT:DNA ternary complex is disrupted by a single mutation at AhR tyrosine 9 (Y9). We previously demonstrated a basic isoelectric point shift that was detected by two-dimensional gel electrophoresis of mouse AhRY9F. This suggests that AhR Y9 is either a phosphorylated residue and/or that Y9 contributes to maintaining normal posttranslational modification at other unidentified residues. Here, we address how Y9 may contribute to phosphorylation of other AhR residues. Kinase assays were performed using synthetic peptides corresponding to the wild-type (MSS GAN ITY ASR KRR KPV QKT VK) and mutant (Y9F or a phosphorylated Y9) mouse AhR residues 1-23. Protein kinase C (PKC)-elicited phosphate incorporation into the Y9F and phosphorylated Y9 peptides was 69% and 36% of the incorporation into the wild-type peptide. An AhR peptide corresponding to residues 383-406, containing two serines and two threonines, did not incorporate a significant amount of phosphate. In addition, compared to AhRY9F, partially purified full-length wild-type AhR was more rapidly phosphorylated specifically by PKC. Overall, these data indicate that Y9 may play a unique role in maintaining a DNA binding conformation of the full-length AhR by permitting proper phosphorylation of serine(s)/threonine(s) by PKC. Furthermore, these data implicate PKC in the regulation of AhR activity as we demonstrate for the first time that PKC can directly phosphorylate the AhR. (Funded in part by NIH Center Grant ES01247, Training Grant ES07026 and Grant ES02515.)

960 IMPACT OF NUCLEOCYTOPLASMIC SHUTTLING AND ACCESSORY PROTEINS ON THE DEGRADATION OF THE ARYL HYDROCARBON RECEPTOR (AHR).

J. Popat, R. Buzzeo and R. S. Pollenz. *Biology, University of South Florida, Tampa, FL.*

The aryl hydrocarbon receptor is a member of the basic-helix-loop-helix/PAS (bHLH/PAS) family of transcription factors. The AHR is an orphan receptor and bind exogenous ligands typified by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). The unliganded AHR exists in the cell in a complex with hsp90, the immunophilin protein XAP2 and p23. Upon ligand binding, the AHR is localized to the nucleus where it binds with ARNT and DNA and then degraded *via* the 26S proteasome. Since the AHR contains both nuclear import and export signals, studies were initiated to determine how the location of the AHR impacted degradation events and whether the unliganded AHR exhibited dynamic shuttling between the nucleus and cytoplasm. Importantly, these studies investigated the endogenously expressed AHR in multiple cell culture models. Western blot and immunohistochemical studies showed that degradation events followed the nuclear accumulation of the AHR in rat smooth muscle (A7), mouse myoblast (C2C12), murine fibroblast (10T1/2) and murine Hepa-1 cells. In all cells, nuclear accumulation was complete within 30-45 minutes of ligand exposure, whereas significant degradation was not observed until 45-90 minutes. To determine whether the AHR was activity shuttling in the absence of exogenous ligand, cells were treated with the nuclear export inhibitor, leptomycin B (LMB). Results showed that treatment with LMB did not affect the cytoplasmic location of the AHR in various Hepa-1 lines, but resulted in the AHR becoming predominately nuclear in A7, C2C12 and 10T1/2 cells within 2 hours. To determine the contribution of hsp90, XAP2 and p23 to the dynamics of AHR location, IP studies were carried out. Results suggest that loss of hsp90, XAP2 and p23 from the core AHR complex is distinct in liganded vs. unliganded translocation events. These studies suggest that the AHR can actively shuttle between the nucleus and cytoplasm and indicate that ligand mediated 26S proteasome degradation can occur within the nuclear compartment. Supported by NIEHS 10991.

961 MOLECULAR MECHANISMS OF DIOXIN INSENSITIVITY IN *XENOPUS LAEVIS* EMBRYOS AND TADPOLES.

J. A. Lavine, B. H. Philips, T. C. Susman, A. J. Rowatt, A. J. Whitington, T. Klimova and W. H. Powell. *Biology, Kenyon College, Gambier, OH.* Sponsor: M. Hahn.

2, 3, 7, 8 tetrachlorodibenzo-p-dioxin (TCDD) is a potent developmental toxicant in most vertebrates. However, several frog species are resistant to TCDD toxicity, especially during early life stages. We have used *X. laevis* as a model for investigating the molecular mechanisms of TCDD insensitivity in frogs. *X. laevis* tadpoles express two distinct transcripts encoding aryl hydrocarbon receptors (AHRs), AHR1α and AHR1β. Biochemical analysis of each AHR indicated that while TCDD enabled sequence-specific DNA interactions, it bound the receptors with an affinity approximately twenty-fold lower than mouse AHR. Furthermore, the signaling activity of the AHRs is attenuated in early life stages. TCDD exposure induced the expression of CYP1A6 and CYP1A7 mRNAs in stage 54 tadpoles (~1 month), but did not alter their expression at stage 48 (~7.5 days) or earlier, despite the expression of both AHR mRNAs. Finally, like other frogs, *X. laevis* embryos eliminate TCDD very rapidly. Embryos (stage 42) exposed to [^{3}H]TCDD eliminated their TCDD burden with a half-life less than one day. While older tadpoles (stage 54 and later) exhibited no TCDD elimination over several days, they were nonetheless remarkably insensitive to TCDD-induced lethality. Taken together, these studies suggest that multiple mechanisms combine to produce TCDD insensitivity in different stages of frog development. Rapid TCDD elimination and repressed AHR activity are likely important in embryos, while weak TCDD binding by AHRs allows insensitivity to persist into the later tadpole stages. Understanding the molecular mechanisms of TCDD insensitivity in different life stages is important for determining the human health relevance of frog embryo toxicity assays (e.g. FETAX). Moreover, the unique features of frog AHR function and expression may provide a novel perspective on the relationship between AHR expression, AHR activity, mechanisms of TCDD toxicity, and the endogenous functions of AHRs during vertebrate development. [NIH R15-ES11130]

962 FUNCTIONALITY OF HUMAN XRCC1 399 AND 194 VARIANT PROTEINS IN CELLS DETERMINED BY AN ULTRA-SENSITIVE AND REAL-TIME SSB ASSAY.

J. Nakamura[1], T. Takanami[2], J. A. Swenberg[1] and Y. Kubota[2]. *[1]Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC and [2]Department of Biochemistry, Medical School of Medicine, Iwate Medical University, Morioka, Iwate, Japan.*

Inherited polymorphisms in DNA repair genes have been suggested as risk factors for cancer susceptibility. The X-ray repair cross-complementing group 1 (XRCC1) has a pivotal role in the coordination of base excision repair (BER) by serving as an essential scaffold protein which interacts with proteins involved in the BER pathway. Although there are conflicting experimental observations, increasing evidence suggests that *XRCC1* codon 399 genotype are associated with increased susceptibility to breast, head and neck, as well as lung cancer. However, the functionality of these polymorphisms has not been fully characterized. The goal of this study was to determine the functionality of XRCC1 variant proteins in cells using a newly established ultra-sensitive single strand break (SSB) assay. We evaluated SSB repair capacity in isogenic XRCC1-deficient Chinese hamster ovary cells expressing wild-type (wt) and two major human XRCC1 variant proteins under oxidative and alkylating conditions. XRCC1-truncated (EM9) cells harboring empty vector or expressing either XRCC1 wt protein, XRCC1 194 or 399 variant proteins were exposed to methyl methanesulfonate (MMS) or hydrogen peroxide (H_2O_2) and intracellular NAD(P)H in these cells was monitored in real time. EM9 cells harboring empty vector showed a significant depletion of intracellular NAD(P)H after either MMS or H_2O_2 exposure. All EM9 cell lines expressing XRCC1 wild-type, 194, or 399 variant proteins after either MMS or H_2O_2 exposure showed a greater resistance to the depletion of intracellular NAD(P)H levels compared to the EM9 cells harboring the empty vector, but there were no significant differences between cells expressing wt and variant proteins. These data strongly suggest that both human XRCC1 194 and 399 variant proteins are as functional as wt XRCC1 protein in facilitating repair of SSBs induced by either alkylation or oxidation.

963 STUDIES ON PROTEIN STABILITY AND METABOLIC ACTIVITY OFHUMAN CYTOCHROME P450 2A6 (CYP2A6) GENETIC VARIANTS.

K. George, X. He and J. Hong. *Joint Graduate Program Toxicology, Rutgers University/UMDNJ and School of Public Health, Piscataway, NJ.*

Human cytochrome P4502A6 (CYP2A6) is a major enzyme for the metabolism of nicotine and for the bioactivation of several tobacco-related carcinogens such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Functional genetic polymorphisms of CYP2A6 could be an important factor in determining an individual's

susceptibility to tobacco-related cancers. Our previous studies showed that the CYP2A6 natural missense variants CYP2A6*2 (Leu160His), CYP2A6*3b (Leu156Pro) and CYP2A6*5 (Gly479Val) are enzymatically inactive. In addition, we observed CYP2A6*2 had a lower protein expression level than the wild-type in a baculovirus/insect cell expression system. The aim of present study is to confirm these observations in mammalian cells. The cDNAs of CYP2A6*2, CYP2A6*3b, CYP2A6*5 and wild-type CYP2A6 were transfected into Chinese hamster ovary (CHO) cells. Analysis of gene expression by reverse transcriptase polymerase chain reaction (RT-PCR) revealed that CYP2A6 mRNA were expressed in all the transfected CHO cells. However, the level of immunodetectable CYP2A6 proteins were significantly lower in the cells transfected with the variant cDNAs than the wild-type CYP2A6, suggesting decreased stability of these missense proteins. In order to analyze the metabolic activity of the variant proteins, the stable transfectants were challenged with NNK and cell *via*bility was assessed by methyl tetrazolium salts (MTS) assay. Preliminary results show that the CHO cells expressing these variant CYP2A6 cDNAs have significantly lower cytotoxicity with NNK at 400-800 μg/μl than those expressing the wild-type CYP2A6. This result is consistent with the role of CYP2A6 in NNK metabolic activation and the reduced metabolic activity of the variant proteins. Further work with circular dichroism analysis of the wild-type and variant CYP2A6 proteins will be conducted to determine the structure-activity relationship.(Supported by NIH grant ES-07148-17 and ROI-ES-10048)

964 SINGLE AND COMBINED GENOTYPES ON LUNG CANCER SUSCEPTIBILITY IN CHILEAN PEOPLE.

L. Gil, V. Martinez and M. Adonis. *Cellular and Molecular Biology, Faculty of Medicine University of Chile, Santiago, Chile.* Sponsor: R. Yang.

It has been suggested that more than 80% of cancers (CA) are produced by chemical carcinogenic compounds. In the year 2000, 10 million of new cases of CA in the world were diagnosed and 6 million of them died. The same year in Chile the number of cases was 2, 174, of which 1, 900 died. The magnitude of lung CA mortality is related to the high rate of incidence and poor survival after the treatment. Although the advances in surgery and other therapies have been important, the 5 years survival rate for lung CA is still low. It is well established that one of the risk factors of CA is the smoking habit. However, the CA is not only related to smoking, suggesting other sources and factors in the CA incidence. Type and aggressiveness of lung CA and degree of response to a specific treatment might be determined by time and level of exposure to chemical carcinogenic agents as well as to genetic susceptibility. Our studies have shown in Santiago as well as in working places very high PM10 levels associated with high PAHs and nitro-PAHs levels and with high mutagenic activity. Additionally, our results have shown that the frequencies of CYP1A1 variant alleles for MspI (m2 or CYP1A1*2A) and null genotype for GSTM1, were higher in lung CA patients than in Chilean healthy controls. The estimated relative risk for combined unfavourable genotypes was particulate high for null genotypes of GSTM1 and m2 variant allele (OR =2.51), especially when the sample was stratified by smoking habit (OR=2.98). If the codon 72 for p53 protein (Pro) polymorphism is included the risk was highly increased (m2+GSTM1+Pro, OR=6.19).In conclusion, considering lung CA aggressiveness, the early detection might play a fundamental role in the rate of survival. If it is considered that the cost of each lung CA treatment is of approximately US$23.000, studies to detect genetic polymorphisms in susceptible individuals would be highly profitable in terms of prevention and improvement in the efficiency of the treatment. F

965 N-ACETYLTRANSFERASE 2, EXPOSURE TO SMALL AMOUNTS OF AROMATIC AMINES, AND BLADDER CANCER.

K. Golka[1], K. Farker[2], W. Weistenhoefer[1], T. Reckwitz[3], V. Prior[4], T. Seidel[5], R. Thier[1], M. Blaszkewicz[1] and H. M. Bolt[1]. [1]*Institute for Occupational Physiology, University of Dortmund, Dortmund, Germany,* [2]*Institute of Clinical Pharmacology, Friedrich-Schiller-University Jena, Jena, Germany,* [3]*Department of Urology, Klinikum Leverkusen, Leverkusen, Germany,* [4]*Department of Urology, Klinikum Dortmund, Dortmund, Germany and* [5]*Department of Urology, Paul-Gerhard-Stiftung, Lutherstadt Wittenberg, Germany.*

The slow NAT2 acetylation state is a risk factor for subjects exposed to aromatic amines at benzidine production sites. We investigated bladder cancer subjects occupationally exposed to aromatic amines at low levels (painters, who started with their apprenticeship decades ago), or non-occupationally exposed (smokers). Sixteen painters with bladder cancer and 26 healthy colleagues were matched for age (± 5 years), gender, and place of residence and investigated for their occupational histories and smoking habits. Additionally, 591 bladder cancer subjects from 3 different industrialized areas with known risk factors for bladder cancer and 196 bladder cancer subjects from the area of Jena without known risk factors were investigated for smoking habits. The NAT2 acetylation state was determined in all subjects. Fourteen of the 16 painters with bladder cancer (88%), but only 17 of the 26 painters without bladder cancer were slow acetylators. Smoking habits and age at first exposure to colorants revealed no relevant differences. In the group of bladder cancer subjects from urological hospitals in areas with known risk factors for bladder cancer, subjects with a smoking history were over-represented, compared with non-smokers: 152 of 240 smokers (63%), but only 70 of 138 non-smokers (51%) were slow acetylators. In the area of Jena, 57% were slow acetylators; from those with known smoking habits (n=69), 65% of the smokers were slow acetylators. The slow acetylation state is a susceptibility factor for bladder cancer also in subjects exposed to low levels of carcinogenic aromatic amines in the past.

966 CORRELATION BETWEEN CATECHOL-*O*-METHYLTRANSFERASE GENOTYPE AND PHENOTYPE.

A. E. Sullivan[1], J. E. Goodman[2], P. M. Silber[3] and J. D. Yager[1]. [1]*Environmental Health Sciences, Johns Hopkins University, Baltimore, MD,* [2]*Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD and* [3]*In Vitro Technologies, Inc., Baltimore, MD.*

A major detoxification pathway for the carcinogenic catechol estrogens is methylation by catechol-*O*-methyltransferase (COMT). A val108met polymorphism in the *COMT* gene has been associated with an increased risk for breast cancer. It had been hypothesized that the met allele ($COMT^L$) codes for a protein with lower activity than that coded by the val allele ($COMT^H$). Other laboratories have shown that the val108met polymorphism is associated with a 3-4-fold difference in COMT activity in homogenates of human erythrocytes, liver and kidney tissue. However, studies conducted in our laboratory using recombinant human COMT protein and the cytosolic fractions of COMT homozygous human breast cell lines revealed no differences between the kinetic parameters of $COMT^H$ and $COMT^L$. Interestingly, the previous studies where differences in activity were found did not normalize COMT activity to COMT protein levels. In order to address this discrepancy we obtained hepatocytes from thirty-one Caucasian female donors and determined their COMT genotype, COMT activity and COMT protein levels. We found that cytosolic COMT activity levels directly correlate with COMT protein levels. Therefore, lower COMT activity seen in tissues and cells with $COMT^{LL}$ genotypes is due to lower COMT protein levels compared with tissues and cells from $COMT^{HH}$ individuals. Protein turnover studies are currently being conducted to determine if $COMT^L$ has a shorter half-life compared to $COMT^H$. Supported by grants R01CA77550 and T32ESO7141.

967 GLUTATHIONE-S-TRANSFERASE POLYMORPHISMS AND ASSOCIATIONS WITH T1DM.

L. M. Bekris[1], C. Shephard[1], F. Farin[1], J. Graham[2], B. Mcneney[2], T. J. Kavanagh[1] and A. Lernmark[1]. [1]*Environmental Health, University of Washington, Seattle, WA and* [2]*Statistics and Actuarial S, Simon Fraser University, Burnaby, BC, Canada.*

Glutathione (GSH) is the major endogenous antioxidant produced by the cell. GSH participates directly in the neutralization of free radicals and maintains exogenous antioxidants such as vitamins C and E in their reduced forms. GSH plays a role in the immune response. Glutathione S-transferases (GSTs) conjugate GSH to free-radicals or xenobiotics. GST activity depletes GSH levels and may either detoxify or enhance the toxicity of a compound. Glutathione-s-transferase mu 1 (GSTM1) located at 1p13.3 and glutathione-s-transferase theta 1 (GSTT1) located at 22q11.2 have polymorphic homozygous deletion (null) genotypes resulting in complete absence of enzyme activity. GST null genotypes have been associated with susceptibility to cancer and protection against chronic pancreatitis. GSTM1 and GSTT1 null genotypes in Caucasian populations have frequencies of approximately 50% and 15-20%, respectively. Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by pancreatic beta cell destruction involving autoreactive T-cells, pro-inflammatory cytokines, reactive oxygen species and loss of insulin. Monozygotic twin studies show only a 20-30% concordance with T1DM indicating strong environmental contributions to the disease. Genetic contributors associated with T1DM include HLA, insulin VNTR, and CTLA-4. The aim of this study was to investigate associations with GSTM1 and GSTT1 polymorphisms in a group of 200 T1DM patients and 200 control subjects who participated in the Combined Swedish Childhood Diabetes Registry and Diabetes Incidence Study. Results show that the GSTM1 null genotype (p=0.07, OR=0.69) but not the GSTT1 (p=0.34, OR=0.75) may protect against T1DM. Taken together, the GSTM1 and GSTT1 null genotypes showed a stronger and statistically significant association with T1DM (p=0.03, OR=0.64). These results suggest that GST null genotypes are negatively associated with T1DM and that susceptibility to T1DM involves GST conjugation and possibly GST mediated GSH depletion.

968 POLYMORPHISMS IN HUMAN SOLUBLE EPOXIDE HYDROALSE.

P. K. Srivastava and D. F. Grant. *Pharmacy, University of Connecticut, Storrs, CT.*

Human soluble epoxide hydrolase (hsEH) and its endogenous substrates have been suggested to play a role in several physiological processes including blood pressure regulation, inflammation, and cell proliferation. It may also play a role in the me-

tabolism of xenobiotics. HsEH consists of two domains, an N-terminus having phosphatase activity and a C-terminus having epoxide hydrolase activity. Recently, we found 6 SNPs in the hsEH gene (EPHX2) in humans. Studies showed that some of these SNPs are associated with changes in epoxide hydrolase activity. In the present study, we examined the effect of these polymorphisms on phosphatase activity, enzyme stability and the ability of the enzyme to dimerize. The results show that mutants Lys55Arg, Arg103Cys, Cys154Tyr, Arg287Gln and the double mutant (Arg287Gln/Arg103Cys) have significantly lower phosphatase activity compared to the wild type hsEH. In addition, the stability of Arg287Gln and the double mutant at 37 degre C are significantly reduced compared to the wild type hsEH. HPLC gel-filtration studies of wild type full-length hsEH show that both dimer and monomer are active, however, the dimer form predominated. On the other hand, the double mutant showed an increased concentration of monomeric form, suggesting that these SNPs disrupt interactions presumably responsible for dimerization. We conclude that several polymorphisms occurring in the human EPHX2 gene affect enzyme specific activity, stability and quaternary structure.

969 POLYMORPHISMS IN ALCOHOL DEHYDROGENASE INFLUENCE TOPICAL CAPSAICINOID ACTIVITY IN HUMAN SKIN.

L. K. Pershing and Y. Chen. *Dermatology, University of Utah, Salt Lake City, UT.* Sponsor: G. Yost.

Topical capsaicin and its analog, nonivamide produce erythema and a stinging/burning sensation in human skin that is influenced by alcohols. Erythema produced in propylene glycol is 3X greater than isopropyl alcohol and highly variable between subjects. Erythema is independent of skin capsaicinoid content. Polymorphisms in genes that encode alcohol dehydrogenase are known to alter the rate of alcohol metabolism. This study evaluated the genetic basis for the variable capsaicinoid-induced erythema as a function of alcohol dehydrogenase genes: ADHIB, ADHIC and ADHIV. Peripheral blood was collected from 8 Asians and 8 Caucasians previously evaluated for erythema to topical capsaicinoid in isopropyl alcohol (IPA), propylene glycol (PG), ethanol (EtOH) and mineral oil (MO) solutions (30 mg/ml). DNA extracted from peripheral blood lymphocytes was PCR-amplified with primers designed to known fragments containing single nucleotide polymorphisms (SNPs) of interest in the three genes. PCR fragments were then denatured and resolved on polyacrylamide gels to detect distinct bands associated with known SNPs. Nucleotide sequencing confirmed band patterns. SNPs in ADHIV (G239C), ADHIC (G815A) and ADHIB (G143A) are associated with slow (0), slow (0), and fast (1) alcohol metabolizing isoforms, respectively. Six ADH haplotypes were associated with altered erythema to topical capsaicinoids in alcohols. Erythema varied with the number of alleles (2 per gene) associated with fast alcohol metabolizing isoforms in the order of importance ADHIV > ADHIC > ADHIB. For example, allele haplotype 110000 produced 2X, 2.2X, 2.6X, 3X and 5X greater erythema to capsaicinoids in PG than haplotypes 101010, 111110, 111111, 111100 and 001000, respectively. These data suggest that ADHIV dominates the resulting altered erythema with alcohols but is negatively modulated by ADHIC and positively modulated by ADHIB. Thus, the apparent alcohol-associated increase in capsaicinoid-induced erythema in human subjects is controlled by ADH genes regulating alcohol metabolism and is genetically determined.

970 ACUTE TOXICITY OF ACETALDEHYDE ON ALDEHYDE DEHYDROGENASE 2 GENE TARGETING MICE: SINGLE DOSE IP STUDY.

T. Isse[1], T. Oyama[1], N. Kunugita[2], K. Matsuno[3], K. Kitagawa[4], A. Yoshida[5], I. Uchiyama[6], M. Ogawa[1], T. Kinaga[1], R. Suzuki[1], T. Yamaguchi[1] and T. Kawamoto[1]. *[1]Environmental Health, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan, [2]Health Science, University of Occupational and Environmental Health, Kitakyushu, Japan, [3]Bio-information Research Center, University of Occupational and Environmental Health, Kitakyushu, Japan, [4] First Department of Biochemistry, Hamamatsu Medical University, Hamamatsu, Japan, [5]Beckman Research Institute of the City of Hope, Duarte, CA and [6]Environmental hygiene, School of technology, Kyoto University, Kyoto, Japan.*

Half of Japanese have inactive aldehyde dehydrogenase (ALDH) 2. Toxicity of acetaldehyde to them and normal people thought to be different in same dose. For estimating the difference of acetaldehyde toxicity to these groups, we compare with 50% of lethal dose (LD50) of acetaldehyde of the Aldh2 inactive gene targeting (Aldh2 KO) mice and that of wild type (Aldh2 WT) mice by single dosage of acetaldehyde intra peritoneal injection. The LD50 of acetaldehyde of the Aldh2 WT mice was 602mg/kg (95% 577-624mg/kg) in male, 598mg/kg (95% 575-625mg/kg) in female, respectively. The acetaldehyde LD50 of the Aldh2 KO mice was 567mg/kg (95% 545-589mg/kg) in male, 587mg/kg (95% 564-616mg/kg) in female, respectively. The acetaldehyde LD50 of the Aldh2 KO mice was smaller than that of the Aldh2 WT. There were no differences in the symptoms of the both mice, though Aldh2 KO mice showed rapidly appearance of the symptoms and latency appearance of the recovery than Aldh2 WT mice. The groups given more than 515mg/kg of acetaldehyde showed the loss activity, wobbling just after the administration. Pale skin, unconsciousness, abdominal raging posture and deeply breath were observed 5 min after the administration. The cases of death were observed from 15 to 30 min after the administration in the Aldh2 WT mice, and from 5 to 15 min in the Aldh2 KO. In the case of the death, dyspnoea, and low body temperature was observed. In the case of survive, couching, low breathing activity, reddishness of skin and standing of fur was observed from 45 min to 120 min after the administration in both mice.

971 ARRESTED LUNG DEVELOPMENT ASSOCIATED WITH CHANGES IN TEMPORAL EXPRESSIONS OF FIBROBLAST GROWTH FACTOR (FGF) RECEPTORS-3 AND -4 IN NEWBORN MICE EXPOSED TO SUBLETHAL HYPEROXIA.

S. E. Welty, M. S. Park, B. L. Schanbacher, L. K. Rogers, A. C. Cook, T. N. Hansen, J. A. Bauer and C. V. Smith. *Pediatrics, Columbus Children, Columbus, OH.*

With improved survival of very premature infants, chronic lung disease of prematurity, also known as bronchopulmonary dysplasia (BPD), is characterized increasingly as arrested lung development. Newborn mice have lungs that are comparable developmentally to lungs in prematurely born human infants and exhibit similarly arrested lung development and alveolarization with exposure to sublethal hyperoxia. In the present studies, we tested the hypothesis that inhibition of normal alveolarization by exposure of newborn mice to hyperoxia would be paralleled by alterations of expressions of FGFR3 and 4, FGF7, and HIF-1a. Newborn FVB mice were exposed to 85% O2 (O2) within 12 h of birth or left in room air. No pup mortality was observed, and body weight gains were not affected by hyperoxia. Pups were sacrificed at 1, 3, 7, or 14 d. Lungs were fixed at 25 cm H2O for morphometry or were freeze-clamped for RNA and protein analyses. Levels of FGFR-3 and -4, FGF7, and hypoxia-inducible factor (HIF)-1a mRNAs were assessed by real time PCR. At P14, lungs of O2 pups had fewer and larger alveoli and smaller alveolar surface-to-volume ratios than did AIR pups (14.3+0.4 vs 20.4+0.8 per HPF[640x]; 2038 vs 1402 mm2; and 0.111+0.002 vs 0.140+0.007 mm-1), respectively (different, $P < 0.05$). Lung FGFR3 and 4 mRNA levels were lower at P3 and higher at P7 in O2 pups than in AIR pups. In AIR mice, lung FGF7 mRNA levels were greater at P14 than at P1, but this increase was not observed in O2 mice. At P14, immunoreactivities of FGFR-3 and -4 were lower in lungs of hyperoxic mice than in controls. Lung HIF-1a mRNA levels decreased to 20% of the P1 level by P14 in both AIR and O2 mice. The arrested alveolar develoment observed in newborn mice exposed to 85% O2 resembles BPD of human preterm infants, and the alterations in the temporally and spatially orchestrated expressions of important growth regulatory genes, such as we observe, potentially contribute to this undesirable outcome.

972 THIOREDOXIN AND THE TOXICITY OF ACROLEIN.

Y. Choi, X. Yang and J. P. Kehrer. *Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.*

Acrolein, an α, β-unsaturated aldehyde, is present ubiquitously in the environment and is also generated endogenously *via* lipid peroxidation. Acrolein exhibits strong electrophilic interactions, particularly with cysteine-containing molecules. While interactions with glutathione are well-documented, interactions with other cellular thiols are less clear. Thioredoxin (Trx), a highly conserved small (12 kDa) peptide with two cysteines at the redox-active catalytic site, is of particular interest because of the important roles it plays in regulating cell signaling and redox sensitive transcription factors. Trx exists in both cytosolic (Trx-1) and mitochondrial (Trx-2) forms. Although both forms provide protection against oxidative toxicities, interaction with, and protection against, electrophile toxicity has been little studied. Acrolein was used as a model electrophilic toxicant. Acrolein rapidly, and dose-dependently, decreased the activity of pure Trx. Using western blotting, acrolein did not affect the immunoreactivity of Trx. Human lung adenocarcinoma A549 cells treated with acrolein showed a loss of Trx activity, but not protein, except at very high doses where crosslinking and/or degradation may have occurred. Interestingly, the expression of Trx protein was induced in A549 cells at later times following acrolein treatment. A549 cells stably overexpressing Trx-1 and Trx-2 have been established. The Trx-2 overexpressing A549 cells showed more cell growth when treated with etoposide indicating that Trx-2 is an important survival/protective factor. The toxicity of acrolein to these cells remains unclear. (Supported by ES09791 and Center Grant ES07784.)

973 EFFECT OF OVEREXPRESSION OF HGSTA4-4 ON OXIDATIVE INJURY AND PROLIFERATION OF HEPG2 CELLS EXPOSED TO 4-HYDROXYNONENAL.

E. P. Gallagher and C. M. Huisden. *Physiological Sciences, University of Florida, Gainesville, FL.*

4-hydroxynonenal (4-HNE) is a major mutagenic aldehyde produced during oxidative injury. Although several aldehyde metabolizing pathways exist in human liver cells that protect against 4-HNE cell injury, the human glutathione S-transferase protein hGSTA4-4 exhibits a particularly high catalytic efficiency toward 4-HNE and other hydroxyalkenals. However, hGST4-4 expression is relatively low in human tissues, and there are other aldehyde metabolizing enzymes (including other GSTs) that also contribute to 4HNE detoxification. In the current study, we determined the effect of stable overexpression of hGSTA4-4 on the rates of HepG2 cell proliferation and sensitivity to 4-HNE injury. HepG2 cells transfected with an hGSTA4-4 vector construct exhibited elevated GST-4-HNE catalytic activities, high steady-state hGSTA4 mRNA expression, but lower glutathione (GSH) concentrations relative to wild type or insert-free vector cells. The hGSTA4-4-transfected cells exhibited a growth advantage relative to control cells when challenged with 4-HNE. Interestingly, exposure to 5 μM or 10 μM 4-HNE caused an increase in GSH concentrations in both control and hGSTA4-4-transfected cells. Collectively, our data indicate that exposure to 4-HNE increases GSH biosynthesis in HepG2 cells, and that overexpression of hGSTA4-4 provides a growth advantage in the presence of 4-HNE. Supported by NIH ES09427.

974 REDUCED EXPRESSION OF 8-OXOGUANINE-DNA GLYSOSYLASE IN THE EKER RAT.

S. S. Lau, S. L. Habib, M. S. Chacko and T. J. Monks. *Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona Health Sciences Center, Tucson, AZ.*

The Eker rat (*Tsc*-$2^{EK/+}$) bears a mutation in one allele of the *Tsc*-2 gene, which predisposes these animals to renal cancer. Treatment of either wild type (Tsc-$2^{+/+}$) Eker rats, or rats carrying the mutant Tsc-2 allele (Tsc-$2^{EK/+}$) with 2, 3, 5-*tris*-(glutathion-S-yl)hydroquinone (TGHQ; 2.5 μmol/kg, i.p. for 4, 8 or 16 wks), a potent nephrotoxic metabolite of HQ, causes oxidative DNA damage, cell necrosis, and proliferation within the outer stripe of the outer medulla (OSOM) in both groups of animals, but renal cell carcinomas only arise in the OSOM of the mutant Tsc-$2^{EK/+}$ strain. Interestingly, constitutive expression of 8-oxoguanine-DNA glycosylase (OGG1) in Tsc-$2^{+/+}$ rats is 2.5 fold higher than in Tsc-$2^{EK/+}$ rats. Moreover, the poor ability of Tsc-$2^{EK/+}$ rats to cope with the TGHQ-induced oxidative stress is exacerbated by their inability to rapidly upregulate this enzyme. The mechanism by which a germline insertion in one allele of the *Tsc-2* gene modulates the expression, and regulation, of the *OGG1* gene is not known. We now report, using RT-PCR, that OGG-1 mRNA expression in Tsc-$2^{+/+}$ rats is 3.3 fold higher than in Tsc-$2^{EK/+}$ rats. Moreover, while OGG1 mRNA is significantly upregulated during 2 and 4 months of TGHQ treatment in Tsc-$2^{+/+}$ rats, OGG1 mRNA levels remain unchanged throughout the 4-months of treatment in Tsc-$2^{EK/+}$ rats. To further investigate the link between *Tsc-2* and *OGG1*, we restored tuberin expression in TGHQ-transformed renal epithelial cells, by transient transfection with Tsc-2 cDNA. Re-expression of tuberin significantly increased OGG1 expression by 120-170%. In humans and rats, the *Tsc-2* (16p13.3 and 10q respectively) and *OGG1* (3p26.5 and 4 respectively) genes are located on different chromosomes. We conclude that the presence of a germline insertion in one allele of the *Tsc*-2 gene indirectly modulates the regulation of *OGG1* gene expression. The mechanism by which tuberin influences *OGG1* expression is under investigation. (GM39338, ES07784)

975 CARBONYL REDUCTASE CATALYZES REDUCTION OF 4-OXONONENAL.

D. R. Petersen[1], J. A. Doorn[1], E. Maser[2] and D. J. Claffey[1]. *[1]Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO and [2]Experimental Toxicology, University of Kiel, Kiel, Germany.*

4-Oxononenal (4ONE) was demonstrated to be a product of lipid peroxidation, and previous studies found that it was reactive toward DNA and protein nucleophiles. Currently, metabolic pathways of 4ONE are unknown. The present study sought to determine whether carbonyl reductase (CR) catalyzes reduction of 4ONE. Recombinant human CR was incubated with NADPH and varying concentrations of 4ONE (pH 7.4, 37 C) and kinetic parameters calculated. Products of the enzyme mediated reaction were identified *via* TLC, GC/MS and LC/MS (ion trap). Aliquots of the CR/NADPH + 4ONE reaction were analyzed *via* HPLC to monitor time-dependent change of [4ONE] and [products]. 4ONE was found to be a substrate with KM and kcat of 345 uM and 220 min-1, respectively. TLC analysis with standards revealed the presence of three products resulting from the CR/NADPH + 4ONE reaction, and two were identified as 4-hydroxynonenal (4HNE) and 1-hydroxynonen-4-one (1HNO). GC/MS analysis confirmed the identity of 4HNE and 1HNO. The unknown product did not correspond to standards representing reduction of both or either 4ONE carbonyls and was not reactive toward N-acetyl cysteine. GC/MS analysis identified the unknown as 4-oxononanal (4ONA). Analysis of oxime derivatives of the reaction products *via* LC/MS (ion trap) confirmed the unknown as 4ONA. The time course for CR mediated, NADPH dependent 4ONE reduction and appearance of 4HNE and 1HNO was determined using HPLC, demonstrating that 4HNE and 1HNO are major (71%) and minor (9.0%) products on a molar basis, respectively. Based on these values, 4ONA was calculated to represent 20% of the total product. Results of the present study demonstrate that 4ONE is a substrate for CR/NADPH and the enzyme may represent a pathway for metabolism of the lipid aldehyde. Furthermore, these findings reveal that CR catalyzes selective reduction of the ketone, but also the aldehyde and C=C of 4ONE, resulting in 4HNE, 1HNO and 4ONA, respectively. (Grants NIH/NIEHS F32ES11937 (JAD), NIH/NIAAA R01AA09300 and NIH/NIEHS R01ES09410 (DRP

976 DNA DAMAGE-INDUCED HISTONE H3 PHOSPHORYLATION DOES NOT INVOLVE SITES NORMALLY ASSOCIATED WITH MITOTIC CHROMOSOMAL CONDENSATION.

K. Cox[1], A. H. Palmer[1], S. S. Lau[3], K. N. Dalby[2] and T. J. Monks[3]. *[1]Pharmacology and Toxicology, University of Texas at Austin, Austin, TX, [2]Medicinal Chemistry, University of Texas at Austin, Austin, TX and [3]Pharmacology and Toxicology, University of Arizona Health Sciences Center, Tucson, AZ.*

Treatment of renal proximal tubular epithelial (LLC-PK1) cells with the reactive oxygen species (ROS)-generating toxicant, 2, 3, 5-*tris*-(glutathion-S-yl)hydroquinone (TGHQ) causes DNA damage and premature chromatin condensation (PCC), which may contribute to cell death. Concurrent with PCC is an increase in the phosphorylation of histone H3. Phosphoamino acid analysis of histone H3 revealed that only serine was phosphorylated in response to TGHQ treatment. Phosphorylation of histone H3 at S10 and S28 is known to contribute to the condensation of chromosomes during mitosis, and these residues are phosphorylated by mitogen- and stress-activated kinase (MSK1). To ascertain whether MSK1 contributes to TGHQ-induced toxicity, LLC-PK1 cells were transfected with wild-type (WT) or dominant-negative (DN) MSK1. [^{32}P]-orthophosphoric acid labeled cells expressing WT or DN MSK1 showed no change in TGHQ-induced histone H3 phosphorylation, compared with control TGHQ-treated cells. Accordingly, neutral red analysis revealed no change in cytotoxicity between WT MSK1, DN MSK1, and control cells. In support of these findings, Western analysis showed a decrease in the phosphorylation of histone H3 at both S10 and S28, following treatment with TGHQ. Together, the data suggest that the site of TGHQ-induced phosphorylation is at alternate serine residue(s) on histone H3. There are three isoform variants of histone H3, each with specific serine residue substitutions. Separation of histone H3 isoforms on acid urea triton gel established that only H3.3 is phosphorylated in response to TGHQ. Histone variant H3.3 contains a serine at position 31, in the highly modified tail region unique to H3.3. We conclude that increases in histone H3 phosphorylation in cells undergoing ROS-induced cell death is not due to phosphorylation of S10 or S28, but a novel site responsive to ROS-induced DNA damage. (ES07784, ES07247, DK59491)

977 GENETIC MUTATION ANALYSIS OF THE GLUTATHIONE REDUCTASE HYPOMORPHIC MICE (GR1A1NEU) INDICATES A GENETIC KNOCKOUT ANIMAL.

C. V. Smith, T. Tamura, L. K. Rogers, B. J. Rogers, T. N. Hansen and S. E. Welty. *Pediatrics, Columbus Children's Research Institute, Columbus, OH.*

Glutathione reductase (GR) is a critical component of GSH-dependent antioxidant defense functions in all mammalian cells and tissues studied to date. Recently, a mutant strain (Neu) of mice with 5 to 10% of control GR activities was generated [Pretsch] by administration of isopropyl methanesulfonate and backcrossing through 15 generations into a C3H background. We obtained, rederived, and have studied progeny of these mice. We used genetic mutation analysis to determine the exact location(s) and extent(s) of the mutation(s). RNAs isolated from the Neu and C3H mice were analyzed by RT-PCR, using nested primers covering all 13 exons of the GR gene. RNA from the Neu mice produced a smaller PCR product than did RNA from the C3H mice. Use of primer sets that spanned smaller portions of the gene revealed that exons 2 through 5 were not present. Genomic DNA was isolated and analyzed, using long PCR and amplification of regions containing exons and introns, and Southern blot analyses. The results indicate that the breakpoints of the deletion are within intron 1 and intron 5, effectively deleting exons 2 through 5 and generating a frame-shift in exon 6, as well as inserting a premature stop codon. The coding region of the GR gene in the C3H mice was identical to the sequence

in 129SV/J mice that we reported previously, but the GR gene in the Neu mice exhibited numerous polymorphisms with the other two strains. The polymorphisms in the GR gene of the Neu mice are identical with 14 out of 17 polymorphisms (2 missense, 12 silent) reported for GR in C57BL/6J mice, the remaining 3 silent polymorphisms are within the deleted region. The data presently available indicate that the Neu mice are functional knockouts for GR. The absence of major phenotypic effects from deletion of a gene so highly conserved through evolution is surprising, suggesting possible adequacy in unstressed cells of crosstalk with thioredoxin/thioredoxin reductase, which probably account for the low GR activities measured in the standard assays employed.

978 THE ROLE OF NRF2 AND ARE IN THE INITIATION AND PROGRESSION OF AMYOTROPHIC LATERAL SCLEROSIS.

J. C. Kern, A. D. Kraft and J. A. Johnson. *Pharmaceutical Sciences, University of Wisconsin-Madison, Madison, WI.*

Amyotrophic lateral sclerosis (ALS) is a disease of muscle atrophy, originating from unknown upstream motor neuron dysfunction. The antioxidant response element (ARE), a cis-acting element present in numerous detoxification enzymes, confers neuroprotective effects in response to oxidative stress and is completely dependent on the transcription factor NF-E2 related factor (Nrf2). By crossing transgenic mice containing ARE-driven human placental alkaline phosphatase (ARE-hPAP) with an ALS mouse model containing a mutation in superoxide dismutase (SOD G93A), we were able to identify temporal and spatial patterns of hPAP activity, indicative of oxidative stress. ARE-hPAP, SOD G93A mice demonstrated ~30-fold hPAP activation at 60 days of age (initiation of ALS symptoms) that increased to ~70-fold activation at 120 days of age (muscle loss and paralysis) in the core tissues of ALS motor neuron degeneration-motor cortex, brainstem, and spinal cord. An adenoviral infection containing Nrf2 (Ad-Nrf2) has previously shown dramatic neuroprotection in cortical primary cultures. Since the muscle is of paramount importance in ALS and has the ability to transport survival factors retrograde to the spinal cord, we directly injected Ad-Nrf2 in the gastrocnemius muscle of ARE-hPAP -/ mice. In ARE-hPAP mice, there was a significant activation of hPAP in the motor cortex and brainstem (>=5-fold) and a >=40-fold activation in the spinal cord and muscle, although the Ad-Nrf2 remained sequestered in the muscle (as detected by RT-PCR). Overall, these data demonstrate a role for a Nrf2-mediated ARE response in ALS disease initiation and progression. It also presents a possible therapeutic intervention in amplifying detoxification mechanisms able to counter oxidative stress and muscle loss in SOD G93A mice.

979 CIGARETTE SMOKE INDUCIBLE HUMAN *FRA-1* EXPRESSION IN AIRWAY EPITHELIAL CELLS IS REGULATED BY EGFR-MEDIATED AND ERK/JNK/P38-DEPENDENT MAP KINASE PATHWAYS.

S. P. Reddy and Q. Zhang. *Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.* Sponsor: K. Steven.

Cigarette smoke (CS) exposure is known to be a major cause of human cancers, especially lung cancer, and other respiratory diseases including emphysema. However, the underlying molecular mechanisms involved in these processes remain enigmatic. An aberrant expression of genes induced by CS can result in an altered phenotype, contributing to the respiratory pathogenesis. Given that the activator protein 1 (AP-1) family of transcription factors regulates genes involved in both physiological and pathophysiological processes, we have investigated the effects of CS on the expression of AP-1 (Jun/Fos) family members in 1HAEO airway epithelial cells. CS strongly up-regulated c-Jun, c-Fos, and Fra-1 expression, whereas it had no effect on Fra-2, Jun-B, and Jun-D. Since Fra-1 is overexpressed in various tumors and upregulates genes associated with tumor progression, we further examined the mechanisms regulating its expression by CS. Upregulation of Fra-1 (mRNA and protein) expression correlatedwell with its DNA binding activity. The *fra-1* induction by CS was regulated primarily at the transcriptional level. Pretreatment of cells with the EGFR inhibitor, AG1498, strongly suppressed the induction. Similarly, inhibitors of the ERK, p38, and JNK MAP kinase pathways had a suppressive effect on CS-induced *fra-1* expression. Consistent with this finding, CS caused a distinct activation of these kinases. The phosphorylation of ERK1 and ERK2, JNK1, and p38 was markedly elevated at 30 min after exposure. In contrast to its effect on JNK1, CS showed no significant effect on JNK2 phosphorylation. Together, these observations suggests that CS distinctly regulates Jun and Fos family members expression in airway epithelial cells. Among them, *fra-1* induction is stimulated *via* EGFR-mediated and ERK/JNK/p38-dependent MAP kinase pathways, further underscoring a potential role for this signaling axis in perturbations of CS-induced gene expression by Fra-1 based AP-1 complexes in the lung.

980 4-HYDROXYNONENAL-MEDIATED INHIBITION OF ENZYME-CATALYZED OXIDATION OF THE REACTIVE ELECTROPHILE 3, 4 DIHYDROXYPHENYLACETALDEHYDE.

J. A. Doorn and D. R. Petersen. *Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO.*

Dopamine (DA) undergoes enzyme-catalyzed deamination to yield 3, 4-dihydroxyphenylacetaldehyde (DOPAL), which is metabolized primarily *via* mitochondrial aldehyde dehydrogenase (ALDH2). Recent work implicates oxidative stress and lipid peroxidation products, e.g., 4-hydroxynonenal (4HNE), as factors in neurodegenerative disease. The goal of this study was to determine whether 4HNE inhibits enzymes that catalyze oxidation/reduction of DOPAL and if DOPAL is a reactive electrophile that can modify protein amines. DOPAL was obtained by incubating DA with rat liver monoamine oxidase. Human ALDH2 and rat brain ALR were incubated with DOPAL and increasing concentrations of 4HNE and activity measured. An amine containing peptide (i.e. RKRSRAE) was treated with DOPAL and DA (37 C, pH 7.4), and the products analyzed *via* mass spectrometry. It was found that at low concentrations of 4HNE (i.e. <10 uM), ALDH2 activity toward DOPAL was significantly inhibited. Concentrations of DOPAL at >50 uM led to a decrease in activity of ALDH2, indicating inhibition of the enzyme. Incubation of RKRSRAE with DOPAL but not DA resulted in adduction of the peptide with two molecules of the aldehyde. The mass of the modifications corresponded to Schiff base containing adducts. Inclusion of sodium cyanoborohydride in the incubation mixture resulted in a shift of 2 Da for the mass of the adducts, confirming the presence of an imine on the DOPAL modification. Treatment of the peptide with equal concentrations of 4HNE and DOPAL (i.e. 100 uM each) resulted only in DOPAL adducts, indicating that DOPAL is a more reactive toward amine nucleophiles than 4HNE. These data indicate that 4HNE can significantly impair enzyme mediated oxidation of DOPAL and ALDH2 is inhibited by DOPAL. Furthermore, results of this study demonstrate that DOPAL is a reactive electrophile that can modify peptide amines, yielding stable Schiff base products. (Grants NIH/NIEHS F32ES11937 (JAD), NIH/NIAAA R01AA09300 and NIH/NIEHS R01ES09410 (DRP)).

981 RAPID ANALYSIS OF HEPATOCYTE TOXICITY USING BD OXYGEN BIOSENSORS.

L. E. Dike[1], H. Xia[1] and M. Timmins[2]. *[1]R&D, BD Biosciences, Woburn, MA and [2]R&D, BD Biosciences, Bedford, MA.* Sponsor: D. Stresser.

Drug toxicity and in particular, the toxicity of xenobiotic metabolites, is a critical area of concern in drug development and is a major reason for compound failure in clinical trials. Toxicity studies in human hepatocytes are problematic because cultured hepatocytes rapidly lose their P450 activity in culture, whereas studies performed with hepatocytes in suspension, while maintaining P450 activity, juxtapose drug-induced toxicity with natural cell death. Such toxicity studies are typically 2 hours in duration and potentially miss slow acting toxins. We present here the use of BD Oxygen Biosensor System (BD OBS) for rat and human hepatocytes in suspension. These studies demonstrate that the BD Oxygen Biosensor System can be utilized for studies of longer duration for toxicity than the standard 2-4 hour assay. Hepatocytes, either fresh or cryopreserved, can be plated onto BD OBS and monitored over a 24 hour period. The hepatocytes appear to form spheroid-like structures, maintain their P450 activity and have minimal background cell death. IC50s have been derived for both direct acting and indirect acting toxins such as tamoxifen, ketoconazole and diclofenac. The BD Oxygen Biosensor System allows for rapid analysis in absence of additional sample manipulations, and thus provides an easy to use platform for obtaining accurate IC50s for hepatocyte toxicity.

982 IDENTIFICATION OF THE POTENTIAL OF I_{Kr} (HERG) BLOCKERS TO INDUCE QT PROLONGATION AND TORSADE DE POINTES: EFFECTS OF TERFENADINE, DL-SOTALOL AND VERAPAMIL IN ANESTHETIZED GUINEA PIGS.

G. P. Thomas, J. G. Morahan and C. B. Spainhour. *Pharmacology and Electrophysiology, Calvert Laboratories Inc., Olyphant, PA.*

Most drugs linked to QT prolongation and Torsade de Pointes (TdP) arrhythmias do so by blocking the rapidly activating component of the delayed rectifier potassium current (I_{Kr}; hERG). However, all I_{Kr} blockers do not prolong QT interval or induce TdP. While dl-sotalol and terfenadine induce QT prolongation and TdP, verapamil, another potent I_{Kr} blocker protects from TdP. The *in vivo* and *in vitro* models currently used in pre-clinical studies do not accurately predict the potential risk of acquired long QT syndrome for drug candidates with I_{Kr} blockade. The aim of the present study was to examine the validity of an anesthetized guinea pig model for identifying the potential of I_{Kr} (hERG) blockers to induce QT prolongation. A

relatively selective blocker of I_{Kr}, dl-sotalol, and terfenadine and verapamil, the two non-selective I_{Kr} blockers with pro- and antiarrhythmic actions, were examined for their ability to prolong QTc interval in anesthetized guinea pigs. Guinea pigs were anesthetized by an intra-peritoneal injection of dialurethane. The trachea and the jugular vein were cannulated for positive pressure artificial respiration and administration of drugs, respectively. Dorsal, Axial and Inferior lead electrocardiograms were recorded on a Hewlett Packard Page Writer II (Model 4755A) prior to and 5, 10, and 20 minutes after each dose administration of each drug. QT interval measurements were made from the ECG strip and were corrected for heart rate using Bazett′s formula. Intravenous administration of the pro-arrhythmic agents, dl-sotalol (5.0 mg/kg) and terfenadine (5.0 mg/kg), caused 32% and 22% prolongation of QTc interval respectively in anesthetized guinea pigs, while the antiarrhythmic agent, verapamil (1.0 mg/kg), had no significant effects on QTc. Our results validate the use of anesthetized guinea-pig model as a useful component in the preclinical assessment of QT prolongation and pro-arrhythmia risk.

983 EVALUATION OF RAT SERUM PROTEIN ELECTROPHORESIS USING THE SEBIA HYDRASYS SYSTEM.

L. Le Sauteur, L. Huard, K. Larocque and Y. Deschamps. *Immunology, CTBR, Senneville, QC, Canada.*

Serum protein electrophoresis patterns are useful biochemical markers that can be used to identify acute or chronic inflammatory reactions, protein deficiencies and renal diseases in safety assessment studies. The objective of this work was to confirm that Sprague-Dawley rat serum proteins could be well separated and quantified using the Sebia Hydrasys system. Precision (intra and inter-assay), accuracy, linearity of dilution, stability of the samples and preliminary reference intervals were determined. Accuracy was determined for each serum protein fraction using the control material (Normal Control Serum and Hypergamma Control Serum) and reagents of the manufacturer. Serum protein separation was done with the agarose gel support (Hydragel). Intra-assay precision was calculated by testing 10 consecutive measurements of the control material and of a fresh Sprague-Dawley rat serum sample while the inter-assay precision was obtained from 5 different analysis of the same samples; for all the samples, %CV (g/dL) was less than 1.0 % for the major albumin fraction and less than 7.5% for all the other protein fractions. Each protein fraction (albumin, alpha1, alpha2, beta, gamma) was well separated from each other. Linearity of dilution was tested for each protein fraction; a correlation coefficient higher than 0.9890 and a slope within 5% of optimal linearity were obtained. Preliminary reference intervals were calculated from 20 rats; the results are consistent with expected physiological levels. Sample stability was verified with 5 rat serum samples; a mean sample difference of less than 1.6 % was obtained following sample storage for 3 days at 2 to 8 deg. C, 31 days at -20 deg. C or 3 freeze-thaw cycles. In conclusion, the accuracy, precision and preliminary reference intervals obtained on the Sebia Hydrasys System for protein electrophoresis of Sprague-Dawley rat serum showed the method to be reliable in providing good separation of the serum protein fractions and excellent precision.

984 CIRCADIAN RHYTHM OF RATS: SHOULD CNS EFFECTS OF SMALL MOLECULES BE EVALUATED DURING THE DARK CYCLE.

K. Cardoza[1], M. Gallacher[1], C. Doherty[2], C. Alden[2], D. Tumas[2] and V. J. Kadambi[2]. *[1]Comparative Medicine, Millennium Pharmaceuticals, Inc., Cambridge, MA and [2]Drug Safety and Disposition, Millennium Pharmaceuticals, Inc., Cambridge, MA.*

As mandated in the ICHS7A Guidance document, motor activity assessment is an integral component of the safety pharmacology core battery. Several studies have documented the circadian rhythm in rats which shows an increase in heart rate, blood pressure and activity during the dark cycle. Traditionally motor activity assessment is conducted during the light cycle when rats are not active. When animals are transferred to new cages for motor activity assessment there is an increase in exploratory behavior, which usually lasts for approximately 30 minutes. However, if the Tmax of the test compound is not reached during the period of exploratory behavior, depressive effects on motor activity may not be adequately captured. Radiotelemetry technology represents an approach to avoid test system artifact. Using the antipsychotic haloperidol, a prototype compound to assess motor affects, at a sub-threshold dose (0.03mg.kg SC) motor activity was assessed during the light cycle using male Sprague-Dawley rats (Charles River Laboratories, Portage, MI) instrumented with telemetry devices (Data Sciences International, St. Paul, MN). The light cycle was then reversed and the circadian rhythm was allowed to adjust over a one week period. Treatment with haloperidol was then repeated during the dark cycle. No change in activity could be detected during the light cycle (animal's resting phase) as activity measured less than 1 count/minute. However, treatment with haloperidol during the dark cycle (animal's active phase) resulted in a 77% decrease in activity, from 4.8 counts/min to 1.1 counts/min. This decrease in activity was observed 2-4 hours post-dosing. Based on the results of this study we determined that consideration should be given to the animal's circadian rhythm and time of administration when evaluating CNS effects of novel small molecules.

985 EVALUATION OF STATISTICAL METHODS TO ANALYZE MOUSE LYMPHOMA ASSAY MUTATION DATA.

M. Moore[1], J. Clements[2], R. Delongchamp[1], A. Thakur[3] and B. Myhr[3]. *[1]NCTR, Jefferson, AR, [2]Covance, Harrogate, United Kingdom and [3]Covance, Vienna, VA.*

The Mouse Lymphoma Assay (MLA) Workgroup of the International Workshop on Genotoxicity Tests (IWGT) is currently developing a new recommendation for the analysis of data. To evaluate the performance of statistical methods the group utilized 398 experimental data from 10 laboratories. Twenty-seven statistical methods were applied to the data sets. For the statistical analysis the dependent variable was defined by either the mutant frequency (MF), log(MF) or rank(MF); the independent variables were either dose, dose categories, dose + dose^2, log(dose) or rank(dose); and the weight was either none or variance of the dependent variable. The presence of a dose response was evaluated by testing for a linear trend and/or a quadratic trend. In studies where there were more than single determinations of each data point, an analysis of variance was performed. When a P-value of less than 0.05 was used to determine whether an experiment was positive or negative, the statistical methods agreed for approximately 40% of the data sets. For the other 60% of data sets the methods gave different positive/negative calls. Graphical representations of the 398 experiments were used to classify the data into various curve shapes. A small subset (about 10%) of the data gave curve shapes that clearly defined positive responses (large dose-related increases in MF), while the rest of the data fell into categories that showed small dose-related increases, no dose-related increase or where it was difficult to visually discern a positive trend. It was this latter set of experiments where the statistical methods gave the most divergent determinations. In these situations the choice of statistical method would determine whether a response was positive or negative. Thus the Workgroup could not recommend a specific statistical method for MLA data analysis. However, because a uniform method is needed for MLA data analysis, the Workgroup is currently evaluating an approach that combines statistical analysis with the variability of the solvent/negative control MF.

986 APPLICATIONS OF AUTOMATED DIGITAL MICROSCOPY IMAGING IN INVESTIGATING MECHANISMS OF TOXICITY.

X. Ying, J. Dwyer, R. A. Schuhl, T. M. Monticello, Z. Jayyosi and P. S. Rao. *Drug Safety Evaluation, Aventis, Bridgewater, NJ.*

Microscopy imaging analysis has been used for the study of apoptosis, mitochondrial dysfunction, and oxidative stress. Our goal was to develop and validate multi-endpoint assays using automated digital microscopy imaging to detect potential toxic liabilities, based on the mechanism of toxicity, in early drug development. Rat primary hepatocytes were treated with model toxicants affecting key biochemical pathways of apoptosis, mitochondrial health, and oxidative stress. Following 24 hours of treatment, the cells were loaded with fluorescent probes, propidium iodide, Hoechst 33342, YO-PRO-1, JC-1 and H2DCFDA, to measure cellular cytotoxicity, early and late-stage apoptosis, mitochondrial membrane potential, and oxidative stress, respectively. The cells with cellular/subcellular fluorescent probes were imaged utilizing automated digital fluorescence and/or brightfield microscopy, with laser microscopic auto-focusing, 12-bits cooled CCD digital camera and Imaging Research's AutoLead software, which ensured high data accuracy in the assessments. Various image analysis toolkits were utilized for image analyses and data processing. All assays were validated with model toxicants. For example, in the apoptosis assay, staurosporine and cyclosporine were used to induce apoptotic bodies which were assessed with Hoechst 33342 staining. Valinomycin was used as a positive control for loss of mitochondrial membrane potential that was detected as a change in aggregate formation using JC-1 dye. Hydrogen peroxide was used to induce reactive oxygen species formation in the oxidative stress assay with H2DCFDA. The validation studies indicate that automated microscopy imaging can be used in mechanistic assessment of toxicity in a chemical series to detect potential liabilities early in the drug development process. The applications also highlight the value of automated digital microscopy for toxicity assessment with high content and sufficient throughput.

987 SAFETY EVALUATION OF SMALL MOLECULES USING RADIOTELEMETRY IN RATS.

M. Gallacher[1], K. Cardoza[1], C. Doherty[2], C. Alden[2], D. Tumas[2] and V. J. Kadambi[2]. *[1]Comparative Medicine, Millennium Pharmaceuticals, Inc., Cambridge, MA and [2]Drug Safety and Disposition, Millennium Pharmaceuticals, Inc., Cambridge, MA.*

Non-clinical testing strategies predict with a high degree of confidence functional perturbations of small molecules prior to clinical testing. Radiotelemetry in conscious, freely moving animals offers a humane way of measuring adverse cardiovascular, respiratory and thermoregulation effects which may have relevance to human safety. We validated the use of a radiotelemetry system (Data Sciences International, St. Paul, MN) in male Sprague-Dawley rats (Charles River Laboratories, Portage, MI) at our facility using reference compounds oxotremorine, scopolamine, captopril and phenylephrine to measure effects on blood pressure, heart rate, core body temperature and baroreceptor reflex. Telemetered male Sprague-Dawley rats received subcutaneous administration of oxotremorine (0.2mg/kg). Methyl scopolamine (1.0mg/kg) was co-administered to block oxotemorine's peripheral effects. This central muscarinic stimulation resulted in elevated blood pressure (+30mmHg) and heart rate (+148BPM) with a decrease in body temperature (-2°C) that persisted 6 hours post administration. Captopril, an angiotensin converting enzyme inhibitor, was administered orally at dosages of 25mg/kg and 50mg/kg resulting in a 23 and 27mmHg decrease in mean arterial pressure respectively. Phenylephrine, an α-adrenergic receptor agonist, was administered intravenously through a jugular vein catheter in incremental doses of 1-15ug/kg to determine the effects on baroreceptor reflex sensitivity. Phenylephrine administration resulted in an acute and reversible increase in mean arterial pressure, with a concomitant reflex decrease in heart rate *via* stimulation of the baroreceptors. We concluded from our studies that radiotelemetry accurately captures physiologic data and is a valuable tool for assessing safety of novel small molecules.

988 MECHANISM FOR SHORTENING PT AND APTT IN DOGS AND RATS: EFFECT OF FIBRINOGEN ON PT AND APTT.

M. Kurata, Y. Sasayama, N. Yamasaki, I. Kitazawa, Y. Hamada and I. Horii. *Worldwide Safety Sciences, Pfizer Global Research & Development, Nagoya Laboratories, Pfizer Inc., Taketoyo, Aichi, Japan.*

Although shortened prothrombin time (PT) and activated partial thromboplastin time (APTT) are frequently observed in pre-clinical toxicity studies, it has not been fully explored. In recent studies on human clinical pathology, shortened APTT was reported to associate with increased coagulation factors, and to be possibly useful to predict thrombosis. It is significant to investigate the mechanism for shortening PT and APTT in laboratory animals for the drug safety evaluation. The present study was therefore undertaken to investigate the mechanisms for shortening PT and APTT, using citrated plasma from dogs and rats *in vitro*, especially with a focus on increasing fibrinogen levels. For the method, PT and APTT were measured in plasma from dogs and rats, under various concentrations of fibrinogen. Clottable activity of fibrinogen was confirmed by measuring the thrombin time (TT). Furthermore, thromboelastogram (TEG) was used to evaluate the hypercoagulable status. As the results, (1) when purified canine fibrinogen was added to citrated canine plasma at the final concentrations of 2, 4 and 8 mg/mL (as added fibrinogen concentration), PT and APTT were significantly shortened. An increased clottable fibrinogen in the test tube was confirmed by a markedly shortened TT. Canine fibrinogen at a final concentration of 4 mg/mL induced an increased rate of clot formation and increased the maximum amplitude on the TEG, indicating a state of hypercoagulation. (2) In the case of citrated rat plasma, while purified rat fibrinogen had no effect on PT or APTT at the final concentrations of 2, 4 and 8 mg/mL, it did shorten TT. The TEG also showed hypercoagulation at a fibrinogen concentration of 1 mg/mL in rats. The present results indicate that an increased concentration of fibrinogen is associated with shortening PT and APTT in dogs, but not in rats.

989 A FLOW CYTOMETRIC ASSAY THAT PERMITS INTEGRATION OF CHROMOSOMAL DAMAGE ASSESSMENT WITH ROUTINE TOXICITY TESTING.

J. T. MacGregor[1], M. E. Bishop[2], S. Dertinger[3], J. McNamee[7], M. M. Moore[2], A. Aidoo[2], S. Harper[4], R. Frobish[5] and M. Hayashi[6]. *[1]USFDA NCTR, Rockville, MD, [2]USFDA NCTR, Jefferson, AR, [3]Litron Laboratories, Rochester, NY, [4]USFDA CFSAN, Laurel, MD, [5]USFDA CVM, Laurel, MD, [6]NIHS, Tokyo, Japan and [7]Health Canada, Ottawa.*

The erythrocyte micronucleus assay, a "core" regulatory assay for assessment of genetic damage, is generally based on microscopic evaluation of micronucleated (MN) bone marrow (BM) reticulocytes ("retics") because the spleen removes MN erythrocytes from the peripheral circulation in most species. We evaluated a flow cytometric method (Dertinger et al., Mutat. Res. 464: 195-200, 2000) that minimizes the effect of splenic selection in the rat, dog, and human, and requires only µl quantities of peripheral blood (PB). Young retics are labeled with FITC-anti-CD71 (transferrin receptor) and micronuclei with propidium iodide (with RNase treatment). We compared this method with microscopic scoring using standard or supravital acridine orange staining, using samples from normal and splenectomized Sprague-Dawley rats treated with a clastogen (cyclophosphamide, CP) or a spindle disrupter (vinblastine). We also report data that demonstrate the sensitivity of the flow cytometric assay in the beagle dog and human. In rats, the flow method did not eliminate the spleen effect on induced MN retic frequency, but spontaneous MN retics were found to be relatively insensitive to the spleen effect. Thus, effects on splenic efficiency would not lead to false positive responses. In eusplenic animals the dynamic range of induced effects was smaller in PB than in BM, but the flow method had superior counting statistics, lower variability, and higher sensitivity than manual scoring. MN retics derived from fragments or whole chromosomes (CP- vs. vinblastine-induced) behaved similarly. With appropriate validation, it appears flow cytometric assessment of micronucleus induction can be integrated into routine testing, eliminating the need for a separate bioassay.

990 EFFECTS OF SUBCHRONIC DERMAL APPLICATION OF BREAK-FREE CLP® IN CD-1 MICE.

D. P. Arfsten[1], A. R. Thitoff[1], E. W. Johnson[1], A. Jung[1], W. W. Brinkley[2], D. Schaeffer[3] and K. R. Still[1]. *[1]Naval Health Research Center Toxicology Detachment, Wright-Patterson AFB, OH, [2]Operational Toxicology, Air Force Research Laboratory, Wright-Patterson AFB, OH and [3]Department of Veterinary Biosciences, University of Illinois at Urbana Champaign, Urbana, IL.*

Studies were conducted to determine the biological effects of subchronic dermal application of Break-Free CLP to the clipped backs of CD-1 mice. Break-Free CLP® is a military and commercial weapons cleaning compound. Groups of 20 CD-1 mice were treated topically with Break-Free (neat), deionized H_2O, 2.5% croton oil (CO) in acetone (positive control), and 99% acetone (vehicle control). Materials were applied to a clipped 2 x 3 cm application site located directly behind the shoulder blades, 50 µL/application, 3 times/week for 90 days. Application sites were left uncovered after treatment; the application site was clipped free of hair once a week every 2 weeks. Mice were sacrificed on Day 90 and histopathology and clinical chemistry profiles were compared between treatment groups. Moderate, persistent erythema occurred in greater than 50% of mice treated with CO; 25% of mice treated with Break-Free developed moderate and persistent erythema. Edema was evident for animals treated with CO but not for animals treated with Break-Free. Serum alkaline phosphatase (ALKP) activity was significantly decreased in animals treated with Break-Free and CO; mean serum glucose was significantly lower for animals treated with CO versus controls. Percent lymphocytes were significantly lower and percent segmented lymphocytes were significantly elevated for animals treated with Break-Free or CO. Histopathological analysis of application site skin sections showed that hyperplasia, hyperkeratosis, and moderate inflammatory cell infiltration was predominate for animals treated with Break-Free or CO; some ulceration was also evident and occurred more frequently for animals treated with CO. mRNA expression analysis of skin and liver sections from animals treated with Break-Free identified a 2-fold or greater increase in gene products involved in irritation response and cell cycle progression.

991 SUBCHRONIC TOXICITY 90-DAY ORAL GAVAGE STUDY OF 8-2 TELOMER B ALCOHOL IN RATS.

G. S. Ladics[1], G. L. Kennedy[1], J. O'Connor[1], N. Everds[1], S. R. Frame[1], S. Gannon[1], R. Jung[2], H. Iwai[3] and S. Shin-ya[4]. *[1]DuPont Haskell Laboratory, Newark, DE, [2]Clariant, GmbH, Sulzbach, Germany, [3]Daikin Industries, Ltd.,, Osaka, Japan and [4]Asahi Glass Co., Ltd.,, Tokyo, Japan.*

8-2 Telomer B Alcohol is a fluorinated chemical intermediate used to manufacture specialty polymers and surfactants. The potential subchronic toxicity and the reversibility of the effects of this chemical were evaluated following approximately 90 days of oral gavage dosing to Crl:CD®(SD)IGS BR rats. A complete toxicological profile including neurobehavioral assessments and hepatic β-oxidation were conducted at selected intervals and a group of rats was included for a 90-day post-dosing recovery period. Dose levels tested were 0 (control), 1, 5, 25, and 125 mg/kg/day. No test-substance-related mortality occurred. Rats at 125 mg/kg/day developed striated teeth such that these animals were switched to ground chow on test day 77. No treatment-related alterations in body weight, food consumption, neurobehavioral parameters, or hematology/clinical chemistry were found. Hepatic β-oxidation was increased in males at 125 mg/kg/day and in females at 25 mg/kg/day. In both males and females, plasma fluoride levels were significantly increased at 125 mg/kg/day and urinary fluoride was elevated at ≥ 5 mg/kg/day. Degeneration/disorganization of enamel organ ameloblast cells was observed in males, but not females at 125 mg/kg/day. Liver weight increases accompanied by focal hepatic necrosis were seen at both 25 and 125 mg/kg/day in males and

chronic progressive nephrotoxicity occurred in females administered 125 mg/kg/day. With the exception of the hepatocellular necrosis observed in males administered 125 mg/kg/day and the incidence and severity of chronic progressive nephropathy in the 125 mg/kg/day female group, which increased after 3 months, all other changes showed evidence of reversibility. The no-observed-adverse-effect level for the 90-day study was 5 mg/kg/day. This study was sponsored by the Telomer Research Program.

992 SAFETY OF TINOSORB® M ACTIVE, A NEW SUNSCREEN INGREDIENT FOR BROAD SPECTRUM UV PROTECTION.

W. F. Salminen[1] and J. R. Plautz[2]. *[1]Product Safety and Regulatory, Ciba Specialty Chemicals Corporation, High Point, NC and [2]PSR, Ciba Specialty Chemicals, Basel, Switzerland.*

Tinosorb® M Active (MBBT; CASRN 103597-45-1: 2, 2'-methylenebis-(6-(2H-benzotriazol-2-yl)-4-(1, 1, 3, 3-tetramethylbutyl) phenol)) is a new active sunscreen ingredient that provides broad spectrum UVA and UVB protection and is very photostabile. MBBT is currently approved for use as an active sunscreen ingredient in the European Union, Switzerland, Australia, and many other countries with the major exception being the US due to the high regulatory hurdles for registering new active sunscreen ingredients. We present here the results showing MBBT to be safe as an active sunscreen ingredient when tested in standard (OECD) protocols. MBBT is of low acute toxicity with rat dermal and oral LD50 values >2, 000 mg/kg. MBBT caused minimal irritation when applied to rabbit eyes and skin. MBBT did not cause skin sensitization, photoirritation, or photosensitization when applied to the skin of guinea pigs or human volunteers. MBBT was not genotoxic or mutagenic in standard *in vitro* and *in vivo* assays and in tests using UV exposure protocols. In an *in vitro* percutaneous absorption assay, MBBT exhibited low penetration (0.14%) across human skin. Rats given doses of radiolabelled MBBT showed very low systemic uptake after a single oral dose (<1% of dose) or after dermal dosing (<1% of dose). In 90-day oral gavage and dermal dosing studies in the rat the NOEL was 1, 000 mg/kg/day, which was the highest dose tested (HDT) in each study. In a developmental toxicity study, the maternal and fetal NOEL was 1, 000 mg/kg (HDT). MBBT did not show binding affinity or competitive inhibition of natural hormone binding to either estrogen or androgen receptors *in vitro* and effects did not occur in the uterotrophic assay with immature rats given MBBT orally. Additional safety tests are currently underway to assess the long-term photo(co)carcinogenicity and dermal carcinogenicity of MBBT. In conclusion, MBBT is a safe UV filter sunscreen ingredient that can be used to make highly photostabile, broad-spectrum sunscreens.

993 VAPOR INHALATION TOXICITY STUDY OF HEXAMETHYLCYCLOTRISILOXANE IN THE RAT.

P. A. Jean, J. M. Tobin and K. P. Plotzke. *Dow Corning Corporation, Auburn, MI.*

Hexamethylcyclotrisiloxane (D3), a low molecular weight silicone used primarily as an intermediate in the synthesis of other silicone materials, was evaluated in an enhanced repeated-exposure toxicity study based on OECD Test Guideline #422 and USEPA Test Guideline 870.360. The study design included reproductive, developmental, and neurotoxicity screening assays. Male and female Sprague-Dawley rats were exposed by whole-body inhalation to 0, 100, 500, and 2500 ppm D3 vapor (6 h/day) for up to 39 days. Exposure to D3 vapor was well tolerated. Hepatomegaly and centrilobular hepatocellular hypertrophy was observed in both sexes exposed to 2500 ppm D3. Protein droplet nephropathy was diagnosed in males exposed to 500 and 2500 ppm D3. An increase in kidney weight and other changes in clinical chemistry parameters were consistent with this finding. In males exposed to 2500 ppm D3 there was a decrease in absolute epididymal weight (10%) and in the absolute and relative seminal vesicle weight (≈30%). Slight seminal vesicle atrophy was identified in 40% of the males. The testes and epididymides were histologically normal. Female rats exposed to 2500 ppm D3 demonstrated decreased litter size (33%), litter weight (27%) and implantation site number (33%). Exposure to 2500 ppm D3 reduced locomotor activity in both sexes and reduced reactivity to handling in females. This study has demonstrated that exposure to high D3 vapor concentrations can elicit adaptive (liver), pathologic (protein droplet nephropathy) and functional/behavioral (reproductive capacity and locomotor) effects in Sprague-Dawley rats. (Study Sponsor: Silicones Environmental, Health and Safety Council of North America)

994 CHRONIC TOXICITY ASSESSMENT OF GENISTEIN IN SPRAGUE DAWLEY RATS.

B. Delclos[1], C. Weis[1], D. Doerge[1], G. Olson[2], R. Trotter[2], N. Sadovova[2] and R. Newbold[3]. *[1]NCTR, Jefferson, AR, [2]Pathology Associates, Jefferson, AR and [3]NIEHS, Research Triangle Park, NC.*

Genistein is the major soy isoflavone and has been of interest as a possible beneficial agent in cancer prevention and the alle*via*tion of adverse consequences of menopause. High human exposures can occur as a result of consumption of soy infant formula and dietary supplements. In the present experiment, 5, 100, and 500 ppm genistein aglycone were administered in a soy- and alfalfa-free diet (Purina 5K96) to Sprague-Dawley rats during three exposure windows: Group 1, conception to 2 years; Group 2, conception to 140 days, followed by control diet to 2 years; Group 3, conception to 21 days, followed by control diet to 2 years. Exposure was monitored by serum genistein measurements in separate animals and found to be considerably lower during the nursing period than during the *in utero* or direct ingestion periods. Body weight gain in females was depressed in the 500 ppm group during periods of genistein consumption, but recovered when the compound was removed. Body weight depression was less in males than females, and there were no treatment-related gross or microscopic lesions in the aged males. The occurrence of aberrant estrous cycles with age started earlier than controls at 500 ppm in Groups 1 and 2 and at all doses in Group 3. Histopathological assessment of lesions, which is pending final review, indicated, as expected for Sprague-Dawley rats, a high spontaneous incidence of benign and malignant mammary tumors in control females and high incidences of pituitary adenomas in control animals of both sexes. There was no treatment effect on pituitary tumors in either sex. Apparent treatment-related effects were a reduction of female mammary gland fibroadenomas at 500 ppm in Group 1 and a marginal increase in mammary gland adenocarcinomas at 500 ppm in Groups 1 and 3. The relevance of these findings for humans consuming high levels of soy-derived products warrants further consideration.

995 HEPAVIR B, A CYP3A4-ACTIVATED PRODRUG OF PMEA, SHOWED BETTER SAFETY THAN HEPSERA IN PRE-CLINICAL STUDIES.

D. Vitarella, S. Dadgostari and C. Lin. *Drug Dev., ICN Pharmaceuticals, Inc., Costa Mesa, CA.*

Hepsera (adefovir dipivoxil), a plasma esterase-activated prodrug of PMEA has been recently approved for the treatment of hepatitis B virus (HBV). However, its recommended dose is sub-optimum due to dose limiting nephrotoxicity. Hepavir B is also a prodrug of PMEA. It is metabolized primarily by CYP3A4 in the liver, but not in the kidney, resulting in 4-fold distribution of PMEA into the liver vs. kidney. Thus, hepavir B may be safer than hepsera. The safety of Hepavir B in general toxicity, genetic toxicity, and safety pharmacology studies was determined. A 28-Day study was conducted in rats at oral doses of 0-300 mg/kg and in monkeys at doses of 0-180 mg/kg. Genetox was evaluated in the Ames, mouse lymphoma, chromosome aberration assay in CHO and human lymphocytes, and in a mouse micronucleus test. Safety pharmacology evaluation included neuropharmacology in rats, cardiovascular in monkeys, renal in rats, respiratory in rats, and GI in mice. The 28-day study in rats demonstrated renal and hepatic effects in males only at hepavir B doses of ≥100 mg/kg. No toxicity was seen in females given up to 300 mg/kg. In contrast, a 28-day study in monkeys demonstrated acceptable safety at doses up to 60 mg/kg. Overall clinical pathology, cardiovascular, ophthalmic, and gross pathology were normal. Previously published data on hepsera indicate toxicity at doses of 12 mg/kg in rats and 8 mg/kg in monkeys. Hepavir B was marginally positive in the lymphoma assay and the CHO test. However it was negative in the Ames test, in the human lymphocyte assay and the micronucleus test in mice. Hepavir B was well tolerated by animals in safety pharmacology evaluations. In conclusion, Hepavir B was well tolerated in rats up to 30 mg/kg, and monkeys up to 60 mg/kg. While the chromosome aberration assay and mouse lymphoma assay were equivocal to positive for genotoxicity, the *in vivo* mouse micronucleus assay was negative. Hepavir B was well tolerated in safety pharmacology Studies. Compared to published data on Hepsera, hepavir B appears to be a safer candidate for HBV treatment.

996 INTRAPERITONEAL ADHESION FORMATION FOLLOWING IMPLANTATION OF HERNIA REPAIR GRAFT MATERIALS:COMPARISONS OF PERMACOL™ WITH SURGIPRO® AND SURGISIS GOLD™ IN THE RAT.

S. L. Saynor[1], G. Hale[1], S. Bloor[2] and C. Curtis[2]. *[1]Covance Laboratories Ltd., Harrogate, United Kingdom and [2]Tissue Science Laboratories Plc, Leeds, United Kingdom.* Sponsor: D. Everett.

Hernia repair grafts commonly used in abdominal surgery can cause potentially dangerous adhesions. A novel biomaterial, a cross-linked stabilised porcine dermal implant (Permacol™ Tissue Science Laboratories Plc) is marketed in the US and Europe for hernia repair and is currently undergoing further investigation to demonstrate its effectiveness in comparison with existing products. Two separate studies in an experimental rat model assessed intraperitoneal adhesion formation following implantation of Permacol™ and compared the performance of two currently marketed repair materials, SurgiPro® and Surgisis Gold™. Under anaesthesia, 1.5 cm x 1 cm samples of Permacol™, Surgipro® and Surgisis Gold™ were sutured to the left and right inner abdominal wall of Sprague-Dawley rats following

a midline incision. After 1 and 3 months, the rats were sacrificed and the extent and severity of abdominal adhesions was assessed using a scoring system (0 to 3). In all cases the Permacol™ scores were significantly lower than the SurgiPro® and Surgisis Gold™ scores ($P<0.05$). Sections through the repair materials were examined histologically. Histology of the Permacol™ sites at 1 and 3 months showed cell infiltration across the implant following the natural pores in the material and 'keying' of the Permacol™ into the peritoneum. SurgiPro® sites on both occasions had a classical foreign body reaction with encapsulation and gross adhesions to bowel. Histology of the Surgisis Gold™ showed various degrees of fragmentation, the original collagen being separated by the inflammatory reaction and cystic spaces. These results demonstrated that adhesion formation was not as frequent or as severe when Permacol™ was sutured to the inner abdominal wall.

997 INTRODUCTION OF THE *IN VIVO* MINI-TOX STUDY AS AN EFFECTIVE TOOL TO IMPROVE CONFIDENCE IN SAFETY IN THE EARLY STAGE OF DRUG DEVELOPMENT.

Y. Sato, M. Nagata, T. Mako, H. Yamada and I. Horii. *Worldwide Safety Sciences, PGRD Nagoya Laboratories, Pfizer Inc., Aichi, Japan.*

Pharmaceutical companies are frequently required to perform a variety of toxicity assays in the early stages of drug development. This presentation will show a novel *in vivo* short-term toxicity assay called the *in vivo* mini-Toxicology study, which was developed at our Nagoya laboratories. In utilizing standard clinical administration routes, normal toxicity studies require relatively large amounts of bulk and large numbers of test animals. However, often only limited exposure to a test compound is required to detect an adverse effect(s) in an *in vivo* study, which is important when dealing with limited, precious bulk. Using jugular-vein cannulated (JVC) rats allows blood for toxicokinetics (TK) evaluation to be collected through the cannula for oral administration or s.c./i.p. injection studies and also allows i.v./infusion administration to be performed with relatively little stress to the test animal. In addition to routine toxicity study items, we can add special examinations to the *in vivo* mini-Toxicology studies in consideration of unique properties of a test compound. Among them we introduce two optional batteries of non-standard test items, which can capture other relevant data simultaneously. The first of these involves neurobehavioral examinations based on a functional observation battery (FOB) and/or locomotor-activity quantitation called Motor Activity. The second is a cardiovascular examination using non-heating tail cuff-type sphygmomanometry, which was recently developed in Japan (BP monitor for mice & rats Model MK-2000, Muromachi Kikai Co., LTD.). These special examination items can be invaluable in revealing any relationship between TK and functional or toxicity changes that may be present at the onset of compound toxicity, which allows greater ability to interpret *in vivo* results. The presentation will illustrate the items employed in the *in vivo* mini-Toxicology study, as well as data obtained from validation studies using chlorpromazine, scopolamine, R-+-Win55, 212-2 and norepinephrine in JVC rats.

998 IS 20% INHIBITION OF HERG CURRENT SUFFICIENT FOR PREDICTING FOR *IN VIVO* QT INTERVAL PROLONGATION.

C. Doherty, C. Claiborne, T. Glyptis, M. Holmqvist, M. Stewart, P. Eddy, V. Sasseville, C. Alden and V. Kadambi. *Millennium Pharmaceuticals, Inc., Cambridge, MA.*

The recent withdrawal of several compounds from the market has led to a call for early testing of compounds for potential to prolong the QT interval *in vivo*. Inhibition of I_{Kr}, the major current responsible for ventricular repolarization, leads to prolongation of the QT interval and is associated with Torsades de Pointes (TdP). Binding to the HERG (human ether à go-go related gene) channel by small molecules has been linked to *in vivo* QT prolongation and TdP in humans. A competitive binding assay, utilizing membranes from HEK 293 cells stably transfected with the HERG channel cDNA and 3H-dofetilide, provides a method of screening for interactions with the HERG channel. Inhibition curves, determined by test compound inhibition of 3H-dofetilide binding, were used to determine both IC_{50} and IC_{20} values. $K_{0.5}$ results from this binding assay showed a good correlation with published electrophysiology values (R2= 0.83, 36 marketed compounds). Traditionally, assessment of HERG inhibition and prediction for *in vivo* QT prolongation has relied on IC_{50} values. A recent manuscript by Redfern et al. (Cardiovas Res, 2003) used ratios of electrophysiology IC_{50} and unbound therapeutic plasma concentration to categorize marketed compounds and correlate safety ratios with clinical incidences of QT interval prolongation and TdP. Calculation of safety ratios using binding $K_{0.5}$ and $K_{0.2}$ categorized marketed compounds in a similar manner. The HERG binding and electrophysiological methods identified false positive and negative compounds, such as verapamil and amiodarone respectively, with similar efficiencies. These results suggest that 20% inhibition of binding to the HERG channel is a sensitive predictor for compounds to prolong the QT interval *in vivo*.

999 THE COMMON MARMOSET (CALLITHRIX JACCHUS) AS A MODEL IN TOXICOLOGY: REFERENCE CONTROL DATA AND COMPARISON WITH MACAQUES.

S. Korte, University. Zuehlke, J. Kaspareit, S. Friedrichs-Gromoll, W. Mueller, F. Vogel and G. Weinbauer. *Covance Laboratories GmbH, Muenster, Germany.*
Sponsor: P. Thomas.

Marmosets have become an important nonhuman primate model as an alternative primate species in the safety evaluation of new drugs (1). Based upon small body size, marmosets have gained particular value for toxicity studies with biotechnology products where compound supply is limited, and there is regulatory acceptance for this species (2). Here we present reference values and ranges for a large array of relevant diagnostic parameters for the normal male and female marmoset, and a comparison is made to macaque monkeys. These reference values are essential for the analysis and interpretation of findings during toxicity studies in the marmoset model. Overall, data were compiled from more than one thousand animals. Among the parameters collected were mortality and morbidity, body and organ weights, clinical chemistry and urine analysis, hematology, cardiology and blood pressure, ophthalmoscopy, respiration, neurology. reproductive physiology and the incidence of spontaneous histopathological lesions. When compared to macaques, marmosets are more sensitive to stress and mortality rate unrelated to test article administration was 2-3%. Digestive problems with secondary body weight loss were seen as the major cause of death, followed by acute infections following minor injuries. Respiratory rate was about two fold and heart rate about 2-3 fold higher than in macaques. Liver enzyme activity was 10-14 fold lower in the marmoset. Further differences were seen for hematology parameters, electrocardiography data, ocular and histopathological background lesions. In summary, an extensive reference data base for a large number of relevant diagnostic parameters is available that supports the use of marmosets in toxicology. 1. Zuehlke University, G Weinbauer 2003 Toxicol Pathol 31:123-127; 2. Smith D, P Trennery, D Farningham, J Klapwijk 2001 Lab. Anim 35:117-130.

1000 DERMAL DOSING OF NEONATAL RATS: EFFECT OF MOTHER-INFANT SEPARATION AND OCCLUSIVE DOSING.

J. F. Barnett[1], D. B. Learn[1], A. M. Hoberman[1], T. G. Osimitz[2] and University. Vedula[3]. *[1]Charles River Discovery and Development Services, Argus Division, Horsham, PA, [2]Science Strategies, LLC., Charlottesville, VA and [3]S.C. Johnson & Sons, Inc., Racine, WI.*

Hazard identification studies for dermal application products intended for use in young children should be conducted using this exposure route and test systems of the same relative developmental status. Mother-Infant separation (MIS) prevents test substance (TS) ingestion by the mother and oral exposure *via* the mother's milk. An occlusive wrap fixes the TS in place and prevents oral ingestion from grooming. However, identification of any MIS and/or occlusion effects is critical. MIS and occlusive dermal dosing (six hours daily, PNDs 9 to 20) were used in four separate groups: pups remaining with the dam (Group I); pups separated, but untreated (Group II); separated, treated with water and occluded (Group III); separated, treated with TS and occluded (Group IV). Daily measurements included body weights and clinical observations. On PND 21, a Detailed Clinical Observation (DCO) evaluation was performed on each pup. No adverse clinical signs were observed. Body weight data indicated: 1) MIS alone had no effect on the pups (Group II v. I); 2) the occlusive procedure caused a statistically significant reduction in body weight gain (Group III v. II); 3) males receiving TS showed statistically significant reduction in body weight gain during PNDs 18 to 21, as compared with pups administered water (Group IV v. III). The use of MIS with occlusive dermal dosing allows precise TS exposure of young pups with only a slight reduction in body weight gain and without adverse clinical responses. This technique provides TS evaluation using age-appropriate test systems and minimizes inappropriate TS exposure.

1001 TAMPON RISK ASSESSMENT: A COMPREHENSIVE APPROACH.

A. E. Hochwalt. *Procter & Gamble, Cincinnati, OH.*

The safety of Tampax tampons is evaluated by a comprehensive, phased, risk assessment approach. Tampon risk assessment (TRA) builds on the principles of risk assessment by including vaginal and tampon-specific endpoints. The TRA assures that new tampons are safe as labeled and minimize the risk of Toxic Shock Syndrome (TSS). TRA begins with evaluation of new tampons to determine design, composition, and/or exposure modifications versus current tampons. TRA then guides the risk assessor to consider potential effects that may occur when using the modified tampon (effects on vaginal condition [vaginal abrasion, ulceration, or

laceration], vaginal microflora, risk of TSS, and local or systemic toxicity) and what data are necessary to support use of the tampon at each product development phase. Historical or newly generated *in vitro* or limited toxicity data support consumer studies. Robust clinical and toxicological data support commercialization. The assessment of Tampax Pearl tampons followed the TRA. Pearl uses the same materials with similar exposures as current Tampax tampons, but has a new shape and absorbent braid. Historical material safety and TSS epidemiology data supported consumer studies; commercialization required additional habits and practices, vaginal condition, and vaginal microflora data to address the design changes and confirmation that TSS risk should not be affected. Studies evaluated Pearl for potential to affect growth and cell density of sentinel vaginal microorganisms *in vitro*, growth of a TSST-1-producing strain of S. aureus and TSST-1 toxin production, vaginal condition, and vaginal microflora population and cell density (*via* in-use clinical studies and clinical assessment of normal vaginal microflora of the lower genital tract). The results of these studies indicated that Pearl did not promote or inhibit growth or cell density of vaginal microflora, promote TSST-1 toxin production, alter consumer habits, or adversely affect vaginal condition versus currently marketed tampons. Therefore consumers may use Tampax Pearl as safely as current Tampax tampons.

1002 COMPARATIVE REPEATED-DOSE TOXICITY OF LIPOSOME-ENCAPSULATED VINCRISTINE SULFATE AND FREE VINCRISTINE SULFATE IN RATS.

P. M. Tam[1], R. Oshanek[1], N. Yasuda[1], R. Namdari[1], A. Janse[1], O. Smals[1], L. Armer[2], J. Daniels[3] and C. Flowers[1]. *[1]Preclincal Development, INEX Pharmaceuticals Corporation, Burnaby, BC, Canada, [2]CTBR Bio-Research Inc., Senneville, QC, Canada and [3]Cantox Health Sciences International, Mississauga, ON, Canada.*

Liposomal vincristine (LV) is vincristine encapsulated into liposomes composed of sphingomyelin and cholesterol (55:45). It was designed to alter the PK and tissue distribution of vincristine (VCR) in order to achieve improved anti-tumor activity. LV is currently being evaluated for the treatment of multiply relapsed or refractory NHL disease. In the present study, the repeated-dose toxicity of LV was evaluated in Sprague-Dawley rats. Rats (15/sex/group) were administered weekly doses of LV (1, 2 and 3 mg/m^2) or VCR (2 mg/m^2) for a total of six treatment cycles. In addition, the liposomal carrier was evaluated by administration of drug-free liposomes corresponding to 3 mg/m^2 LV. LV induced dose-dependent toxicities that included body weight loss, peripheral neurotoxicity and toxicities to the bone marrow, developing dentition, and testes. No CNS toxicity was observed. Peripheral neurotoxicity was associated with secondary skeletal muscle injury. Dental toxicity was manifested as broken incisors that were associated with lesions to the abnormal dentition and lesions to ondontoblasts. Qualitatively VCR elicited the same toxicity profile as LV. However VSLI was less toxic to the bone marrow but was somewhat more toxic to the PNS. Toxicokinetic evaluation demonstrated that VSLI mediated significantly prolonged duration of exposure to vincristine than VCR. Drug-free liposomes were not toxic to rats at 60 mg/m^2. In conclusion, the toxicity of VSLI and VCR was qualitatively similar and any differences in the degree of toxicity were attributed to the prolonged duration of exposure mediated by LV.

1003 CONSTRUCTION OF A HUMAN ADVERSE EFFECTS DATABASE FOR MODELING QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS (QSARS).

N. L. Kruhlak[1], J. L. Weaver[2], R. Benz[1], J. F. Contrera[1] and E. J. Matthews[1]. *[1]USFDA, Rockville, MD and [2]USFDA, Laurel, MD.*

FDA/CDER's Spontaneous Reporting System (SRS) database contains reports of the post-market adverse effects of pharmaceuticals in humans not detected during pre-market trials. The database contains over 1.6 million ADR reports for 8620 drugs and biologics that are listed for 1191 Coding Symbols for Thesaurus of Adverse Reaction (COSTAR) terms of adverse effects. FDA/CDER's Informatics and Computational Safety Analysis Staff (ICSAS) has developed a database specifically for modeling quantitative structure-activity relationships (QSARs). The ICSAS Adverse Effects database links the trade names of pharmaceuticals to generic names and molecular structures for a subset of 1515 organic chemicals that are suitable for modeling with commercially available QSAR software packages. ADR reports from the SRS database are pooled for the first five years that a pharmaceutical was marketed. To estimate patient exposure during this period, the number of cases shipped for each pharmaceutical were used as a denominator, resulting in the creation of the Adverse Effect Index (AEI), where AEI = (# ADR reports/# cases shipped) × 1, 000, 000. We have identified the optimal conditions for defining a significant adverse effect by generating QSAR models for a subset of toxicologically related COSTAR endpoints using the MCASE/MC4PC software package in conjunction with a set of human expert data analysis rules. A pharmaceutical defined as active for a single COSTAR endpoint is characterized by ≥4 ADR reports, >20, 000 cases shipped and an AEI ≥4.0 during the first five years of marketing. Furthermore, test chemicals evaluated as active must contain a statistically significant structural alert, called a decision alert, at two or more toxicologically related endpoints. We also report the use of a combination QSAR module which pools observations from two or more toxicologically related COSTAR term endpoints to provide signal enhancement for poorly represented adverse effects.

1004 THE FERRET AS A MODEL OF EMETIC SENSITIVITY.

S. Mason, H. Penton and P. Mansell. *CTBR, Senneville, QC, Canada.* Sponsor: M. Vezina.

In order to provide an alternative to the established ipecac model in dogs for emesis research and testing of anti-emetic agents using smaller animals in a GLP compliant setting, an emetic sensitivity model was established in the ferret using several common emetic compounds with differing actions. Male ferrets (Mustela putorius furo), Marshall Farms, NY, approximately 1.2 kg were administered Cisplatin (10 mg/kg IV) or Ipecac Syrup USP (1 mL/kg PO) to assess an emetic response. Animals were food deprived overnight, and approximately 30 minutes prior to dosing, received ~50g commercially available cat food. Following dosing, animals were monitored and observed for episodes of emesis. The time prior to the first episode of retching or vomiting was recorded (latent period), together with the total number of retching and vomiting periods through the observation period. Episodes of retching and vomiting, characterized by rhythmic abdominal contractions with or without oral expulsion of solid or liquid material from the gastrointestinal tract were observed following both test article doses, the latent period for Cisplatin treated animals being significantly longer than that of Ipecac treated ferrets. The total number of retching and vomiting episodes for both sets of animals was similar, although resolution of effects was much earlier for Ipecac treated animals, consistent with its acute effects seen in the clinical setting. For assessment of anti-emetic activity, animals were treated and observed in the same manner as the emetic response assessment, however approximately one hour prior to vehicle/test article dosing received 0.5 mg/kg Ondansetron PO. Pretreatment with the 5-HT3 receptor antagonist Ondansetron completely inhibited the vomiting response to both Ipecac and Cisplatin, again consistent with the clinical use of this compound in treatment of emesis induced by chemotherapeutic agents. This model is therefore considered acceptable for use as an indicator of potential emetic or anti-emetic activity, in combination with an appropriate positive control.

1005 EFFECTS OF 3, 3', 4, 4'-TETRACHLOROBIPHENYL (PCB 77) ON THE DISTRIBUTION AND METABOLISM OF SELENIUM IN RATS.

D. N. Stemm[2], L. W. Robertson[3], J. C. Tharappel[1] and H. P. Glauert[1]. *[1]Grad. Center for Nutritional Sciences, University of Kentucky, Lexington, KY, [2]Graduate Center for Toxicology, University of Kentucky, Lexington, KY and [3]Department of Occupational and Environmental Health, University of Iowa, Iowa City, IA.*

Selenium is an essential element that may influence the development of cardiovascular diseases and cancer. Environmental contaminants, like polychlorinated biphenyls (PCBs), produce oxidative stress, reduce selenium utilization, and affect antioxidant systems, including glutathione peroxidase, a selenoprotein. PCB 77, a dioxin-like, coplanar PCB, has been shown to reduce glutathione peroxidase activity in the liver. The aim of this study was to determine the effect of PCB 77 on the metabolism and distribution of selenium. Following PCB 77 i.p. bolus dose administration (300 µmole/kg), the daily excretion and 21-day post treatment tissue distribution of selenium in Sprague-Dawley rats were examined. We measured selenium levels in tissues (liver, kidney, heart, brain, spleen, thymus, adrenal, muscles, and intestine), urine, and feces using graphite furnace atomic absorption spectrometry after microwave digestion of samples. Selenium concentrations in the heart and adrenal tissues of the PCB 77 treated group were increased while in the other tissues, selenium concentrations were not significantly different from the control group. Interestingly, the selenium level in the liver at 21 days after PCB 77 treatment was not affected. Daily urinary selenium levels were significantly decreased from Day 1 to Day 9 following PCB 77 treatment, after which selenium excretion increased to levels similar to the control group except for Day 17, when selenium excretion was increased. These results indicate that PCB 77 affects urinary selenium excretion patterns and may be influencing distribution of selenium in tissues. (Supported by ES 97380 and ES 07266 from NIH).

1006 DEVELOPMENT OF SOFTWARE THAT INTERFACES WITH THE FOOD ANIMAL RESIDUE AVOIDANCE DATABANK TO ESTIMATE EXTENDED WITHDRAWAL INTERVALS FOR VETERINARY DRUGS.

R. Gehring, J. Wang, X. Lou, R. E. Baynes and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Estimation of extended withdrawal intervals following extralabel use of veterinary drugs requires knowledge of label withdrawal times, tolerances for marker residues and rate of depletion of marker residues from target tissues. This information is

contained in databases of the Food Animal Residue Avoidance Databank (FARAD). A software application was developed to interface with these databases and extract necessary information, which is then entered into an algorithm to calculate an extended withdrawal interval by extrapolation of the drug's depletion curve. Ideally, the elimination half-life of the drug is extracted from the FARAD database, but pharmacokinetic data is not available for all drugs or multiple pharmacokinetic studies have been performed, resulting in numerous database entries. The algorithm offers two options for the calculation of extended withdrawal intervals. The first option (Estimator A) uses actual tissue or plasma half-lives. The second option (Estimator B) estimates the tissue half-life from the label withdrawal time by assuming that 5 half-lives are required for the concentration of most drugs to deplete to tolerance levels. The software was used to calculate extended withdrawal intervals for selected antimicrobial drugs. Estimators A and B gave identical results for drugs with shorter label withdrawal times (<8 days) (n=8). For drugs with longer label withdrawal times, Estimator A gave more conservative estimates for all drugs except spectinomycin (>10 days) (n=5). The software is a useful tool for extracting information from various FARAD databases. Application of an algorithm ensures that reliable and repeatable extended withdrawal intervals are calculated considering all available pharmacokinetic data. Further research is required to develop and validate methods to meta-analyze pharmacokinetic data for drugs with multiple database entries. (Supported by USDA/CSREES 2002-45051-01362)

1007 EVALUATION OF THE SUBCHRONIC TOXICITY OF A TRANSGENIC MAIZE.

I. Lamb[2], S. A. MacKenzie[1], L. A. Malley[1], N. E. Everds[1] and J. F. Hansen[2]. *[1]DuPont Haskell Laboratory, Newark, DE and [2]Pioneer Hi-Bred International, Inc., Johnston, IA.*

Five groups of young adult male and female Crl:CD®(SD)IGS BR rats (12/sex/group) were administered diets containing 33% TC1507 (transgenic), 33% 33P66 (non-transgenic, near-isogenic control), 33% 33J56 (non-transgenic, commercial), 11% TC1507, or 11% 33P66 maize for approximately 90 days. All diets contained comparable levels of proximate analytes, fiber/energy, amino acids, minerals, vitamins, and heavy metals. The transgenic protein, Cry1F, was only detected in the 33% and 11% TC1507 diets, using Cry1F ELISA and/or European corn borer (ECB) bioassay. Analysis of these diets near the beginning and end of the study demonstrated that Cry1F was stable over the course of the study. No diet-related differences were observed among groups fed the different diets, with respect to clinical signs, ophthalmological observations, neurobehavioral assessments, clinical pathology (hematology, clinical chemistry, coagulation, or urinalysis parameters), organ weights, and gross or microscopic pathology. Statistically significant lower food consumption was observed in male rats fed 33% 33P66, compared to those fed 33% TC1507, but was not considered toxicologically significant as there were no meaningful differences between these groups in body weight gain or food efficiency. Under the conditions of this study, exposure of male and female rats to diets containing a transgenic strain of maize (TC1507) produced no adverse effects, compared to rats fed diets containing a non-transgenic, near isogenic strain of maize (33P66) or a non-transgenic, commercial strain of maize (33J56).

1008 HEALTH HAZARD ASSESSMENT FOR PEANUT ALLERGY.

S. A. Assimon and P. M. Bolger. *CFSAN, USFDA, College Park, MD.*

About 0.6 % of the general population in the US is allergic to peanut. For those affected, it is most often a persistent, life-long affliction. The hazards of exposure to peanut in this sensitive group can be serious and significant. The allergic response involves signs and symptoms associated with a number of physiological sites and systems. Severe reactions begin within minutes after peanut ingestion and can progress very rapidly. Allergy to peanut is the major cause of reported fatal and near-fatal anaphylaxis reactions to food. Avoidance of peanut is the principal tool for management of adverse effects. Thus, the determination of the tolerable intake level of peanut protein in sensitized individuals is relevant. A health hazard assessment of the allergenicity of peanut on this sensitive subpopulation was performed. The critical effects were derived from a published double-blind placebo-controlled food challenge study that administered peanut to sensitive adults and that included dose-response information on adverse effect levels. The no (NOAEL) and low (LOAEL) observable adverse effect level for both subjective (self-reported symptoms) and objective (observed symptoms) were identified. Utilizing an uncertainty factor of 10 for intra-individual differences and the NOAELs for subjective and objective adverse symptoms, estimations of the tolerable acute intake of peanut were determined. The tolerable intake for subjective symptoms and for objective symptoms are 5 and 100 μg peanut protein per person, respectively. The nature of individual sensitivities and responses suggests additional considerations in addressing uncertainty in this assessment may be warranted. Factors like the magnitude of intra-individual differences, severity of the effect, and limitations in the study subjects able to safely participate were also examined. The possibility exists that the tolerable acute intakes levels as derived above may very well provide a lower margin of safety than is normally considered appropriate on the basis of established severe effects in humans. Thus, in this case, additional adjustments in deriving the margin of exposure may be a reasonable consideration.

1009 METABOLISM AND PHARMACOKINETICS OF NEOTAME IN HUMAN VOLUNTEERS.

P. Aikens[1], D. Kirkpatrick[1], D. Mayhew[2], W. Stargel[2], G. Wright[2] and J. Allen[2]. *[1]Huntingdon Life Sciences, Huntingdon, Cambs, United Kingdom and [2]The NutraSweet Company, Evanston, IL.*

Neotame, N-[N-(3, 3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine l-methyl ester is a new high intensity sweetener. As part of the safety testing, studies were conducted to evaluate the absorption, pharmacokinetics, metabolism and excretion of neotame in healthy, adult male volunteers. For this purpose, neotame was labelled with C-14 at the 1-position in the dimethylbutyl side chain and with C-13 in the 2 terminal methyl groups of the same side chain. In one study volunteers ingested a single dose of the labelled test substance in water at a level approximately equivalent to 0.25 mg/kg, which corresponds to the amount of neotame needed to sweeten one litre of beverage. Neotame was rapidly, but incompletely absorbed and rapidly excreted. A mean of 98% of the administered radioactivity was recovered in urine and faeces, mostly within 72 hours of dosing. Mean plasma concentrations of neotame peaked at 0.4 hour post-dose and declined with a half-life of 0.6 hour. The major metabolite of neotame was de-esterified neotame formed by hydrolysis of the methyl ester group. Mean plasma concentrations of this metabolite peaked at 1 hour post-dose, were approximately 2.5 times higher than neotame concentrations and declined with a half-life of 1.5 hours. De-esterified neotame represented a mean of approximately 80% of the excreted dose. Two other metabolites were detected at greater than 1% of the dose. One, that was a mean of about 4.9% of the dose, was found in the faeces and was identified as N-(3, 3 dimethylbutyl)-L aspartic acid. The other metabolite was in urine and was identified by LC/MS/MS, NMR and original synthesis as a carnitine ester of 3, 3-dimethylbutanoic acid. All metabolites of neotame present at 1% or greater of the dose have been shown to occur in the species used in safety studies, confirming the safety of these metabolites. A comparison of the metabolism and pharmacokinetic data in humans with those in animals indicates that the animal species are relevant for predicting human safety.

1010 EFFECTS OF DIETARY EXPOSURE TO POLYPHENOLIC COMPOUNDS IN DIFFERENT BERRIES ON COLON CANCER IN RATS.

J. H. Exon and T. Taruscio. *Food Science and Toxicology, University of Idaho, Moscow, ID.*

Small fruits such as berries are a good source of polyphenolic chemicals which are thought to have bioactive properties toward a number of diseases including cancer. The effects of these chemicals vary however between studies. The bioactive chemical profile of nine different types of the Vaccinium sp of berries have been recently characterized in our laboratory. The profiles show the different levels of four major groups of polyphenolics, flavanols, flavonols, phenolic acids and anthocyanidins, and the individual chemicals which make up these major groups. Three different profiles were chosen to study the effects on a colon cancer model in rats. The highbush blueberries contained high levels of phenolic acids, the huckleberries contained high levels of anthocyanidins (and the greatest antioxidant activity) and the cranberries contained intermediate levels of all major groups. Three groups of year-old male rats were exposed to dietary levels of individual berry groups at levels of 3%dw in the diet for nine weeks. One group served as the control. All rats were injected ip with azoxymethane to induce preneoplastic lesions, aberrant crypt foci (ACF), in the colon. Rats exposed to the huckleberry diet had significantly greater numbers of ACF compared to the control. Rats receiving the blueberry or cranberry diets tended to have increased numbers of total ACF but not significantly different from the control. These results indicate that diets high in anthocyanidins, phenolic acids and antioxidant activity did not protect against the development of ACF and in fact enhanced the effects of the colon carcinogen.

1011 VITAMIN E ANALOGUE LEVELS IN TISSUES OF OLD AND YOUNG RATS FED D-α-TOCOPHERYL SUCCINATE.

T. G. Taruscio[1], J. H. Exon[1], G. D. Clifton[2] and M. W. Fariss[2]. *[1]Food Science and Toxicology, University of Idaho, Moscow, ID and [2]Pharmaceutical Science and Pharmacotherapy, Washington State University, Pullman, WA.*

Recent studies have shown that different forms of vitamin E may have different bioactive properties. Female Sprague-Dawley rats, 20 (OLD) or 2 mo (YNG) of age, were fed commercial rodent chow or the same feed supplemented with either 1

(YNG) or 2 (OLD) g d-α tocopheryl succinate (αTS)/kg diet. The animals were sacrificed after 49 days and tissues were collected for analysis of α-tocopherol (αT), γ-tocopherol (γT) and α-tocopheryl succinate (αTS). Serum triglycerides and percent body fat were also measured. There was no significant difference in the intake of αTS between YNG and OLD animals on a mg/kg body wt basis. All rats within each age group treated with αTS had significantly greater levels of αT in all tissues than the rats not treated with αTS. OLD rats treated with αTS retained significantly higher levels of αT in all tissues compared to the YNG rats treated with αTS. Detectable levels of γT were only found in serum, heart, lung and colons of OLD rats not given αTS and in the colons of OLD group of rats given αTS. No detectable levels of γT were found in any tissues of YNG rats. Levels of αTS were found in all tissues except the brain of OLD and YNG rats which were treated with αTS. The αTS levels in all tissues except colon were 2-fold higher in the OLD animals compared to the YNG group. The OLD group of rats had significantly greater percent body fat and serum triglycerides than the YNG group. This is the first study to show that dietary exposure to αTS will result in residues of the intact form in various tissues. It also shows that older rats accumulate more αTS, αT and γT than younger rats. This may be related to the greater fat content and serum triglyceride levels in old rats. The retention of γT and αTS in particular in tissues may be important in disease prevention that may be unique to these forms of vitamin E.

1012 METABOLISM AND PHARMACOKINETICS OF NEOTAME IN RATS AND DOGS.

D. Mayhew[2], P. Aikens[1], D. Kirkpatrick[1], W. Stargel[2], G. Wright[2] and J. Allen[2]. *[1]Huntingdon Life Sciences, Huntingdon, Cambs., United Kingdom and [2]The NutraSweet Company, Evanston, IL.*

Neotame, N-[N-(3, 3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine l-methyl ester is a new high intensity sweetener. As part of the safety testing, studies were conducted to evaluate the absorption, distribution, pharmacokinetics, metabolism and excretion of neotame in laboratory rats and dogs. For this purpose, neotame was labelled with C-14 at the 1-position in the dimethylbutyl side chain and was administered to animals at doses of 15 or 120 mg/kg bodyweight. In rats and dogs, oral doses of neotame were rapidly, but incompletely absorbed and rapidly excreted with no evidence of potential for accumulation. In rats, absorbed C-14 was mainly associated with the gastrointestinal tract and organs of metabolism and excretion (liver, kidney and bladder). Almost no neotame was detected in (stabilised) plasma or excreta samples after oral dosing to rats. This was probably due to high activity of plasma esterases. The major metabolite of neotame was de-esterified neotame formed by hydrolysis of the methyl ester group. In rats, mean plasma concentrations of this metabolite peaked at 0.5 hour post-dose and declined with a half life of 1 hour. In dogs, which have a lower level of plasma esterase activity, neotame was detected in plasma and excreta after oral dosing. Mean plasma concentrations of neotame peaked at 0.5 hour post-dose and declined with a half-life of 0.4 hour. De-esterified neotame represented a mean of approximately 70-80% of excreted oral doses in both rats and dogs. Other metabolites detected included N-(3, 3 dimethylbutyl)-L aspartic acid (in rats and dogs about 2% of the dose) and a β-glucuronide conjugate of 3, 3-dimethylbutanoic acid (in rats and dogs about 5% of the dose). In addition the carnitine ester of 3, 3-dimethylbutanoic acid was present in the urine of female rats. The safety of these metabolites has therefore been assessed in toxicology studies conducted with neotame in these species. A comparison of the metabolism and pharmacokinetic data in animals with those in humans indicate that the animal species are relevant for predicting human safety.

1013 DOCOSAHEXAENOIC ACID DOSE-DEPENDENTLY SUPPRESSES DEOXYNIVALENOL INDUCED IGA NEPHROPATHY, INTERLEUKIN-6 AND CYCLOOXYGENASE-2 GENE EXPRESSION.

Q. Jia[1, 2] and J. J. Pestka[1, 2]. *[1]Department of food science and human nutrition, Michigan State University, East Lansing, MI and [2]Institute for enviromental toxicologcy, Michigan State University, East Lansing, MI.*

Dietary exposure to trichothecene deoxynivalenol (DON, vomitoxin) may induce experimental IgA nephropathy (IgAN) in mice by modulating mucosal immune function. Interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2) may play important roles in the upregulation of IgA production *in vivo*. Both docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) can retard progression of IgAN in this model, with DHA being more effective. The purpose of this research was to optimize the regime for DHA treatment and to test DHA effects on proinflammatory genes (IL-6 and COX-2) expression. Mice were fed for 18wks with AIN-93G diet containing DON and different concentration of DHA (0.2-6%). DHA dose-dependently inhibited serum IgA elevation, serum IgA immune complex and kidney mesangial IgA deposition. Although IL-6 mRNA and heterogeneous nuclear IL-6 RNA (hnIL-6) were not induced by DON in the chronic feeding study they were inhibited by DHA (1, 6%). COX-2 mRNA level was enhanced by DON and inhibited by DHA (6%). Both DON and DHA treatment did not affect CD40, CD40 ligand (T, B cell activation) or activation-induced cytidine deaminase (AID, IgA isotype class-switch related) gene mRNA level *in vivo*. Pre-feeding mice with 6% DHA for 4 wks significantly blocked induction of IL-6 and COX-2 mRNA by acute DON exposure, but failed to block mitogen activated protein kinase (MAPKs) activation triggered by DON. Together, the results suggested that DHA may retard DON-induced IgA superinduction and then IgA nephropathy progression by blocking IL-6 and COX-2 gene induction independent of MAPK activation. (Supported by NIH grants DK58833 and ES03358)

1014 EXPOSURE FOR ONE YEAR TO A METABOLIC NUTRITION SYSTEM CONTAINING EPHEDRA AND CAFFEINE DOES NOT ALTER SERUM CHEMISTRY PROFILE OR TARGET ORGAN HISTOPATHOLOGY OF B6C3F1 MICE.

S. D. Ray[1, 2], S. Stohs[2] and R. Hackman[3]. *[1]Pharmacology & Toxicology, Long Island University, Brooklyn, NY, [2]Coll. of Pharmacy and Health Professions, Creighton University, Omaha, NE and [3]Department of Nutrition, University of California, Davis, CA.*

Nutritional value and therapeutic benefits coupled with presumed safety have heightened interest in the use of custom designed dietary supplements. Their use has increased substantially since passage of the 1994 Dietary Supplement Health education Act. However, few well-controlled studies have been conducted to assess the safety and potential adverse effects of dietary supplements. MNSO (MNS Orange-AdvoCare) is a unique combination of vitamins, minerals, omega-3 fatty acids and herbal extracts designed to provide a strong foundation of nutritional support, enhance thermogenesis, and boost energy levels and immune status. This investigation was designed to explore the safety and toxic effects, if any, of 12 months continuous exposure to ephedra and caffeine containing MNSO on serum chemistry (enzyme, lipid, carbohydrate, electrolyte profiles) and histopathology of seven vital target organs in female B6C3F1 mice. MNSO is enriched with the extracts of citrus, ephedra, gingko, green tea and Ocimum. In this study, mice were fed control (-MNSO) or MNSO (1X-10X, 1X=daily human dose) diets. Blood was collected from control and variously treated animals every four months for serum chemistry analysis. Target organs (brain, liver, kidney, lung, spleen, duodenum and heart) were collected for histopathology. Food consumption and body weight changes were also monitored biweekly. Data shows that 12 month exposure of 10X-MNSO did not significantly influence organ histopathology or normal serum chemistry profile. MNSO-exposed animals were more active, consumed more food, and were relatively leaner compared to controls. This study indicates that a caffeine and ephedra containing metabolic nutrition system is nontoxic in mice at up to 10X the human consumption dose for ephedra. [Supported by AdvoCare International Carrolton, TX]

1015 SPHINGOSINE KINASE CONFERS RESISTANCE TO FUMONISIN B_1 APOPTOSIS IN HUMAN RENAL CELLS.

N. sharma and R. P. Sharma. *Physiology and Pharmacology, University of Georgia, Athens, GA.*

Fumonisin B_1, a mycotoxin produced by *Fusarium verticillioides*, causes a variety of diseases in animals and humans, including cancer. Fumonisin B_1 interferes with sphingolipid metabolism due to its similarity to the long-chain backbone of sphingolipid bases. Sphingoid bases are toxic in cells and their accumulation is known to produce cellular apoptosis after fumonisin B_1 exposure. Fumonisin B_1 blocks *de novo* ceramide biosynthesis in all cells causing accumulation of free sphingoid bases; however, only some cell types are sensitive to its toxic effects. It was reported that African green monkey kidney cells (COS-7) and rat primary hepatocytes are resistant to the apoptotic and antiproliferative effects of fumonisin B_1. Exposure to 50 μM fumonisin B_1 of human embryonic kidney (HEK-293) cells for 48 hours did not induce apoptosis while it did so in porcine renal cells. We hypothesized that HEK-293 cells may be resistant to apoptotic effects of fumonisin B_1 due to reduced levels of sphinganine and sphingosine because of their conversion to respective phosphates *via* sphingosine kinase. Coincubation with 5 μM DL-*threo*-dihydrosphingosine (DHS), an inhibitor of sphingosine kinase, considerably increased sensitivity of HEK-293 cells to 25 μM fumonisin B_1. Annexin V, Hoechst 33258 and Tunnel staining confirmed both apoptosis and necrosis of cells. Sphingoid bases, sphinganine or sphingosine, added to cultures were only slightly cytotoxic; apoptosis was enhanced when coexposed with DHS or fumonisin B_1. These findings indicate that sphingoid base metabolism is a critical event in the mechanism of rendering certain cells resistant to fumonisin B_1-induced apoptosis. (Supported by ES 09403 from PHS).

1016 DOWNREGULATION OF mdr1b mRNA EXPRESSION IN THE KIDNEY OF NEXT GENERATION EXPOSED TO TRIBUTYLTIN CHLORIDE.

K. Kobayashi[1], T. Watanabe[2] and Y. Sugita-Konishi[1]. *[1]Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan and [2]Division of Food, National Institute of Health Sciences, Tokyo, Japan.*

We investigated the change of mdr1b mRNA expression in the kidney of offspring exposed to tributyltin chloride (TBTC) *via* dams by real-time reverse transcription-polymerase chain reaction. Pregnant ICR mice were given water containing TBTC (0, 15 and 50 mg/mL) *ad libitum* from first of pregnancy to weaning (exposure *via* placenta and lactation) or from parturition to weaning (exposure *via* lactation). TBTC exposure did not inhibit the growth of the offspring in any concentrations used in this study. In the kidney of the offspring exposed to TBTC *via* placenta and lactation, mdr1b mRNA expression was significantly lower than that of the control group, whereas TBTC exposure *via* lactation showed a tendency to decrease the expression as compared with the control group. These findings suggest that TBTC exposure through mother, in particular through placenta and lactation, may impair the ability of offspring to protect itself from environmental toxic compounds.

1017 NAVIGATING THE REGULATORY WATERS AT FDA-TOXICOLOGY SAFETY REVIEWS OF FOOD AND COLOR ADDITIVES.

D. B. Carlson, T. L. Taras and T. Thurmond. *FDA, College Park, MD.*

Food and color additives that become components of our food supply, either directly or indirectly, are required to undergo pre-market safety review by the US Food and Drug Administration. Changes initiated by the Food and Drug Modernization Act resulted in the 2001 reorganization of the Center for Food Safety and Applied Nutrition's "Office of Premarket Approval" into the "Office of Food Additive Safety". While the safety standards and review criteria for potential food and color additives have not changed, under this Act there are now more options for industry and citizen submissions for new food and color additive uses. The toxicological testing standards for determining the safety of food and color additives are driven by the potential toxicity of the additive. The possibility for human toxicity can be estimated by coupling consumer exposure estimates with chemical structural alerts, determined by comparing the chemical moieties of the additive to known toxicant structures. Ultimately, the safety of potentially toxic substances can be supported by *in vitro* and/or *in vivo* toxicity tests. Well designed and executed toxicity studies are important, but a well prepared petition is equally critical to effectively navigate the regulatory process for new food or color additives. To facilitate an efficient and transparent review of petitions, the Office of Food Additive Safety encourages communication and consultation between stakeholders and the FDA throughout the submission and review process. The most efficient way to obtain approval of safe new uses of additives is to consult with FDA personnel, including toxicologists, *prior* to submitting a petition. Helpful guidelines for toxicology tests that ensure the safety of these additives can also be found in various published and online sources, such as the "Toxicological Principles for the Safety Assessment of Food Ingredients" (i.e. the "Redbook"). Proper toxicity testing and preparation of petitions are critical components for assuring both the safety of food and color additives and a smooth and efficient petition process.

1018 NATIONAL RESIDUE PROGRAM (NRP) AS A FOOD SAFETY TOOL.

R. Kishore, P. Zervos, A. Brown, C. Deyrup, R. Sutton and J. Vodela. *Residue Branch, USDA/FSIS, Washington DC, DC.*

The Food Safety and Inspection Service is a public health regulatory Agency of the USDA, which protects consumers health by regulating meat, poultry, and egg products. FSIS enforces the tolerances and action levels set by the Food and Drug Administration (FDA) and Environmental Protection Agency (EPA). The main component of FSIS residue prevention activities is the NRP, a multi-component analytical testing program for residues in domestic and imported meat, poultry and egg products. The NRP is designed to monitor, detect and identify drugs, pesticides and other chemical contaminants in meat, poultry and egg products in order to reduce and control chemical residues in animals. The Zoonotic Diseases and Residue Surveillance Division annually publishes the residue plan and the analytical results. The NRP also uses national data on chemical residues to support risk assessment, enforcement, and educational activities. In 2002, FSIS sampled and tested 12 compound classes of drugs and pesticides, comprising approximately 59 residues. Under the Domestic Monitoring Plan 26, 028 samples were analyzed. The preliminary results indicate 75 residue violations consisting of 23 antibiotics, 16 avermectins/milbemycins, 10 sulfonamides, two flunixins, 23 zeranol, and one chlorinated hydrocarbon/chlorinated organophosphate. No residue violations were found in the testing of arsenicals, chloramphenicol, and ractopamine. The most common cause of violations of approved drugs in livestock, poultry, and egg products is a failure to allow an adequate withdrawal time for the drugs to clear the animal system. The illegal residues are usually concentrated in the kidney, liver, or fat rather than muscle. The FSIS sampling focuses on kidney and liver tissues, since most FDA limits are established for these tissues.

1019 TOXICOLOGICAL EVALUATION AND ANTIOXIDANT POTENTIAL OF A NOVEL BOTANICAL EXTRACT FOR USE IN AMELIORATING ALLERGIC RHINITIS.

M. Bagchi[1], A. Amit[3], V. S. Saxena[3], N. Pratibha[3] and D. Bagchi[2, 1]. *[1]R & D, InterHealth Research Center, Benicia, CA, [2]Pharmacy Sciences, Creighton University Medical Center, Omaha, NE and [3]R & D, Natural Remedies Research Center, Bangalore, India.*

A standardized botanical formulation (Aller-7/NR-A2), developed from 7 medicinal plants, was shown to ameliorate the symptoms of allergic rhinitis by mast cell stabilization, lipoxygenase and hyaluronidase inhibition, as well as potent anti-histaminic, anti-inflammatory, anti-spasmodic activities. The present study was designed to evaluate the acute oral, acute dermal, dermal irritation and eye irritation toxicities of Aller-7 *in vivo*. Furthermore, the concentration-dependent inhibitory effect of Aller-7 was assessed against superoxide anion, hydroxyl radicals and nitric oxide, the key inflammatory mediators in pulmonary damage and bronchoconstriction. The oral LD50 of Aller-7 was found to be greater than 5, 000 mg/kg b.w. in both male and female rats, and no mortality or signs of toxicity were observed at this dose. The acute dermal LD50 was found to be greater than 2, 000 mg/kg b.w. The skin irritancy index was 0.0 in rabbits and classified as minimal irritant to eye in rabbit. The superoxide scavenging IC50 values of Aller-7, gallic acid and catechins, as demonstrated by PMS-NADH non-enzymatic system, were found to be 24.65, 21.15 and 82.15 μg/ml, respectively. The nitric oxide scavenging IC50 values of Aller-7 and curcuminoids, as determined spectrophotometrically at 546 nm using Greiss reagent, were found to be 16.34 and 33.63 μg/ml, respectively. The hydroxyl radical scavenging IC50 values of Aller-7 and catechins, as determined spectrophotometrically at 532 nm using deoxyribose assay, were found to be 741.73 and 2193.39 μg/ml, respectively. In addition, Aller-7 was found to be significantly effective in protecting rat erythrocytes, as demonstrated by AAPH-induced lysis of rat RBC, against free radical injury in the concentration of 20-80 μg/ml as compared to BHA 15-50 μg/ml, respectively. These results demonstrate the safety and novel antioxidant potential of Aller-7 for use in treating allergic rhinitis.

1020 SAFETY STUDIES OF A NOVEL, NATURAL EXTRACT OF (-)-HYDROXYCITRIC ACID, A SUPPLEMENT FOR WEIGHT MANAGEMENT.

D. Bagchi[1, 2], M. Shara[1], S. E. Ohia[1], T. Yasmin[1], M. Bagchi[2], A. Chatterjee[1] and S. J. Stohs[1]. *[1]Pharmacy Sciences, Creighton University Medical Center, Omaha, NE and [2]R&D, InterHealth Research Center, Benicia, CA.*

Garcinia cambogia-derived (-)-hydroxycitric acid (HCA) is a competitive inhibitor of the enzyme ATP citrate lyase, a building block for fat synthesis. Results have shown that HCA-SX (a 60% calcium-potassium salt of HCA, Super CitriMax) promotes fat oxidation, enhances serotonin release and availability in the brain cortex, normalizes lipid profiles, and lowers serum leptin levels in obese subjects. Acute oral, acute dermal, primary dermal irritation and primary eye irritation toxicity demonstrated the safety of HCA-SX. HCA-SX did not induce mutagenic effect in the bacterial reverse mutation test in five Salmonella typhimurium strains (TA98, TA100, TA1535, TA1537 and TA102), either with or without metabolic activation. Similarly, HCA-SX did not induce mutagenic effects in the mammalian cell gene mutation test in L5178Y mouse lymphoma cells TK(+/-), either with or without metabolic activation. We evaluated the dose- and time-dependent effects of HCA-SX in Sprague-Dawley rats on body weight, selected organ weights, hepatic lipid peroxidation and DNA fragmentation, histopathology, hematology and clinical chemistry over a period of 90 days. The animals were treated with 0, 0.2, 2.0 and 5.0% HCA-SX of feed intake and were sacrificed on 30, 60 or 90 days of treatment. A significant reduction in body weight was observed in treated rats as compared to control animals. An advancing age-induced marginal increase in hepatic lipid peroxidation was observed in both male and female rats, while no such difference in hepatic DNA fragmentation was observed as compared to the control animals. Furthermore, selected organ weights individually and as a % of body weight and brain weight at 90 days of treatment exhibited no significant difference between the groups. The results show that 90 day treatment of HCA-SX results in a reduction in body weight, and does not cause any changes in major organs or in hematology, clinical chemistry, and histopathology.

1021 THE USE OF STRUCTURE ACTIVITY RELATIONSHIP (SAR) ANALYSIS IN THE SAFETY ASSESSMENT OF FOOD CONTACT NOTIFICATIONS.

A. J. McDougal, M. Twaroski, R. Chanderbhan, N. Braier, K. Arvidson, J. Mayer, M. Cheeseman and A. Bailey. *Division of Food Contact Notifications, OFAS, USFDA, College Park, MD.*

The food contact notification (FCN) process was established by Section 309 of the FDA Modernization Act of 1997. For the past 4 years, the FCN process has been the primary method in the US for the evaluation and authorization of new uses of food additives that are food contact substances (FCSs). The FCN process is divided into 2 phases: Phase 1 to verify that the FCN is complete regarding the regulatory, chemical, toxicological, and environmental information necessary to evaluate the FCS and its constituents, and Phase 2 to carefully analyze the manufacturing, exposure, and safety data, in order to reach a safety decision. SAR analysis is currently used in both phases as a tool to assist in the safety evaluation. Phase 1 SAR analysis focuses on identifying structural alerts and close analogs for which relevant mutagenicity and carcinogenicity data are available. The goal is to decide if additional testing should be recommended to address safety concerns. This decision incorporates data from commercial and in-house software and databases. For a constituent of a FCS determined in Phase 1 to be of potential concern, Phase 2 SAR analysis can, in some cases, assess the safety without the need for additional testing. If sufficient bioassay data are available for chemical analogs that adequately represent the structure of the constituent, a quantitative estimate may be determined of the upper-bound lifetime cancer risks for the analogs; these risks can then be used to extrapolate a range of associated risk for the untested constituent that may be useful in the safety assessment. Currently, analogs are identified using a structurally searchable version of the Carcinogenic Potency Database (CPDB), as well as in-house and publicly available databases.

1022 TOXICOLOGICAL EVALUATION OF JOALA, A HOME-BREWED BEVERAGE, PREPARED FROM CORN CONTAMINATED WITH *FUSARIUM VERTICILLIOIDES* CULTURE MATERIAL.

K. A. Voss[1], L. H. Couch[2], P. C. Howard[2], N. P. Keller[3], M. Mabathoana[4] and C. W. Bacon[1]. *[1]Toxicology & Mycotoxin Research Unit, USDA Agricultural Research Service, Athens, GA, [2]NCTR, USFDA, Jefferson, AR, [3]Plant Pathology, University of Wisconsin, Madison, WI and [4]Consultant, Maseru, Lesotho.*

Fumonisins are water-soluble mycotoxins produced by *Fusarium verticillioides* and related fungi. They are liver and kidney toxins and the most common analogue, fumonisin B_1 (FB_1), is carcinogenic to rodents. Fumonisins have been implicated as a risk factor for esophageal cancer and, more recently, neural tube defects in some human populations heavily dependent on fumonisin-contaminated corn as a dietary staple. Fumonisins are found in foods and have been detected in commercial beers. Joala is a type of home-brewed beverage consumed in rural southern Africa that is made from corn, malted sorghum, yeast, and water. The effect of the Joala making process on the fate and toxicity of fumonisins is unknown. Rats (n = 6/group) were dosed by gavage twice daily for two weeks with 10ml/kg of distilled water (negative controls), Joala made from sound corn (Joala-SC), Joala made from sound corn spiked with culture material of *F. verticillioides* (Joala-FC), or a water extract of the spiked corn (WE-FC). Decreased body weight gain and food consumption were found in the Joala-FC and WE-FC groups. Decreased kidney and liver weights and microscopic kidney and liver lesions typical of short-term fumonisin exposure were also found in animals given Joala-FC or WE-FC. The Joala and water extracts made from the spiked corn had similar FB_1 concentrations of about 0.1 mg/mL (determined by HPLC). Thus, fumonisins are readily extracted into Joala beer and the Joala making process did not affect their toxicity. The findings suggest that Joala and similar home-brewed beverages can be a significant source of fumonisin exposure in populations depending on foodstuffs made from contaminated homegrown corn.

1023 FUMONISINS IN MAIZE IN GUATEMALA AND A PRELIMINARY ESTIMATE OF DAILY INTAKES.

R. T. Riley[1], E. Palencia[2], O. R. Torres[2], A. E. Glenn[1] and M. Fuentes[3]. *[1]Toxicology and Mycotoxins Research Unit, USDA-ARS, Athens, GA, [2]Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala and [3]Institute of Agricultural Science and Technology, Guatemala City, Guatemala.*

Maize samples were collected from highlands (> 1700 m) and lowlands (< 360 m) of Guatemala in 2000 to 2002. Samples were analyzed for fumonisin B1 (FB1) by high performance liquid chromatography (HPLC) in 2000 and 2001 and by LC ion trap mass spectrometry (LCMSMS) in 2002. The detection limit for HPLC and LCMSMS methods were 0.3 ppm and 0.05 ppm, respectively. The LCMSMS method also detected FB2 and FB3. Samples in 2002 that had detectable FB1 but were < 0.3 ppm were assigned a value of 0 ppm. The mean FB1 level (2000 to 2002) in maize from the lowlands (1.2 ±0.3 ppm, n=205) was significantly higher than the maize from the highlands (0.26 ±0.18 ppm, n = 142). The incidence of FB1 positive samples was significantly greater in the lowland maize (109/205) compared to that from the highlands (13/142). In the maize from the highlands, 9% of the samples contained ≥ 0.3 ppm FB1 with the highest being 7.3 ppm, whereas, in the lowland samples 53% were ≥0.3 ppm FB1 and 2.4 % were ≥10 ppm with one sample being 21 ppm. Analysis of the samples from 2002 by LCMSMS revealed that most FB1 positive samples contained FB2 and FB3 at a ratio of 1:0.4:0.3. In addition, approximately 92% (104/113)of the samples from the lowlands in 2002 contained detectable levels of FB1, whereas, all but 5 (5/92) of the samples from the highlands contained no detectable fumonisins. Based on a recall study in women conducted in the Central Highlands, a preliminary assessment of daily intake of total FBs was estimated. Consumption of nixtamalized maize products made from lowland maize could result in exposure exceeding the provisional maximal tolerable daily intake (2 μg total fumonisins/kg bw) with over 50% of the maize samples. At the highest level, 2.4% of the samples would provide a daily intake that was on average 58 μg/kg bw (support: USDA FAS grant X01-4510-62-751071-4; ILSI NA Technical Committee on Food Toxicology and Safety Assessment).

1024 SAFETY EVALUATION OF A NATURAL TOMATO OLEORESIN EXTRACT DERIVED FROM TOMATOES.

R. A. Matulka, A. M. Hood and J. C. Griffiths. *Burdock Group, Vero Beach, FL.*

Experimental and epidemiological studies suggest that consumption of tomato products containing high levels of the carotenoid lycopene is associated with lowered cancer risk. The protective effects of lycopene may be related to its antioxidant potential, being the carotenoid with the highest singlet oxygen radical quenching ability. Lycopene has been demonstrated to inhibit oxidation in both DNA and lipid structures. A proprietary, natural tomato oleoresin extract (NTOE), is a purified tomato oleoresin containing 6% lycopene produced from tomatoes. The objective of this series of experiments was to analyze the safety assessment of NTOE. The acute, oral LD50 of NTOE in Sprague-Dawley rats was greater than 5000 mg/kg bodyweight. In a 13-week oral (*via* gavage) study at doses of 0, 45, 450 and 4500 mg NTOE/kg/day (addition of 0, 2.7, 27 and 270 mg/kg/day lycopene, respectively) in male and female CD rats, no changes in the bodyweight gains of the treated animals were noted. Plasma concentrations of lycopene at the end of the study were similar at 450 and 4500 mg NTOE/kg/day (57.33 and 57.43 μg/l lycopene, respectively), but lower at the 45 mg/kg/day dose level (a range from 0 to 29.37 μg/l lycopene). Urinalysis investigations at Week 11 revealed no treatment-related abnormalities. Hematological and histopathological analysis at the end of the subchronic study found no significant treatment-related effects by NTOE. NOAEL was determined to be 4500 mg/kg/day NTOE. Mutagenic potential was determined *via* the Ames assay utilizing five strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537 and TA1538) and one strain of Escherichia coli (WP2uvrA-). There was no evidence of mutagenicity up to 5000 μg/plate; either with or without enzyme mix (S9) addition. Consumption analysis of lycopene-containing foods in the US population was performed, with the estimated mean daily intake of lycopene calculated at 8.2 mg/day. These results demonstrate the inability of NTOE to produce oral or mutagenic toxicity at doses greater than 300 times the normal human consumption of lycopene.

1025 POLYUNSATURATED FATTY ACIDS INHIBIT TOXIN-INDUCED ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINASES.

Y. Shi[1] and J. J. Pestka[1, 2]. *[1]Food Science and Human Nutrition, Michigan State University, East Lansing, MI and [2]Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.*

Activation of the mitogen activated protein kinase (MAPK) cascade by environmental toxins, such as the mycotoxin deoxynivalenol (DON), lipopolysaccharide (LPS) and H2O2, can affect cellular differentiative, proliferative and apoptotic pathways. We investigated the effects of n-3 polyunsaturated fatty acids (PUFA) on activation of MAPKs using the mouse macrophage RAW 264.7 cell line. Serum-deprived cells were incubated for 24 hours with 100 uM PUFA (arachidonic acid [AA, C20:4 n6], eicosapentaenoic acid [EPA, C20:5 n3] and docosahexaenoic acid [DHA, C22:6 n3]) complexed with bovine serum albumin and then phospholipids were extracted for fatty acid analysis. Gas chromatography revealed that each PUFA was incorporated into cell membrane effectively. Incubation with AA, USEPA or DHA increased their respective contents in cells from 1.96% to 6.89%, undetectable to 5.41%, and undetectable to 7.33% respectively. For analysis of MAPKs, DON (250 ng/ml), LPS (100 ng/ml) or H2O2 (0.5 mM) was added to the medium after fatty acid treatment and MAPK phosphorylation was analyzed by Western blot. JNK 1/ 2 phosphorylation was increased 5.7-, 2.7-, 4.0- fold; ERK 1/

2 phosphorylation was increased 1.7-, 3.0-, 1.3- fold; and p38 phosphorylation was increased 8.1-, 2.4-, 1.5- fold by DON, H2O2 and LPS, respectively. The three PUFAs inhibited DON and H2O2 activation of: (1)JNK 1 / 2 by 52-89%; (2) ERK 1 / 2 by 64-93%; and (3) p38 by 35-73%. PUFA inhibited LPS induced JNK 1 / 2 activation by 29-34%, but ERK1 / 2 and p38 were not affected. Taken together, the results suggested that the MAPK responses to toxins were differentially modified by PUFA and that no difference was observed between the effects of the n-3 and n-6 PUFA. (Supported by NIH grant DK58833)

1026 A 24-MONTH DIETARY CARCINOGENICITY STUDY OF DAG (DIACYLGLYCEROL) IN MICE.

J. B. Kirkpatrick[1], C. P. Chengelis[1], R. H. Bruner[1], O. Morita[2], Y. Tamaki[2] and H. Suzuki[2]. *[1]WIL Research Laboratories, Inc., Ashland, OH and [2]Kao Corporation, Haga Tochigi, Japan.*

This study evaluated the possible carcinogenic effects of DAG, when given in the diet at levels up to 6.0% (60, 000 ppm) for 24 months to mice. DAG is a cooking oil which contains >80% diglycerides, <20% triglycerides and <5% monoglycerides. A special diet was prepared so that all of the dietary fat could be provided by DAG and/or the control oil, TG (triacylglycerol), at various concentrations. The TG contained >85% triglycerides, <10% diglycerides and <5% monoglycerides. The fatty acid composition for DAG and TG was closely matched. Dietary concentrations (% DAG/% TG) of 0%/6.0% (TG control), 1.5%/4.5%, 3.0%/3.0%, and 6.0%/0% were presented ad libitum, seven days per week, for 104 weeks. An additional control group (group 1) received the standard basal diet (Certified Rodent LabDiet 5002), which has a fat content of 4.5%. Each group consisted of 50 male and 50 female Crl:CD-1(ICR)BR mice. The clinical condition of the animals, body weights, body weight gains, gross and histopathologic findings were unaffected by DAG. The effects in the DAG-treated groups were no different than those observed in the TG control group. However, the 6.0% dietary fat level is 33% higher than the standard basal diet (4.5% fat). Both TG and DAG, when presented separately or together in the diet at a total fat level of 6.0%, caused some changes relative to the basal diet control (decreased survival, higher body weights, lower food consumption, and increased incidences of macroscopic and microscopic findings) related to the higher dietary fat content and/or the semi-purified diet. Thus, DAG at dietary concentrations up to 6.0% for 24 months produced no signs of systemic toxicity and had no effect on the incidence of neoplastic findings.

1027 COMBINATION TOXICITY OF ACRYLAMIDE AND HEAVY METALS – ADDITIVE EFFECTS OR MORE.

J. B. Schulze[1] and C. Siegers[2]. *[1]Office of the Dean, Frankfurt/Main, Germany and [2]Inst. Exp. CLin. Pharmacology Toxicol., Luebeck, Germany.*

Acrylamide is a potent neurotoxicant; additionally it has been classified as a potential carcinogenic compound. Despite an extensive body of literature for acrylamide effects, scant data are available investigating acrylamide effects in combination with other toxicants. We investigated the combined cytotoxicity of acrylamide together with heavy metals, widely occuring in the environment, in the kidney cell line LLC–MK2, the hepatic cell line Hep G2 and an ocular cell line I–407. All three cells showed cytotoxicity at acrylamide concentrations above 100 ng/ml (no cytotoxicity observed at this concentration). For $HgCl_2$ alone IC_{50} values ranged from 4.3 to 9 µg/ml. When combining 60 ng/ml acrylamide with 1 to 30 µg/ml $HgCl_2$, IC_{50} values for $HgCl_2$ were unaffected for I–407 cells and slightly lower by 20% for kidney and hepatic cells. However, when exposing these cells to 10 µg/ml $HgCl_2$ inducing a considerable cytotocix effect, IC_{50} values for additionally applied acrylamide dropped to 3.4 to 8 ng/ml, i.e. by a factor of appr. 100. A similar drop in IC_{50} values for acrylamide was seen when I–407 cells where simultaneously exposed to 6 µg/ml $CdCl_2$ (IC_{50} value for $CdCl_2$ alone 14 µg/ml). In highly exposed groups, up to 500 ng/ml Hg has been detected in blood samples (Vahter et al. 1997 Int Arch Occup Environ Health 70 p.282), with blood values in the general US population of about 1 ng/ml blood. No serum concentrations for acrylamide have been published. Extrapolating from a daily uptake of 80 ng/kg bw per meal and a distributioon volume of 0.6 (for water soluble compounds), 0.13 ng/ml acrylamide may be reached as peak serum levels. We conclude that acrylamide has no major cytotoxic effect on healthy cell cultures but cytotoxic effects are drastically increased in cells already damaged. This implies that the combination toxicity of acrylamide may be high under conditions when the target cells are stressed by other factors.

1028 A 24-MONTH CARCINOGENICITY STUDY OF DAG (DIACYLGLYCEROL) IN RATS WITH DIETARY OPTIMIZATION.

C. P. Chengelis[1], J. B. Kirkpatrick[1], R. H. Bruner[1], O. Morita[2], Y. Tamaki[2] and H. Suzuki[2]. *[1]WIL Research Laboratories, Inc., Ashland, OH and [2]Kao Corporation, Haga Tochigi, Japan.*

This study evaluated the possible carcinogenic effects of DAG, when given in the diet at levels up to 5.5% (55, 000 ppm) for 24 months to rats. DAG is a cooking oil which contains >80% diglycerides, <20% triglycerides and <5% monoglycerides. A special diet was prepared so that all of the dietary fat could be provided by DAG and/or the control oil, TG (triacylglycerol), at various concentrations. The TG contained >85% triglycerides, <10% diglycerides and <5% monoglycerides. The fatty acid composition for DAG and TG was closely matched. Dietary concentrations (% DAG/% TG) of 0%/5.5% (TG control), 1.0%/4.5%, 2.75%/2.75%, and 5.5%/0% were presented daily, seven days per week, for 104 weeks. An additional control group (group 1) received the standard basal diet (Certified Rodent LabDiet 5002), which has a fat content of 4.5%. Groups 1-5 (basal diet plus four DAG and/or TG-treated groups) each consisted of 50 male and 50 female Crl:CD(SD)IGS BR rats and were maintained on a daily dietary optimization schedule. Groups 6 -7 (5.5% DAG or TG) each consisted of 65 rats/sex and were fed ad libitum. The clinical condition of the animals, body weights, body weight gains, food consumption, body composition, gross and histopathologic findings were unaffected by DAG. While there were no differences between the effects of TG and DAG, both caused some changes relative to the basal diet control (decreased survival, higher body weights, and increased incidences of macroscopic and microscopic findings) related to the higher dietary fat content and/or the semi-purified diet. These changes were more marked in the ad libitum-fed groups than in the restricted diet groups. Thus, DAG at dietary concentrations up to 5.5% for 24 months produced no signs of systemic toxicity and had no effect on the incidence of neoplastic findings.

1029 *IN VITRO* SCREENING FOR BIOLOGICAL ACTIVITY ASSOCIATED WITH FUMONISINS IN NIXTAMALIZED FOODS.

L. D. Williams[1, 2], K. A. Voss[2], W. P. Norred[2], D. S. Saunders[3] and R. T. Riley[2, 1]. *[1]Environmental Health Sciences, University of Georgia, Athens, GA, [2]Toxicology and Mycotoxin Research Unit, USDA, Athens, GA and [3]Department of Food Safety, Frito-Lay, Inc., Plano, TX.*

Fumonisins (FB) are inhibitors of ceramide synthase, a key enzyme in the sphingolipid biosynthetic pathway. Nixtamalization, the alkali processing of corn, reduces the total FB in corn products. However, it is unclear whether hidden or more toxic FB metabolites are formed. To address this issue, the biological activity of FB contaminated corn before and after nixtamalization was determined *in vitro*. Sphinganine elevation was used as a biomarker for FB inhibition of ceramide synthase in LLC-PK1 and Vero cells. However, clean corn extracts, but not extracts of nixtamalized products, were toxic to cells. The objectives of this study were 1) determine a non-toxic exposure for FB-free corn extracts and 2) determine if a reduction in total detectable fumonisins by nixtamalization results in a similar reduction in biological activity. LLC-PK1 cells were treated for various times with various amounts of residues of corn extracts dissolved in growth medium. There was a dose and time dependent decrease in ATP-dependent dome formation and tight junction integrity based on visual observation. A 6h exposure to the equivalent of 0.086 g corn/ml of growth medium was used for subsequent experiments. In Vero cells exposure was for 48 h at 0.04 g corn equivalents/ml growth medium. Ceramide synthase inhibition by extracts of contaminated corn and nixtamalized products was determined. There was a significant decrease in sphinganine elevation in cells exposed to nixtamalized products compared to cells exposed to the FB contaminated corn used to make the products. These results indicate that nixtamalization of FB-contaminated corn does not produce increased fumonisin-like biological activity; results consistent with the conclusion that unknown or more toxic FB metabolites are not formed during the nixtamalization process.

1030 COMBINATIVE TOXIC EFFECTS OF AFLATOXIN-B_1 AND MICROCYSTIN-LR IN HUMAN CELL LINES AND VERTEBRATES.

M. Billam, L. Tang, C. McKean, H. Luo, M. Tang and J. Wang. *EnvTox/TIEHH, TTU, Lubbock, TX.*

Aflatoxin-B_1 (AFB_1) is a potent hepatocarcinogen and Microcystin-LR (MCLR) is a strong liver tumor promoter. Human exposure to these two toxins through diet and drinking water has been found in certain parts of the world, especially in high risk areas of liver cancer in China. Cocarcinogenic effect of these two toxins on rodents was also reported. In order to investigate mechanisms involved in their action, combinative toxic effect was studied in vertebrates and human liver and lung cells. Healthy mosquito fish (*Gambusia affinis*) were divided into 6 groups and each group treated with combinative doses of 1/8, 1/4, 3/8, 1/2, 1 and 0 of LC_{50} of AFB_1 (LC_{50} is 681.0 ppb) and MCLR (LC_{50} is 135 ppb). The combinative LC_{50} is 206.5 ppb with 95% CI at 151.7-304.8 ppb (K = 1.98). Young male F-344 rats were divided into 7 groups, each of 8 rats. Each group was treated respectively with 1.0, 3/4, 1/2, 3/8, 1/4, 1/8, and 0 of LD_{50} of AFB_1 orally (LD_{50} of 2.71mg/kg) and MCLR intra-peritoneally (LD_{50} of 72 µg/kg) and observed for 14 days. The combinative LD_{50} is 1.21 mg/kg with 95% confidence limit at 0.95-1.54 mg/kg (K = 1.154). Combinative cytotoxicity with these two toxins was tested in human

HepG2 and BEAS-2B cells using tetrazolium dye-based WST assay. IC_{50} of MCLR in BEAS-2B cells is 76.7 μM (95% CI at 60.5 to 100.6 μM), and 1 μM for AFB_1 in HepG2 cells. MCLR has no apparent cytotoxic effect on HepG2 cell lines while AFB_1 has no cytotoxicity to BEAS-2B cells. Combinative cytotoxicity study was done in HepG2 cells using a fixed concentration of 50 μM MCLR with various concentrations of AFB_1 (1/8, 1/4, 3/8, 1/2 and 3/4 IC_{50} values). The combinative IC_{50} with the mixture is 50.81μM. Similar study carried out in BEAS-2B cells using fixed concentration of 100 μM AFB_1 and varied concentrations of MCLR (1/8, 1/4, 3/8, 1/2 and 3/4 IC_{50} values) showed synergistic cytotoxicity with IC_{50} of 100.30μM. The results show that AFB_1 and MCLR when ingested together may have additive or synergistic toxic effects in animals and humans. Supported by SBCCOM, USArmy Research Contract (DAAD13-02-C-0070).

1031 RECALL AND TRACE BACK: FEDERAL, STATE AND FOOD INDUSTRY COOPERATIVE EFFORTS TO ENSURE FOOD SAFETY.

A. Tawadrous[1], A. M. Kadry[2], E. Jensen[2], W. Schlosser[2], J. Kause[2] and C. Maczka[2]. *[1]Recall Management Divsion, Food Safety and Inspection Service Department Of Agriculture, Washington, DC and [2]Risk Assessment Divison, Food Safety and Inspection Service Department Of Agriculture, Washington, DC.*

The Food Safety and Inspection Service (FSIS) of the US Department of Agriculture (USDA) inspects and regulates meat poultry and egg products. FSIS is responsible for ensuring that these products are safe, wholesome, and accurately labeled. Recalls are voluntary product withdrawals carried out by food industry firms in cooperation with Federal and State agencies. Products may be recalled if they are found to be contaminated, adulterated, or mislabeled. FSIS recall database was analyzed. Between January 1990 and December 2002, FSIS conducted 684 recalls of a total of 262, 966, 547 lbs of meat, poultry and egg product. The maximum quantity recalled in any one episode was 35, 000, 000 lb. Sixty-eight percent of the recalls were Class I (cases presenting a reasonable probability of serious adverse health consequences), 23% were Class II (remote probability), and 9% were Class III (no adverse health consequences). Recalls were associated with a variety of contaminants. E. coli O157:H7 in raw ground beef prompted fifteen percent of all recalls, constituting a total of 58, 903, 051 lbs of meat and poultry withdrawn. Listeria monocytogenes in ready to eat products (RTE)was the reason for 31% of the recalls (137, 367, 610 lbs).Salmonella in RTE accounted for 25 recalls (3, 848, 073 lbs), while bacterial spoilage organisms resulted in 11 recalls (4, 928, 018 lbs). Ninety six recalls (18, 471, 925 lbs) were due to contamination with extraneous material (e.g. metals, bone, plastic or glass). There were also 14 recalls each due to chemical contamination (1, 972, 681 lbs) and mislabeling (2, 975, 653 lbs). The overall average number of recalls per year was 48 +/- 24. Analysis of the data showed nearly as many recalls between 1999 and 2002 as between 1990 and 1998 (306 vs. 316 combined, respectively). Various reasons for these recalls are discussed.

1032 EFFECTS OF SIX DIETARY PHYTOCHEMICALS ON AFLATOXIN B_1-MEDIATED GENOTOXICITY AND GENE EXPRESSION IN HUMAN HEPATOCYTES AND HEPG2 CELLS.

K. Gross-Steinmeyer[1], K. M. Bradley[1], P. L. Stapleton[1], F. Liu[1], J. H. Tracy[1], T. K. Bammler[1], R. P. Beyer[1], S. C. Strom[2] and D. L. Eaton[1]. *[1]Department Environment & Occup. Health Sciences, University Washington, Seattle, WA and [2]Department Pathology, University Pittsburgh, Pittsburgh, PA.*

Six dietary phytochemicals, curcumin (CUR), 3, 3'-diindolylmethane (DIM), isoxanthohumol (IXN), 8-prenylnaringenin (8PN), phenethyl isothiocyanate (PEITC) and sulforaphane (SFN) protect animals against chemically induced tumors. The ability of these phytochemicals to reduce DNA-adduct formation of the hepatocarcinogen, aflatoxin B_1 (AFB), was examined in cultures of human primary hepatocytes (PH) and human hepatoma cells (HepG2). In PH, SFN (50 uM) significantly decreased, whereas DIM (10 uM) and 8PN (10 uM) increased AFB-DNA adduct levels. In HepG2 cells, PEITC (5 uM) and SFN (3 uM) also decreased, and DIM (3 uM) also increased AFB-DNA adduct levels. We determined the effects of phytochemicals on mRNA expression of enzymes involved in biotransformation of AFB (e.g. CYPs 1A1, 1A2, 3A4, 3A5, 3A7, GSTM1) by RT-PCR. CYP1A1/2 mRNA was up-regulated by all six chemicals at 10 and 50 uM. DIM induced CYP1A1 (up to 474-fold) and 1A2 (up to 113-fold) in a dose-related manner from 0.1-50 uM, and this was generally reflected in protein levels (Western blot) and enzyme activity (MROD). CYP3A4, 3A5 and/or 3A7 transcription was down-regulated by all phytochemicals whereas GSTM1 mRNA was not altered. CUR and DIM, but not SFN, inhibited human CYP1A2-mediated MROD activity *in vitro*. Inhibitory effects of PEITC and IXN on hCYP1A2 activity were reported previously by others. Microarray analysis in human hepatocytes revealed that mRNA levels of many genes were affected. For example, DIM significantly increased mRNA of more than 80 genes, including CYPs 1A1, 1A2, 2B6, UGT1A9, and SULT1A3. Collectively, these data suggest that each compound exhibits its AFB-DNA damage modulating effects *via* different mechanisms. The increase in DNA damage by DIM raises potential safety risks involved in consuming dietary supplements of indole-3-carbinol, the precursor of DIM (Supported by NIH grants R01 ES05780 and P30 ES07033)

1033 EFFECTS OF INCREASING PERCENTAGE OF DIETARY MAIZE ON RATS.

S. A. MacKenzie[1], N. E. Everds[1], L. A. Malley[1], G. S. Ladics[1], J. F. Hansen[1], I. Lamb[2] and G. Dana[2]. *[1]DuPont Haskell Laboratory, Newark, DE and [2]Pioneer Hi-Bred International, Inc., Johnston, IA.*

The objective of this study was to assess the maximum percent of dietary maize that could sustain adequate health and growth in rats. Six groups of male and female Crl:CD®(SD)IGS BR rats (10/sex/group) were fed diets containing 33, 55, 70, 80, 90, or 100% maize. An additional control group was fed PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 (contained 33% maize; referred to as 0%). Diets were supplemented to provide adequate nutrients, where possible. Rats were evaluated for body weights, food consumption, clinical signs, neurobehavioral, ophthalmological, clinical and anatomic pathology parameters, and allergenicity. The 100% group was sacrificed after 4 weeks due to mortality. In groups fed up to 90%, no effects were observed on survival, clinical or ophthalmological parameters, food consumption, hematology, urinalysis, neurobehavioral, anatomic pathology (females only), or allergenicity parameters. The NOEL in females was 90% maize, based on body weight loss and mortality in the group fed 100%. The NOAEL in males was 80% maize, based on lower body weight gain and food efficiency, clinical chemistry changes, and increased hepatic fatty change in groups fed 90% maize. The NOEL in males was considered to be 55% maize, as minimally increased prothrombin and activated partial thromboplastin times were observed in individual rats in groups fed 70% and above; however, the increased coagulation times were not associated with any correlative adverse effects in males fed up to 90% maize.

1034 IMPACT OF 30-DAY ORAL DOSING WITH N-ACETYL-L-CYSTEINE ON SPRAGUE-DAWLEY RAT PHYSIOLOGY.

E. W. Johnson[1], A. R. Thitoff[1], A. E. Jung[1], J. S. Eggers[2], S. L. Lohrke[1], A. J. Bobb[1] and D. P. Arfsten[1]. *[1]Naval Health Research Center Toxicology Detachment, Wright-Patterson AFB, OH and [2]Air Force Research Laboratory, Brooks AFB, TX.*

Clinical trials have shown that administration of N-acetyl-L-cysteine (NAC) has beneficial effects in persons afflicted with HIV, Sjogren's Syndrome, Alzheimer's disease, and acute lung injury. Studies in animals have demonstrated a protective effect of NAC when given prior to toxic chemical exposure. This effect may be attributed to free radical quenching by NAC or to NAC serving as a cysteine source for glutathione (GSH) synthesis. The long-term impact of NAC intake on liver, kidney, and skin GSH/GST levels, GI tract, liver, and kidney histopathology, and serum chemistries were evaluated in adult Sprague-Dawley (SD) rats. Groups of 20 SD rats (10 male, 10 female), 8 weeks of age, were dosed daily by oral gavage for up to 30 days with deionized H2O (negative controls) or NAC at a rate of 600 or 1, 200 mg/kg. Animals were euthanized on study days 5 or 30 at 6 hours after treatment. There were no significant differences in final body weights or weekly average weight gain between treatment groups at both the 5- and 30-day study endpoints. At study day 30, serum alanine aminotransferase (ALT) activities were significantly elevated ($p \leq 0.05$) for animals treated with NAC as compared with controls. Serum creatinine concentrations from animals treated with 600 mg/kg for 30 days were nearly 2 times higher ($p \leq 0.05$) than those for animals treated with dH_2O or 1, 200 mg/kg NAC; 50% of these animals had concentrations above the normal cut-off of 0.65 mg/dl. No treatment-related histopathology was found in analysis of tissues from animals treated for 5 or 30 days. Repeat oral treatment with NAC did not increase skin, liver, or kidney GSH levels or GST activity. Further studies are planned to determine if NAC treatment possibly results in an increase in cysteine concentrations present in these tissues.

1035 COMBINATIVE TOXICITY OF MULTIPLE MYCOTOXIN MIXTURES IN ANIMALS AND HUMAN CELLS.

C. Mckean, L. Tang, M. Billam, H. Luo, M. Tang, C. Theodorakis, R. Kendall and J. Wang. *EnvTox/TIEHH, TTU, Lubbock, TX.*

Ubiquitous co-contamination of multiple mycotoxins in dietary components has been widely reported and is believed to play an important contributing role in high incidence of primary liver and esophageal cancers in certain areas of the world. Aflatoxin B_1 (AFB_1), T-2 Toxin (T2) and fumonisin B_1 (FB_1) are important foodborne mycotoxins that historically caused a variety of acute mycotoxicoses in ani-

mals and humans. In this study the combinative toxicity of AFB_1, T-2, and FB_1 was tested in F-344 rats, Mosquitofish (*Gambusia affinis*), and the immortalized human cell lines HepG2 and Beas-2B. This was accomplished by obtaining the LD_{50}, LC_{50} and IC_{50} for individual toxins in each model respectively, followed by testing the combinations beginning with two and based on those results finally three mycotoxins. Male F344 rats were divided into four treatment groups and dosed with 1/2, 1/3, 1/4, and 1/6 LD_{50} values for AFB_1 and T-2 respectively, while FB_1 remained constant at 10 mg/kg. Mortality in the highest dose group reached 83.33%, while no other deaths were recorded. Surviving animals had apparent toxic symptoms with the exception of the lowest dose. Healthy mixed sex mosquito fish (approx. 1:1 ratio) under optimized condition were divided into four treatments dosed with 1/3, 1/4, 1/6, and 1/12 LC_{50} AFB_1, T-2, and FB_1. Observation extended for 7 days resulting in a mortality of 83.34%, 62.52%, 25.02%, and 12.49% respectively. The cytotoxicity of treated HepG2 and BEAS-2B were assessed. Treatment of cells with 3/4, 1/2, 3/8, 1/4, and 1/6 IC_{50} of AFB_1, T-2, and FB_1 yielded *via*bilities of 67.3%, 73.2%, 78.1%, 98.1%, and 93.2%. All models demonstrated a significant dose response in observed parameters to treatment. Overall, however, the combination of three toxins demonstrated decreased toxicity over our predicted values and results of two combination trials. These results will provide foundational knowledge for future studies on long-term combinative toxic and health effects of these mycotoxins. (Supported by the research contract DAAD13-02 C-0070 from the SBCCOM, US Army).

1036 APPLE JUICE EXTRACT AND CERTAIN POLYPHENOLS IN APPLE JUICE ACT AS INDUCERS OF CYP1A1 AND MRP2.

C. Pohl[1], V. Emmerlich[1], H. Schmitz[1], F. Will[2], H. Dietrich[2] and D. Schrenk[1]. *[1]Food Chemistry and Environmental Toxicology, University of Kaiserslautern, Kaiserslautern, Germany and [2]Forschungsanstalt Geisenheim, Geisenheim, Germany.*

Flavonoids represent a group of phytochemicals exhibiting a wide range of biological activities such as antioxidant properties and their ability to modulate several enzymes. A number of reports have shown that flavonoids can induce the expression of several cytochromes P450 (CYPs) and ABC export pumps and inhibit or stimulate their activity. In this study we incubated the human colon carcinoma cell line Caco 2 with an apple juice extract (AJE) and also with single polyphenolic components of this extract (quercetin, rutin, phloridzin). In parallel experiments, the antioxidants ascorbate (0.25 mM) and a-tocopherol (0.05 mM) were added. AJE was cytotoxic at high concentrations (24 h; 1 mM as phloridzin), whereas the pure polyphenols and the glycoside rutin were cytotoxic at much lower concentrations: quercetin (1 h; > 50 μM), phloridzin (1 h; > 5 μM), rutin (1 h; > 5 μM), measured as significant inhibition of MTT reduction. These effects were completely abolished by the antioxidants indicating the formation of reactive oxidation products of the polyphenols as cytotoxic mechanism. After 48 hours of incubation with AJE or pure polyphenols (with ascorbate/tocopherol) mRNA and protein levels of CYP 1A1, 1A2, 1B1, 3A4, P-glycoprotein (Pgp), and Multidrug resistance protein (MRP) 1 and 2 were determined. CYP1A1 mRNA was not detectable in untreated cells but was induced significantly with AJE (> 50 μM; as phloridzin). MRP2 mRNA was detectable in untreated cells and was induced about 3-fold with 100 μM AJE (as phloridzin). All other genes tested were expressed in untreated cells but were not induced with AJE or the pure polyphenols. The polyphenols also induced CYP1A1 and MRP2. The effects at the mRNA level were paralleled by induction at the protein level. Calculations suggest that induction by AJE is almost exclusively due to phloridzin, one of the major polyphenolic constituents of apple juice.

1037 ONCOGENICITY EVALUATIONS OF SOY ISOFLAVONES AND BOWMAN-BIRK INHIBITOR IN $p53^{(+/-)}$ MICE.

D. McCormick[1], W. Johnson[1], R. Selby[1], L. Dooley[1], R. Morrissey[2], L. Arp[2] and J. Crowell[3]. *[1]IIT Research Institute, Chicago, IL, [2]Pathology Associates, Chicago, IL and [3]National Cancer Institute, Bethesda, MD.*

Epidemiologic data suggest that soy consumption may protect against cancer induction in several tissues. Although the soy components responsible for this activity remain unidentified, soy isoflavones (*e.g.*, genistein) and soy-derived protease inhibitors (*e.g.*, Bowman-Birk Inhibitor [BBI]) demonstrate chemopreventive activity in several experimental cancer models. As part of the preclinical development of soy isoflavones and BBI for cancer prevention, oncogenicity studies were conducted in $p53^{(+/-)}$ (heterozygous p53 knockout) mice. In each study, groups of 25 $p53^{(+/-)}$ mice per sex received daily gavage exposure to a soy component or vehicle for 6 months. PTI G-2535 (a soy isoflavone mixture containing 45% genistein, 23% daidzein, and 4% glycitein) was administered at 0, 250, 1000, or 2500 mg/kg/day in 0.5% aqueous carboxymethylcellulose. PTI G-2535 doses were selected using data from a subchronic dose tolerance study. BBI was administered at 0, 500, 1000, or 2000 mg/kg/day in distilled water. BBI dose was limited by viscosity. *p*-Cresidine (400 mg/kg/day) was used as a positive control article in both studies. Daily administration of PTI G-2535 to $p53^{(+/-)}$ mice induced a dose-related suppression of body weight gain in males only. Both sexes demonstrated increased liver and spleen weights, decreased RBC counts, and increased RBC volume and mean corpuscular hemoglobin. Females demonstrated increased plasma cholesterol and triglycerides. Daily administration of BBI to $p53^{(+/-)}$ mice had no effect on body weight, food consumption, clinical pathology, or organ weights in either sex. Neither agent induced any clinical evidence of toxicity, and gross pathology was unremarkable in both studies. Histopathologic evaluation of tissues demonstrated no increases in the incidence of either benign or malignant tumors in any group exposed to PTI G-2535 or to BBI. Neither PTI G-2535 nor BBI demonstrated any evidence of oncogenicity in the $p53^{(+/-)}$ mouse model. (NCI-N01-CN-15142 and N01-CN-95140)

1038 A SIMPLE CYTOTOXICITY AND GENOTOXICITY ASSAY FOR ENVIRONMENTAL MONTORING USING NOVEL PORTABLE INSTRUMENTATION.

A. W. Knight[2, 3], P. O. Keenan[1, 3], N. J. Goddard[2], P. R. Fielden[2] and R. M. Walmsley[1, 3]. *[1]BMS, UMIST, Manchester, United Kingdom, [2]DIAS, UMIST, Manchester, United Kingdom and [3]Gentronix Ltd., Manchester, United Kingdom.*
Sponsor: L. Walsh.

A simple assay capable of simultaneously measuring both general toxicity and more subtle genotoxicity, in aqueous environmental samples, will be described. The assay uses eukaryotic (yeast) cells, genetically modified to express a green fluorescent protein (GFP) whenever DNA damage, as a result of exposure to genotoxic contaminants, is repaired. A measure of cell proliferation is used to characterise general toxicity producing familiar EC50 and LOEC data. The assay protocol has been developed for proposed use in the field and hence employs dedicated, portable instrumentation, which will be described. A range of environmentally relevant substances has been evaluated using the assay, including solutions of metal ions, solvents and pesticides. Preliminary data comparing the response of the yeast assay to that of a standard Daphnia test in the analysis of the toxicity of 34 varied industrial waste effluents will be presented. The sensitivity to a wide range of substances suggests the assay should be useful for environmental toxicity monitoring.

1039 THE EFFECT OF URBAN PARTICULATE MATTER ON HUMAN LUNG CELLS.

A. Jalbert[3], N. Gordon[2], S. Langley-Turnbugh[3] and J. P. Wise[1]. *[1]Wise Laboratory of Environmental and Genetic Toxicology, Center for Integrated and Applied Environmental Toxicology, University of Southern Maine, Portland, ME, [2]Department of Chemistry, University of Southern Maine, Portland, ME and [3]Department of Environmental Science and Policy, University of Southern Maine, Portland, ME.*

Maine has the fastest growing asthma rate in the nation. The environmental factors behind the increasing rate and those that trigger asthma episodes are poorly understood. One factor is airborne particulate matter. We assessed the constituents of particulate matter that may be important in triggering asthma episodes. We evaluated the cytotoxic effect of, and the amount of metal uptake from, Urban Particulate Matter (UPM), in WTHBF-6 cells, a human lung cell line. UPM induced concentration-dependent cytotoxicity in cells grown in DMEM/F12 + CCS medium. The relative survival was 81, 65, 54, and 54% after exposure to 10, 50, 100 and 200 ug/cm^2 UPM respectively. We extracted UPM using 0.12 M NaCl solution (the largest component of the growth medium) at pH 7.0 for 24+ hours at 25oC or for 4 hours at 37°C. The metal levels were, in ug/mg (25°C values in parentheses): Mn 0.20 (0.18), Cd 0.036 (0.029), Cu 0.100 (0.088), Cr 0.004(0.003), Ni 0.022 (0.019), Zn 0.986 (0.466), and Pb 0.058 (0.034). Because the growth medium contains proteins that may extract and bind metals, we extracted using DMEM/F12 +CCS instead of the 0.12 M NaCl. The metal levels were, in ug/mg (25°C values in parentheses): Mn 0.053 (0.067), Cd 0.032 (0.032), Cu 0.451 (0.451), Cr 0.006(0.006), Ni 0.024 (0.027), Zn 1.63 (1.37), and Pb 0.084 (0.203). Based on these results a solution of metals was prepared to reflect the predicted metal concentrations for the 200 ug/cm^2 UPM concentration. This solution induced 61% relative survival. Metal uptake and cytotoxicity studies of human lung cells treated with the same UPM concentrations showed increasing levels of Cu, Zn and Pb in the medium as the particle concentrations increased, but only increased levels of Zn and Pb inside the cells. This suggests that these biosoluble metals may be toxic to human lung cells and a triggering factor in asthma episodes. This work was supported by NIEHS grant 1R01 ES 10838-01(J.P.W.).

1040 EFFECTS OF MOTORCYCLE EXHAUST INHALATION EXPOSURE ON CYTOCHROME P450 2B1, ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN RAT LIVER AND LUNG.

T. Ueng, C. Hung and H. Wang. *Institute of Toxicology, National Taiwan University, Taipei, Taiwan.*

The effects of motorcycle exhaust (ME) on metabolic and antioxidant enzymes and lipid peroxidation were determined using male rats exposed to 1:10 diluted ME by inhalation 2 h daily for 4 wk. For microsomal cytochrome P450 (P450) enzymes,

ME resulted in 3- and 6-fold increases of 7-ethoxyresorufin O-deethylase activities and a 3-fold increase and an 80% decrease of pentoxyresorufin O-dealkylase activities in liver and lung, respectively. The results of immunoblot analysis of microsomal proteins revealed that ME increased liver and lung P450 1A1 with minimal effects on P450 2E1. ME increased P450 2B1/2 proteins in liver but decreased P450 2B1 in lung. Similar to ME, treatment with an ME constituent m-xylene intraperitoneally at 0.5 g/kg/d for 4 d increased liver but decreased lung pentoxyresorufin O-dealkylase activity and P450 2B1 protein. For phase II enzymes, ME resulted in 53% and 2-fold increases of cytosolic NAD(P)H:quinone oxidoreductase activities and no effect and a marginal increase of microsomal UDP-glucuronosyltransferase activities in liver and lung, respectively. For antioxidant enzymes, ME produced 23% and 35% decreases of superoxide dismutase, 9% and 27% decreases of catalase, and no changes of glutathione peroxidase activities in liver and lung cytosols, respectively. For lipid peroxidation, the results of thiobarbituric acid assay showed that ME resulted in 2- and 3-fold increases of formation of malondialdehyde by liver and lung microsomes incubated with $FeCl_3$-ADP, respectively. The present study demonstrates that ME inhalation exposure differentially modulates P450 2B1 and antioxidant enzymes and increases susceptibility to lipid peroxidation in rat liver and lung.

1041 INTERACTION OF SIMULATED HUMAN BODY FLUIDS WITH MERCURY MINE WASTES.

J. E. Gray[1], P. L. Higueras[2], P. L. Hageman[1], G. S. Plumlee[1] and T. L. Ziegler[1]. *[1]US Geological Survey, Denver, CO and [2]Universidad de Castilla-La Mancha, Almaden, Spain.*

Abandoned Hg mines are found worldwide and human activities on these mines are generally unrestricted. Of particular concern, local road constructions sometimes utilize Hg mine wastes and farming activities are found in areas contaminated by such wastes. This study was designed to evaluate potential human exposure to Hg when such mine wastes are inhaled or ingested. Waste materials were studied from mines at Almaden, Spain, the worlds largest Hg district, which has produced about 250, 000 t of Hg, and from abandoned mines in the Terlingua district, Texas, which produced about 7, 000 t of Hg. The mine wastes contain abundant Hg sulfide, elemental Hg, and Hg salts, which constitute primary ore or compounds formed during Hg extraction. Mine waste samples were leached with simulated human body fluids to evaluate their capacity to release Hg. Leaching was carried out at 37o C for 24 hours (20:1, v/w) with a simulated gastric fluid, simulated lung fluid, protein-enriched fluid (RPMI-1640 with fetal bovine serium), and deionized water. The highest Hg concentrations were found in the simulated gastric fluid leachates, and then Hg decreased in the protein-enriched fluids, simulated lung fluids, and deionized water leachates, respectively. Gastric fluid leachates of mine wastes from the Almaden district were especially elevated in Hg, as high as 460 mg/L. Soot collected from the Terlingua mine retorts also produced gastric fluid leachates with elevated Hg concentrations (up to 9.5 mg/L). Protein-enriched leachates contained Hg as high as 81 mg/L; whereas simulated lung fluid leachates contained Hg as high as 48 mg/L. Data suggest that the Hg leaching capacity is most strongly controlled by the mineralogy of the mine wastes, and leachates with the highest Hg contents result from samples containing elemental Hg and Hg salts. Results indicate that ingestion or inhalation of even small amounts of Hg mine waste may lead to increased Hg contents in the human body, especially gastric fluids.

1042 THE TOXICOLOGICAL GEOCHEMISTRY OF DUSTS, SOILS, AND OTHER EARTH MATERIALS: INSIGHTS FROM *IN VITRO* LEACH TESTS.

G. S. Plumlee, P. J. Lamothe, G. P. Meeker, S. J. Sutley and T. L. Ziegler. *US Geological Survey, Denver, CO.*

Exposure to dusts, soils, and other earth materials results in chemical reactions between the materials and body fluids encountered along the exposure route such as lung or gastrointestinal fluids. *In vitro* physiologically-based leach tests provide useful insights into these chemical reactions and their potential toxicological implications. We have conducted such leach tests on asbestos, volcanic ash, dusts from dry-lake beds, mine wastes, wastes left from the roasting of mercury ores, mineral processing wastes, coal dusts and coal fly ash, various soils, and complex dusts generated by the World Trade Center collapse. Size-fractionated samples of well-characterized earth materials are reacted at 37°C for periods from 2 hours up to multiple days, with various proportions of simulated lung, gastric, intestinal, and/or plasma-based fluids (SLF, SGF, SIF, and PBF). Results show that different earth materials have different solubility and dissolution behavior *in vivo*. For example, biodurable asbestos and volcanic ash particles bleed off low levels of metals into the SLF, including metals such as Fe and Cr that may help generate reactive oxygen species and resulting DNA damage *in vivo*. In contrast, dry-lake dusts and concrete-rich dusts are highly alkaline and bioreactive, and cause substantial pH increases and other chemical changes in the SLF, SGF, and PBF. Many of the earth materials tested contain a variety of metals that can be quite soluble (bioaccessible). For example, acidic SGF readily solubilize metals (Hg, Pb, Zn, others) that form strong chloride complexes; although these metals partially reprecipitate in near-neutral SIF, complexes with organic ligands (amino, carboxylic acids) enhance their solubility. These metals are also quite soluble in near-neutral, protein-rich PBF because they form strong complexes with the proteins. In contrast, metalloids (As, Cr, Mo, W, V) that form oxyanion species are commonly more soluble in near-neutral pH SLF than in acidic SGF.

1043 THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES OF AMPHIBOLE FROM LIBBY, MT.

T. L. Ziegler[1], J. D. Hyde[2], G. P. Meeker[1], S. J. Sutley[1], P. J. Lamothe[1], I. K. Brownfield[1], T. M. Hoefen[1], G. S. Plumlee[1] and M. L. Witten[2]. *[1]US Geological Survey, Denver, CO and [2]The University of Arizona, Tucson, AZ.*

Fibrous amphibole minerals are suspected of being the causative factor in the high incidence of respiratory diseases in the residents of Libby, MT. Analysis of these materials has revealed a range of amphibole compositions, classified primarily as winchite with lesser amounts of richterite and tremolite. Because winchite and richterite are not among the 6 types of asbestos cited in the current OSHA regulations, their potential links to extensive health problems suggest that the mineralogical definitions currently used to regulate asbestos may be in need of refinement. The USGS has characterized a representative suite of 30 amphibole samples obtained from the site of the former vermiculite mining operations near Libby. Toxicological analysis of these samples, utilizing an alveolar epithelial type II cell model, indicates that the level of toxicological indicators is significantly greater than those measured on the toxicological standards of 5 types of asbestos minerals currently cited in OSHA regulations. Furthermore, the toxicological response varied among the 30 Libby samples. Extensive toxicity studies and geochemical solubility studies in biological fluids (simulated lung fluid, protein enriched, gastric fluid) are in progress. In addition to the range of amphibole mineral identity, a variety of morphologies was identified in the Libby amphiboles including asbestiform fibers, acicular crystals and prismatic crystals. The role of these varying morphologies in the toxicity of the Libby area amphiboles is uncertain but clearly in need of detailed study. Future data from this project will enhance the current state of knowledge about the mineralogical, geochemical, and toxicological perspective of asbestos amphibole and may provide a foundation for the reexamination of current definitions and analytical procedures.

1044 FLUORIDE MODIFIES ADHESION OF STREPTOCOCCUS PYOGENES.

J. Cao[1], J. luengpailin[2] and D. Ron J.[2]. *[1]Stomatology, The Great Wall Hospital, Beijing, China and [2]Microbiology, University of Louisville, Louisville, KY.* Sponsor: G. Jiang.

Human streptococcal isolates are confronted with numerous environmental challenges, one of which is the necessity that they adhere to one or more of the varied mucosal surfaces between their points of entry into the host and the site where physiological conditions favor colonization. To maintain themselves in these environments, the streptococci must be able to interact with different receptors on the distinct surfaces. These interactions are important in promoting bacterial attachment to the mucosal surface. Fluoride in drinking water has been acclaimed as one of the greatest public health achievements of the 20th century. Although the exact mechanisms of action of fluoride have not been defined, it is thought that the anion acts on cariogenic streptococci as well as by promoting remineralization of hydroylapatite. Recent study shown that Streptococcus sobrinus grown in medium supplemented with fluoride anion resulted in loss of glucan-binding lectin from the cell. These results have prompted a study on the effects of fluoride on adhesion of group A streptococci. Our results shown that fluoride inhibit the growth of S. pyogenes. 10 - 200 mM of NaF prevent the attachment of several stereotypes of S. pyogenes to buccal cells and to surfaces coated with collagen, fibronectin and laminin. NaF inhibited the adhesion of 4 strains S. pyogenes to buccal cells in a dose-related manner. When cells were grown in ^{3}H-thymidine, in the presence and absence of fluoride, and test the adhesion to Ln, Fn and Cn, it was showed that NaF inhibited the adhesion of S. pyogenes M5 to Cn, Fn and Ln. NaCl has no effect on adhesion. Our results also indicated that fluoride increases the secretion of M3 and M5 proteins and affects the synthesis of several proteins. Importantly, when the bacteria are grown in 0.5-3.0 mM sodium fluoride, the bacteria lose abilities to adhere to buccal cells and to plastics coated with extracellular matrix proteins. These results provide a valuable clue to investigate the effects and mechanism of fluoride in treating streptococcal infection.

1045 DEVELOPMENT OF SCREENING VALUES FOR SOIL AND SEDIMENT BASED ON PROTECTION OF FLORIDA WILDLIFE.

H. Ochoa-Acuna and S. M. Roberts. *Ctr. Env. Human Toxicology., University of Florida, Gainesville, FL.*

The current ecological risk assessment paradigm relies on first comparing site soil and sediment concentrations to screening values that are typically based on direct toxicity of the chemical(s) to invertebrates. When these criteria are exceeded, exposure and risks to wildlife (usually a *via*n and mammalian species) are modeled. It has been our experience that there is little consistency in the modeling of wildlife risks, even when the same sources of information are ostensibly used. In an effort to develop reproducible, defensible modeling of wildlife risks from contaminated soils and sediments at sites in Florida, a database of information relevant to exposure and risks for Florida species was constructed. Toxicity data for approximately 400 organic and inorganic chemicals were collected, and uptake factors were developed for each through modeling based on approaches developed by the USEPA, the Dutch Ministry of the Environment, and published reports. Also, exposure factors were developed for 30 Florida wildlife species. Toxicity data were collected from bioassays in which survival, reproduction, or developmental effects were monitored endpoints. Toxicity values for each of the 30 modeled species were calculated taking into account allometric differences. Bioaccumulation factors for sediments were estimated through modeling based on approaches endorsed by the USEPA. For contaminants with measured bioaccumulation values available in the literature, the model predictions are within the variability observed for empirical data. For soils, accumulation factors for earthworms were developed using a physiologically-based model. Correction factors were used to account for the lesser uptake of some insects. For plant matter, uptake was estimated based on a partitioning model. The wide range of species included in the database allows selection of the most applicable screening values based on the habitats present at a site. This work was supported in part by a contract from the Florida Department of Environmental Protection.

1046 DETECTION OF ORGANOCHLORINE CONTAMINANTS IN THE SHED SKIN OF SNAKES.

S. D. Holladay and D. E. Jones. *Veterinary Medicine, Virginia Tech, Blacksburg, VA.*

Corn snakes (Elaphe guttata) were fed thawed mice once per week. On the first feeding of each month, the thawed mice were injected, into the peritoneal cavity, with vehicle or vehicle containing a mixture of the organochlorine (OC) compounds: chordane, lindane (gamma-hexachlorocyclohexane), and a polychlorinated biphenyl (PCB) mixture, arochlor 1254. Snakes were weighed prior to feeding of contaminated mice, after which chemicals were injected into mice to result in exposure to the snakes as follows: chlordane: 2 mg/kg; lindane: 8 mg/kg; PCB: 4 mg/kg. Remaining weekly feedings each month were with non-contaminated mice. Shed skins from snakes exposed to vehicle or the OC mixture were collected for a period of six months and pooled by animal. Skins were finely chopped into small pieces and then ground to a fine grain consistency using mortar and pestle. OC contaminants were extracted from ground skins using solvents, and analyzed by gas chromatography equipped with electron capture. OC contaminants were readily detected in the shed skins: chlordane: 0.155 to 0.213 ppm; lindane: 0.028 to 0.042 ppm; PCB: 3.49 to 7.01 ppm. These levels of OCs are similar to previous levels detected in livers of wild-caught turtles. All snakes exposed to vehicle only had non-detectable levels of OC. These data suggest that the shed skin of snakes may serve as an elimination route for chemical contaminants, and as such may have utility as a non-invasive, non-destructive indicator tissue for assessing local environmental contamination.

1047 PATHOLOGY OF OCHRATOXISIS IN BROILERS.

A. -. Muthuswamy[1] and G. -. -[2]. [1]*Toxicology, University of Kentucky, Lexington, KY and* [2]*Department of Pathology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India.* Sponsor: M. Vore.

In India Ochratoxin A contamination in feed is widespread and causes massive reduction in production in the poultry industry. The present work was carried out, to characterize the pathology of Ochratoxin A in broilers. Broiler chicks (96 day-old) were randomly divided into four groups (24 each) and were given Ochratoxin A in feed at 0, 1.5, 2.5 and 4 ppm for 4 weeks. All birds were weighed on day-one and at weekly intervals. Six from each group were sacrificed from 1st to 4th week. A highly significant ($P < 0.01$) reduction in body weight was shown from 2nd week in birds treated with 4 ppm of Ochratoxin A. In toxin treated groups, there was significant reduction ($P < 0.05$) in values of packed cell volume (4 ppm), haemoglobin (all groups) and total erythrocyte count (all groups) from that of control birds. The values of serum total protein, albumin, cholesterol, uric acid, creatinine and serum alkaline phosphatase showed highly significant difference (all groups) from that of control birds. Clinical signs like listlessness, reduced feed intake, polydipsia, depression of growth and diarrhea were exhibited by Ochratoxin A treated birds. Grossly, kidneys were enlarged, pale in colour; liver was enlarged with distended gall bladder; spleen was atrophic and bursa of Fabricious showed reduction in size from first week onwards. Microscopically, kidneys showed tubular dilatation, foci of mononuclear cell infiltration, vacuolar degeneration, apoptotic bodies and thickening of glomerular basement membrane. Liver showed focal mononuclear cell collection, Kupffer cells hypertropy, degeneration of hepatocytes, biliary epithelial hyperplasia and microgranuloma formation. Spleen, bursa of Fabricious and caecal tonsils showed lymphoid depletion. Pancreas showed acinar cell degeneration, hyperplastic and hypertrophic changes in islets. The histopathologic changes were found to be duration dependent, with the increased severity with increased time.

1048 MERCURY AFFECTS NEUROCHEMICAL RECEPTOR BINDING CHARACTERISTICS IN THE CEREBRAL CORTEX AND CEREBELLUM OF WILD RIVER OTTERS.

N. Basu[1], K. Klenavic[2], A. M. Scheuhammer[3] and H. M. Chan[4, 5]. [1]*Natural Resource Science, McGill University, Montreal, QC, Canada,* [2]*Environmental and Resource Studies, Trent University, Peterborough, ON, Canada,* [3]*Canadian Wildlife Service, Ottawa, ON, Canada,* [4]*Center for Indigenous Peoples' Nutrition and Environment, McGill University, Montreal, QC, Canada and* [5]*Dietetics and Human Nutrition, McGill University, Montreal, QC, Canada.*

Fish-eating wildlife, such as river otters (Lutra canadensis), can bioaccumulate high levels of mercury (Hg) through their natural diets. Hg can impair the neuro-behaviour of these wildlife, but few studies have explored the sub-clinical changes in neurochemistry that may precede permanent behavioural outcomes. The objective of this study is to determine the relationship between neurochemical receptor binding characteristics and mercury exposure in river otters. River otters were collected in Ontario and Nova Scotia (Canada) by local trappers, and concentrations of total Hg and methyl Hg (MeHg) were measured in the cerebral cortex and cerebellum. Receptor-binding assays for the cholinergic muscarinic acetylcholine (mACh) receptor were conducted to calculate the receptor density (Bmax) and ligand affinity (Kd). In the cerebral cortex, there was a significant correlation between Hg and mACh receptor density (total Hg: $r = 0.72$, $p<0.001$; MeHg: $r = 0.82$, $p<0.001$). In contrast to the cerebral cortex, a significant negative correlation was calculated between Hg and mACh receptor density in the cerebellum (total Hg: $r= -0.55$, $p<0.05$; MeHg: $r = -0.61$; $p<0.05$). Collectively, these results show that environmentally relevant concentrations of Hg may affect mACh receptor density in specific brain regions of river otters, and the physiological and ecological implications of these changes need to be addressed. In conclusion, neurochemical receptor binding characteristics (i.e. mACh and dopaminergic receptors) represent potentially novel biomarkers of Hg exposure and effect in wildlife.

1049 THYROID AXIS INHIBITION IN *XENOPUS LAEVIS*: GENE EXPRESSION CHANGES IN THE BRAIN.

S. J. Degitz[1], J. J. Korte[1], G. W. Holcombe[1], P. A. Kosian[1], J. E. Tietge[1], C. M. Bailey[2], N. Veldhoen[2], F. Zhang[2] and C. C. Helbing[2]. [1]*MED, USEPA, Duluth, MN and* [2]*Univesity of Victoria, Victoria, BC, Canada.* Sponsor: J. Nichols.

Previously, we have proposed an amphibian-based thyroid axis screening assay in which NF stage 54 tadpoles are exposed for 14 day and disruption of the axis is determined by measuring developmental rate and thyroid morphology. Using this approach we have conducted experiments establishing comprehensive dose responses for three model thyroid axis antagonists methimazole, 6-propylthiouracil, and perchlorate. In the current experiments we have examined gene expression changes in the brain following exposure to these inhibitors using cDNA and oligonucleotide arrays specific to *Xenopus laevis*. Stage 54 tadpoles were exposed to methimazole (25 mg /L), 6-propylthiouracil (20 mg/L) and perchlorate (1000 ug/l) for 24, 48, or 96 hr, the brains were removed and processed to gene array analysis. We have previously shown that these chemical concentrations have comparable impacts on both developmental rate and thyroid gland morphology. A couple of the most interesting changes observed include those with myelin basic protein and myelin proteolipid protein. Both genes were up regulated by all three inhibitors in a time dependent fashion. These inhibitors are known to have their direct action on the thyroid gland and it is likely that these changes observed in the brain are a consequence of decreases in circulating T4, rather than a direct action of the chemicals. From these experiments we conclude that these genes show promise as biomarkers of thyroid axis inhibition in developing amphibians. This abstract does not necessarily reflect EPA policy.

1050 AQUATIC MICROBIAL POPULATION AND COMMUNITY RESPONSES TO SELECT SSRIS.

B. W. Brooks[1], D. Fadelu[1, 2], E. A. Glidewell[1] and R. Massengale[2]. [1]*Environmental Studies, Baylor University, Waco, TX and* [2]*Biology, Baylor University, Waco, TX.* Sponsor: M. Kanz.

Select selective-serotonin reuptake inhibitor (SSRI) pharmaceuticals are reported to affect microorganisms, perhaps by inhibiting efflux pumps. Because microbial communities form the base of stream ecosystem food webs, such a potential effect

of SSRI on aquatic microorganisms is of particular interest. Recent research indicates that pharmaceutical and personal care products are found in effluent-dominated streams. Our objectives were to assess response of a model bacterium, Vibrio fischeri, and microbial communities following individual and combined exposures to the commonly prescribed therapeutics fluoxetine, fluvoxamine and citalopram. Community responses were assessed with carbon-utilization pattern analysis of aquatic microbial populations collected from an uncontaminated regional reference stream. Temporal community responses to SSRI exposure of 0, 10, 100, 1000, 10000 ng/L, which represent environmental realistic concentrations, were determined using Biolog ECOplates. For fluoxetine, carbon-utilization analyses indicated significant differences in carbon-utilization following exposure for 24-96 hours. Furthermore, analysis of average well-color development indicated that utilization of 5 carbon sources was significantly inhibited with increasing fluoxetine concentration. This inhibition was observed with fluoxetine concentrations as low as 1 ng/L suggesting that ambient aquatic microbial population carbon-utilization is suppressed by environmentally realistic fluoxetine exposures. Based on acute studies with three SSRI and a model marine microorganism, aquatic microbial responses to SSRI mixtures are suggested to be additive. However, future studies are required to assess microbial community responses to SSRI mixtures. Our preliminary data suggest that carbon-utilization patterns provide a valuable technique to study specific environmental or non-target effects of SSRIs on aquatic microorganisms.

1051 INHIBITION OF GERMINAL VESICLE BREAKDOWN (GVBD) IN XENOPUS OOCYTES *IN VITRO* BY A SERIES OF SUBSTITUTED GLYCOL ETHERS.

D. J. Fort[1], J. H. Thomas[1], J. H. Thomas[1], P. D. Guiney[2] and J. A. Weeks[2]. *[1]Fort Environmental Laboratories, Stillwater, OK and [2]Product Safety, Toxicology & Environmental Assessment, SC Johnson & Son, Inc., Racine, WI.*

A 24-h *in vitro* Xenopus oocyte maturation assay was used to screen chemicals for endocrine disruption activity. Under normal conditions, Xenopus oocytes undergo final maturation which is induced by progesterone *via* a membrane bound receptor (OMPR) or androgens *via* a classical intracellular receptor (AR). Final maturation is marked morphologically by germinal vesicle breakdown (GVBD). Because the Xenopus oocyte progesterone receptor is membrane bound, chemicals in the medium may directly interact with the OMPR. The progestin/antiprogestin activities of a series of substituted glycol ethers including: ethylene glycol monomethyl ether (EGME), -monoethyl ether (EGEE), -monopropyl ether (EGPE), -monobutyl ether (EGBE), -monophenyl ether (EGPhE), diethylene glycol monomethyl ether (DGME), and triethylene glycol monomethyl ether (TGME) were screened using the *in vitro* Xenopus oocyte GVBD assay. Results suggested that each of these test materials were capable of inhibiting GVBD *in vitro* in a concentration dependent manner with the following relative potency: EGPhE > EGME >> EGEE > EGPE >> EGBE >> DGME > TGME. Potency, expressed as an IC25, ranged from 1.0 ug/L for EGPhE to >10 mg/L for TGME. In each case, androstenedione was capable of reversing the inhibition. These results, combined with previous OMPR binding studies, suggest that effects of this glycol ether series may be mediated through the OMPR, and not the AR. Overall, results suggest that the Xenopus oocyte GVBD assay could be useful in high throughput progestin/androgen screening.

1052 TOXICOKINETICS OF PCBs IN TADPOLES OF THE GREEN FROG (*RANA CLAMITANS*): DOES METAMORPHOSIS AFFECT ELIMINATION RATES.

J. L. Leney, D. G. Haffner and K. G. Drouillard. *Great Lakes Institute for Environmental Research (GLIER), University of Windsor, Windsor, ON, Canada.* Sponsor: R. Letcher.

In recent decades, worldwide declines in amphibian populations have been observed. One of the possible explanations for this phenomenon is the increase in levels of toxic chemicals in the environment. In order to determine the relative hazard of various chemicals to amphibians, a life cycle exposure model must be developed. Such a model could be used to identify critical exposure periods during the amphibian life cycle. The focus of the present study was to investigate the exposure dynamics of polychlorinated biphenyls (PCBs) in tadpoles of the green frog (*Rana clamitans*). This investigation involved determining the elimination rates of 79 PCB congeners from dosed tadpoles. Tadpoles were dosed by feeding fish food spiked with a 1:1:1 mixture of Aroclors 1248, 1254, and 1260. Over a period of 7 days, approximately 20 g of PCB-spiked fish flakes were placed into a tank containing *R. clamitans* tadpoles. The total PCB concentration in the food was found to be 229.1 mg/g (SEM=26.9 mg/g, n=3). Spiked tadpoles were then placed into clean water along with control tadpoles. Control and dosed tadpoles were kept separate by mesh dividers. Tadpoles were sampled on various dates over a 44-day period as they underwent metamorphosis. Sampled tadpoles were analyzed to determine tissue residue concentrations for 79 PCB congeners. The average initial total PCB concentration in the dosed tadpoles was 11.0 mg/g (SEM=1.3 mg/g, n=6). At many sampling dates, some tadpoles were undergoing metamorphosis, while others had not yet started to transform. PCB elimination rates were determined separately for metamorphosed and non-metamorphosed tadpoles and results compared. The loss of lipid that occurs as tadpoles become frogs is predicted to increase the fugacity of PCBs and cause increased mobilization of these chemicals during metamorphosis. Results confirmed that metamorphosed frogs could be distinguished from tadpoles based on PCB congener profiles.

1053 EFFECT OF TEMPERATURE ON TOXICITY OF HEAVY METALS IN AQUATIC INVERTEBRATES.

M. A. Khan. *Biological Sciences, University of IL at Chicago, Chicago, IL.*

The effect of acute thermal stress and thermal acclimation on toxicity of copper, zinc, cadmium, and lead was studied in selected freshwater micro- and macro-invertebrates. The rate of oxygen consumption increased in zebra mussels (Dreissena polymorpha) by 100% during a rise in temperature from 20° to 25°C and in crayfish (Procambrus clarki) by 60% during a rise from 15° to 23°C. In the water flea (Daphnia magna) the rate of heart beat increased by 25% during a rise from 15° to 23°C. The acute lethal limits were 30°C for all these species. Thermal stress increased the acute toxicity of metals to these species. In zebra mussels, a rise from 20° to 25°C reduced the 24-hour LC50 value of copper from 774 to 238 mg/L and of zinc from 45 to 23 mg/L. The toxicity to daphnids increased more than 2-fold for cadmium and lead, 5-fold for copper, and 10-fold for zinc by 8°C-rise in water temperature. The toxicity of these metals to crayfish increased only about 2-fold by an 8°C rise in temperature. Lead, which caused 100% mortality in 14 hr at 28°C, took more than a week to cause this at 15°C. The toxicity of these metals and their negative effect on metabolism increased by raising temperature, or prolonging the duration of exposure of animals. Acclimation at respective temperatures for 2 weeks or longer, lowered the intensity of the effect on metabolism observed during thermal stress, as seen above. However, thermal effect on metal toxicity was still observed. These data indicate that the mechanism/s of toxicity of these metals are sensitive to temperature and may not be directly related with their effects on metabolism. These bio-indicators demonstrate that the global warming of bodies of water can make sub-lethal concentrations of these metals become lethal to and, thus, threaten the survival of aquatic food-chains.

1054 A DETERMINATION OF ARSENIC CONCENTRATIONS IN FISH FROM THE NORTH FORK OF AMERICAN FORK CANYON, UTAH.

W. Ball, T. Jewkes, J. Contreras and E. Revenaugh. *Environmental Epidemiology Program, Utah Department of Health, Salt Lake City, UT.*

The North Fork of the American Fork Canyon is located in the Wasatch Mountains approx. 40 miles SE of Salt Lake City, UT. In Aug 2002, three species of fish were collected for tissue sampling from the North Fork to assess the potential impacts from abandoned mining operations. Fifteen trout from the North Fork and five control fish from the Utah State Fish Hatchery in Springville, UT were collected and delivered to the Trace Element Research Laboratory at Texas A&M University for analysis. The fish were filleted (skin on) and analyzed for total arsenic and inorganic arsenic. Total arsenic was determined by inductively coupled plasma-mass spectroscopy. Inorganic arsenic was determined using hydride generation-atomic fluorescence spectroscopy. Arsenic concentrations were determined for two trout species native to the North Fork (brown and cutthroat trout) and for transplanted rainbow trout. Total arsenic concentrations in brown trout ranged from 0.098-0.612 mg arsenic per kg fish wet weight (mg/kg)with 0.9-6.9% inorganic arsenic. Cutthroat trout total arsenic was 0.062-0.137 mg/kg with 1.9-12.5% inorganic arsenic. Total arsenic in rainbow trout ranged from 0.065-0.244 mg/kg with <0.6-7.4% inorganic arsenic. Fish collected from the fish hatchery served as the control. The hatchery fish displayed higher concentrations of total arsenic, ranging from 0.413-0.748 mg/kg, but had a much lower percentage of inorganic arsenic, ranging from 0.3-0.7%. The hatchery trout presented the highest mean total arsenic concentration at 0.535 mg/kg. Brown trout had the highest mean inorganic arsenic concentration at 0.005 mg/kg. The mean percent inorganic arsenic content was comparable between the species; 3.15% for brown trout, 4.58% for rainbow, and 4.66% for cutthroat. The mean percent inorganic arsenic measured in hatchery trout was 0.52%. The percent of inorganic arsenic decreased with increasing concentrations of total arsenic. (This study is supported by Cooperative Agreement Number U61/ATU886120 from the Agency for Toxic Substances and Disease Registry)

1055 NONSPECIFIC IMMUNE RESPONSES IN TWO POPULATIONS OF ATLANTIC TOMCOD (MICROGADUS TOMCOD) EXPERIMENTALLY EXPOSED TO PCB 126: CONTAMINATED HUDSON RIVER VS. REFERENCE MIRAMICHI RIVER.

E. A. Berg, J. T. Zelikoff, I. I. Wirgin, N. K. Roy, J. E. Duffy and E. A. Carlson. *Environmental Medicine, New York University, Tuxedo, NY.*

The Hudson River (HR) estuary (NY) is severely polluted with polychlorinated biphenyls (PCBs), a class of compounds that bioaccumulates in many wildlife species, and alters immune function in both fish and mammals. Resistance to PCB-induced toxicity has been observed in Atlantic tomcod (Microgadus tomcod) from the HR (compared to reference site fish, Miramichi River [MR] in Canada). This study seeks to determine the effects of a coplanar PCB congener on the nonspecific immune response of laboratory-reared Atlantic tomcod bred from feral parents caught either in the HR or the MR, and to explore the role of resistance in PCB-mediated immunotoxicity. Fish were injected i.p. with either the vehicle control, 0.01, 0.1 or 1.0 ug PCB 126 / g BW, and examined after 7 and 14 d. Nonspecific immune function was evaluated by measuring intra- and extracellular superoxide ($O_2^{\cdot-}$) production and phagocytic ability, by kidney phagocytes. Hepatic cytochrome P450 1A1 (CYP1A1) mRNA induction was quantified by slot blot hybridization. After 7 d, phagocytosis was significantly depressed in MR tomcod exposed to PCB at the highest dose (compared to controls). Extracellular $O_2^{\cdot-}$ production was also decreased after 7 d, but only in HR fish exposed to 1.0 ug/g PCB; exposure to PCB had no effect on intracellular $O_2^{\cdot-}$ production in either population of fish. By 14 d, no effects were observed in either fish population for any endpoint. Moreover, no differences were observed between populations. The results demonstrate that the innate immune system of M. tomcod is only modestly sensitive to PCB 126-induced toxicity, and illustrates the importance of post-exposure duration for demonstrating immunotoxicity. Studies are currently ongoing to uncover the role of HAH-resistance in tomcod humoral and cell-mediated immune responsiveness. NIEHS Center Grant No. ES00260 and DOD Grant No. DAMD 17-99-2-9011.

1056 SEX STEROID HORMONES IN A WATERSHED DOMINATED BY CONCENTRATED ANIMAL FEEDING OPERATIONS.

E. Oberdoerster, P. Gravel, B. North, A. Dongell and J. H. Easton. *Southern Methodist University, Dallas, TX.*

The goal of this study was to determine the impact of Concentrated Animal Feeding Operations (CAFOs) on the Bosque River, TX. The levels of three sex steroid hormones, 17β-estradiol (E2), Progesterone (P), and Testosterone (T) were measured in the streams receiving effluent from the dairy CAFOs. In addition, largemouth bass were exposed to the levels of hormones found in the river to determine induction of vitellogenin (VTG) and changes in plasma hormone levels. Water samples were collected monthly from five sites on the Bosque River, four sites on tributaries, and one reference site. The Bosque sites are approximately 20 river miles apart, and one of the Bosque sites also receives municipal sanitary sewage discharge from the town of Stephenville, TX. Each month water samples were collected, sterile filtered, and extracted over a C18 disc, usually within 48 hours of collection. The hormones were eluted from the disc and analyzed using EIA. Hormone levels in the Bosque and it's tributaries showed a similar pattern each month, with hormone levels being highest near the northern end of the river (where the majority of the CAFOs are located), and then tapering off downstream. The reference site always had the lowest levels of hormones. The maximal hormone levels in the Bosque were 5 ng/L E2, 25 ng/L P, and 1 ng/L T. Juvenile bass were exposed in the laboratory to either 3 or 39 ng/L E2; 14 or 113 ng P; or a combination of 40 ng E2 and 13 ng P (measured doses). After two weeks, fish were sacrificed and plasma VTG, E2 and P were measured by ELISA and EIA, respectively. Under these conditions, there was no elevation of plasma VTG, E2 or P in the fish. Positive control fish that were injected with E2 had elevated VTG, E2 and P. In conclusion, sex steroid hormone levels were found at elevated levels in the Bosque River near the CAFOs. The hormone levels found in the Bosque were not high enough to induce VTG in a two-week fish exposure study. Others have shown that levels of E2 as low as 5 ng/L can induce VTG in other species of fish after a longer exposure period.

1057 LOW SPECIFIC ANTIBODY RESPONSES AND ELEVATED BIOINDICATORS OF INNATE IMMUNITY IN CREOSOTE-ADAPTED MUMMICHOGS, *FUNDULUS HETEROCLITUS*.

L. A. Frederick and C. D. Rice. *Biological Sciences, Clemson University, Clemson, SC.*

Several populations of the mummichog, *Fundulus heteroclitus*, exhibit characteristics of resistance or adaptation to high levels of priority pollutants along their east coast North America range. One such population inhabits the upper Elizabeth River estuary in Virginia at the Atlantic Wood site (AW). AW is a superfund site heavily contaminated with creosote containing a mixture of polycyclic aromatic hydrocarbons and some organometallics. Robust individuals comprise a thriving population at this site. Virtually all adults eventually develop various hepatic tumors. These fish are known to be resistant to BAP-induced CYP1A protein and EROD activity and over-express a form of GST, as well as p-glycoprotein multidrug resistance (MDR) protein. Although PAHs are known to be immunotoxic, the resident mummichog population seems to thrive. Mummichogs from the AW site and from a reference site, Kings Creek, Virginia were examined for circulating antibody titers against ubiquitous marine bacteria, including *E. coli, M. marinum, V. anguillarum, V. parahemolyticus*, and *V. natriegens*, as well as total immunoglobin and circulating lysozyme protein. The expression of lymphoid lysozyme protein and cycloxygenase 2 (COX 2) were also examined. Compared to high responses in reference fish, AW mummichogs had very low antibody titers against all bacteria examined, however expressed higher levels of circulating lysozyme. Lymphoid cells in the AW mummichogs also expressed more lysozyme and COX 2, which may indicate a state of activation and possible resistance. Taken together, our findings suggest that this chemical environment may be activating components of innate immunity, while suppressing humoral immune responses, thus compromising the AW mummichogs immune system. Present studies are underway to pathogen-challenge AW and KC mummichogs to further characterize possible differences in disease susceptibility and to determine possible mechanisms of action associated with overall differences in immune function between these two populations.

1058 ESTROGENIC COMPOUNDS IN FISH BILE IDENTIFIED WITH BIOASSAY-DIRECTED FRACTIONATION.

C. J. Houtman, A. M. van Oostveen, M. H. Lamoree, A. Brouwer and J. Legler. *Institute for Environmental Studies, Vrije Universiteit, Amsterdam, Netherlands.*

In fish, conjugates of many estrogenic chemicals, endogenous as well as xenobiotic, are excreted *via* bile into the intestine. Therefore, measurement of estrogenic activity in bile could yield useful information about the animal's internal exposure to (xeno-)estrogens. Although estrogenic activity in male fish bile has been reported before, the contribution of natural hormones and xenobiotic substances to this activity is unknown. To elucidate compounds responsible for estrogenic activity, we developed a bioassay-directed fractionation method for the identification of estrogenic chemicals in fish bile. In this approach, the *in vitro* reporter gene ER-CALUX assay (Estrogen Responsive Chemical Activated Luciferase Gene Expression) was used to assess estrogenic activity in deconjugated bile samples and to direct RP-HPLC fractionation and chemical analysis (by GC-MS/MS) of estrogenic compounds. The method was applied to bile from male breams (*Abramis brama*) collected at three locations in the Netherlands. At one of these locations, the small River Dommel, extremely high levels of plasma vitellogenin and high prevalence of intersex gonads in male bream have been observed, indicating the exposure of these fish to high levels of estrogens. In our study, we detected many xenobiotic chemicals in the bile fractions. Nevertheless, natural hormones (17β-estradiol, estrone and estriol) accounted for the majority of estrogenic activity in male bream bile. At the Dommel River, the synthetic contraceptive pill component ethynyl estradiol was detected in effective concentrations as well. Observed natural and synthetic hormones probably originate from an anthropogenic source, e.g. a sewage treatment plant, and may be responsible for the estrogenic effects observed in wild bream.

1059 EFFECTS OF ACUTE, SUB-LETHAL SODIUM ARSENATE EXPOSURE ON MIGRATORY BIRD MODELS: MITOCHONDRIAL FUNCTION, OXIDATIVE STRESS AND TIME OF FLIGHT.

J. M. Brasel[2, 1], R. Cooper[2, 1] and C. A. Pritsos[1, 2]. [1]*Nutrition, University of Nevada, Reno, NV and* [2]*Environmental Sciences and Engineering, University of Nevada, Reno, NV.*

Arsenic is distributed in the environment at a wide range of concentrations. It is primarily transported in the environment by water and these concentrations vary due to both natural and anthropometric factors. Human factors contributing to higher than normal arsenic levels include: arsenical pesticide application; mining, and metal fabrication. Exposure of migratory birds to arsenic occurs as the result of agricultural runoff into surface waters and accumulation in mine waste ponds. The impact of this exposure has not been thoroughly investigated. We tested the impact of acute, sub-lethal exposure to arsenate on mitochondrial function, oxidative stress and time of flight using surrogate migratory bird species. Na-Arsenate toxicity at 0.0, 20.0, and 40.0 mg/kg *via* gavage were performed on mallard ducks as a surrogate species of migratory birds. Arsenic disrupts normal mitochondrial function, which can result in diminished intracellular ATP levels and also induce oxidative

stress. Brain, heart, and liver tissues were excised and analyzed two hours post exposure for mitochondrial respiratory control ratios (RCRs), antioxidant enzyme activities and lipid peroxidation. At 20mg/kg significant decrease (P<0.05) in RCRs were seen in both heart and brain tissues. A dose-dependent increase in superoxide dismutaste (SOD) activity was observed in all tissues, however, notable changes were not observed in catalase, glutathione peroxidase, or glutathione reductase. A dose-dependent increase in lipid peroxidation was also noted in heart and brain tissues with significance (P<0.05) in the brain at 40mg/kg. Significant increase (P<0.05) in sera creatine kinase (CK) indicating biological damage was demonstrated 24 hours following 20mg/kg exposures. Currently, studies with our Homing Pigeon Model are underway to determine if low-level acute exposures to arsenic have an affect on a*via*n time of flight. This project was funded in part by USDA Hatch Grant NEV 00727 and the Nevada Ag. Experiment Station.

1060 ASSESSING ENDOCRINE-ACTIVE COMPOUNDS IN A JAPANESE QUAIL REPRODUCTION STUDY DESIGN.

L. Brewer[1], J. Stafford[1], E. Mihaich[2] and R. A. Becker[3]. [1]*Springborn Smithers Lab., Inc., Snow Camp, NC,* [2]*Rhodia, Raleigh, NC and* [3]*American Chemistry Council, Arlington, VA.*

A reproduction study in an a*via*n species is part of the EPA planned endocrine screening program. Japanese Quail, (Coturnix coturnix japonica, CCJ) offer advantages compared to other a*via*n species. A single generation study in CCJ was designed to follow OECD guidance & included 4 additional endocrine endpoints to see if this enhanced design could improve assessment of endocrine-active compounds. Endpoints added were measurements of: 1) excreted steroid hormones; 2) egg yolk estrogen levels; 3) oviduct growth & development in 14-day hatchlings exposed to the test substance post-hatch ; 4) circulating estrogen & testosterone levels. Methoxycholor (MXC), a prototypical estrogenic substance, was administered in dosed feed at 0, 5, 20, 80 & 320 ppm ad libitum for 6 weeks (commencing 2-weeks after demonstrated egg production). MXC clearly suppressed estrogen levels in fecal/urate/urine samples collected at 3-day intervals from breeding pairs. Excreted testosterone levels seemed unaffected. Egg yolk estrogen was highly variable in all groups & was not significantly different among groups. MXC-treated chick oviduct weight increased, but not as much as in those of positive control birds exposed to estradiol, & the increase did not show a clear dose-response relationship. Body weight of MXC-exposed chicks increased at all exposure levels except 300 ppm (indicating systemic toxicity at this high dose). Mean blood estradiol & testosterone levels were not significantly different at 3 time points: acclimation, lights up & study termination. Monitoring excreted steroid hormone levels & 14-day oviduct & body weights in combination appear to be the most sensitive endpoints in this study. This study demonstrates the relative effectiveness of different endocrine-specific endpoints & the feasibility for using these with a CCJ reproduction study design within an endocrine assessment program. Additional evaluations with other estrogenic substances are recommended before final conclusions are made about the added value of these endpoints.

1061 THE AFRICAN FISH EAGLE: DEVELOPING A BIOSENTINEL MODEL TO STUDY ENVIRONMENTAL POLLUTION IN UGANDA.

W. K. Rumbeiha[1], S. Hollamby[1], J. Sikarskie[4], C. Dranzoa[2], J. Kaneene[1] and W. Bowerman[3]. [1]*Pathobiology and Diagnostic Investigagtion, Michigan State University, East Lansing, MI,* [2]*Wildlife and Animal Resource Management, Makerere University, Kampala, Uganda,* [3]*Environmental Toxicology, Clemson University, Pendelton, SC and* [4]*Small Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

Uganda, like other developing countries, has in the past used persistent organic compounds in the agricultural, public health and industrial sectors. Besides, some of these compounds can drift across continents. There is no environmental biosentinel model to monitor enironmental pollution and to assess the impact of such pollutants on environmental health in this Great Lakes region of Africa. The objectives of this study were to develope the African Fish Eagle (Haliaeetus vocifer) as a biosentinel model in this region, and secondly to determine the concentrations of persistent organochlorinated pollutants (POPs) and their metabolites, and of total mercury in blood plasma and/or feathers collected from birds in the proximity of an industrial area (Entebbe) on the shores of Lake Victoria and a control site (Lake Mburo) in South Western Uganda. The hypothesis tested was that Fish eagle samples collected from Entebbe contain higher concentrations of POPs than those from L. Mburo. Samples were collected from 33 Fish eagles at the two study sites (15 at Entebbe and 18 at Lake Mburo) and analyzed for chlorinated pesticides, PCBs, or total mercury. Mercury concentration was significantly higher in samples collected from Entebbe than those from L. Mburo (p< 0.05). Five samples from Entebbe tested positive for 4, 4'-DDE at concentrations ranging from 0.001-0.005 ppm wet weight. No PCBs were detected in any samples at the limit of detection of 0.003 ppm. Considering the limited sample size, these results suggest that there is more POP pollution on the shores of Lake Victoria than Lake Mburo.

1062 DETECTION OF A CRITICAL PERIOD NECESSARY FOR ATRAZINE-INDUCED MAMMARY GLAND DELAYS IN RATS.

J. L. Rayner[1] and S. E. Fenton[2]. [1]*Department of Environmental Sciences & Engineering, University of North Carolina, Chapel Hill, NC and* [2]*Reproductive Toxicology Division, NHEERL, ORD, USEPA, Research Triangle Park, NC.*

In utero exposure during gestational days (GD) 15-19 to 100 mg/kg atrazine (ATR) was shown to delay mammary gland (MG) development in the female offspring of Long Evans (LE) rats. To determine if there is a critical period of ATR exposure, dams were gestationally dosed at varying 3-day intervals and their offspring evaluated. Timed-pregnant LE rats (N= 8/group) were gavaged GD13-15, 15-17, 17-19, or 13-19 with 100 mg ATR/kg body weight (BW) or vehicle (controls, C) GD13-19. BW and MG development were compared on postnatal day (PND) 4, 22, 25, 33, and 46. No treatment effects on pup BW were detected at these times. MG whole-mounts were subjectively scored (2 scorers) for development (branching, lobules, end buds) and epithelial areas were measured (mm^2). MG taken from GD15-17, 17-19, and 13-19 offspring (N>4dams/group, d/g) at PND4 displayed significant delays in epithelial development compared to C ($p<0.002$), and all exposed MG were smaller in area ($p<0.001$). At PND22 (N=3 d/g), MG taken from offspring in GD17-19 and 13-19 groups were developmentally delayed ($p<0.009$ vs. C), and areas were reduced in MG from those groups and GD15-17 ($p<0.008$). MG from animals in all groups were developmentally delayed at PND25 (N>5 d/g), 33 (N>6 d/g), and 46(N>4 d/g); $p<0.05$, $p<0.0002$, and $p<0.0004$ respectively, when compared to MG taken from C. Due to observed MG delays, we hypothesized that ATR-exposed animals may have difficulties in nursing their young. Females exposed *in utero* to either ATR (all groups as defined above) or C (N>3 in separate litters/group) were bred on PND68 and allowed to rear their offspring. Both female and male F2 fromGD17-19 and GD13-19 groups were significantly smaller in BW than those of C ($p<0.0001$, litter means, for both sexes) at PNDs 4 and 11, suggesting a need for further studies to clarify a possible adverse effect of ATR on MG development and function. *(This abstract does not necessarily reflect EPA policy; Supported by NHEERL-DESE, USEPA CT826513.)*

1063 DIFFERENTIATION OF MAMMARY TISSUE IS SEVERELY IMPAIRED IN PREGNANT MICE TREATED WITH TCDD.

B. A. Vorderstrasse[1], S. E. Fenton[2], A. A. Bohn[3], J. A. Cundiff[1] and B. Lawrence[1, 3]. [1]*Pharmaceutical Sciences, Center for Reproductive Biology, Washington State University, Pullman, WA,* [2]*RTD, NHEERL, USEPA, Research Triangle Park, NC and* [3]*Veterinary Clinical Sciences, Washington State University, Pullman, WA.*

The arylhydrocarbon receptor (AhR) is expressed in mammary epithelial cells, and inappropriate activation of the AhR during fetal development causes defects in mammary gland formation that persist into adulthood. However, it is not known whether the extensive differentiation of mammary tissue that occurs during pregnancy is also sensitive to disruption by AhR activation. To examine this, we exposed pregnant C57Bl/6 mice to a non-fetotoxic dose (5 μg/kg) of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) on days 0, 7 and 14 of pregnancy. Examination of mammary glands on days 9, 12, and 17 of pregnancy and on the day of parturition showed severe defects in development, including stunted growth, decreased branching, and poor formation of lobular alveolar structures. This impaired differentiation was biologically significant, as expression of milk protein in the gland was suppressed, and all pups born to TCDD-treated dams died within 24 hours of birth. Analysis of circulating progesterone, prolactin, and estradiol suggested that hormone production was impaired by TCDD exposure. However, hormone levels were affected only very late in pregnancy. Given that the observed defects in gland development preceded these hormonal effects, altered hormone levels are an unlikely mechanistic explanation for impaired mammary development. This novel finding that exposure to TCDD during pregnancy disrupts mammary gland development raises questions about the susceptibility of mammary tissue to direct injury by endocrine disrupting agents and the potential for AhR-mediated signaling to adversely affect lactation and breast tissue development in human populations.

1064 ONE-GENERATION REPRODUCTIVE TOXICITY STUDY OF FENITROTHION IN RATS.

N. Okahashi[1], K. Miyata[1], M. Sano[2], S. Tamano[2], H. Higuchi[1], Y. Kamita[1] and T. Seki[1]. [1]*Environmental Health Science Laboratory, Sumitomo Chemical Company, Ltd.,, Osaka, Japan and* [2]*Daiyu-Kai Institute of Medical Science, Ichinomiya, Japan.* Sponsor: T. Yamada.

Fenitrothion is a broad-spectrum organophosphate insecticide. It has been reported recently that Fenitrothion showed anti-androgenic activity in some *in vitro* and *in vivo* screening assays, while other reports exhibited no such effects in Hershberger

or prepubertal assays.To provide a comprehensive and higher tire assessment of the effects of Fenitrothion on reproductive and endocrine system, especially on anti-androgenic activity, we conducted a one-generation reproductive toxicity study in rats. Fenitrothion was administered to Crj:CD(SD)IGS rats in the diet at concentrations of 10, 20, and 60 ppm. Parental animals (P) were exposed for 10 weeks prior to mating, and through gestation and lactation until euthanasia. Their offspring (F1) were exposed from weaning until maturation at the age of 10 weeks. In P generation, brain cholinesterase activity was remarkably inhibited with statistical significance at 60 ppm in males and at 20 and 60 ppm in females. The inhibition in maternal animals was severe (-19% and -73% at 20 and 60 ppm, respectively). There were no effects on clinical signs, body weights and food consumption with exception of slightly depressed male food consumption in the early treatment period. Reproductive performance, organ weights, histopathology, and sperm analytical parameters were not affected. In F1 generation, no effects were observed on general toxicity, anogenital distance, retained areolae/nipples, age at the onset of puberty (vaginal opening, or preputial separation), organ weights, organ histopathology, and sperm analysis. In conclusion, Fenitrothion had no effects on reproductive and endocrine systems of P and F1 generations and on development of F1 offspring even at the toxic doses that induced severe inhibition of brain cholinesterase activity in P animals. The parental systemic no-observed-adverse-effect level (NOAEL) was estimated to be 10 ppm (0.64 mg/kg/day). The reproductive and developmental NOAELs were estimated to be at least 60 ppm (3.81 mg/kg/day).

1065 EFFECTS OF 20 WEEK EXPOSURES IN FEMALE SPRAGUE-DAWLEY (S-D) RATS TO THE DRINKING WATER DISINFECTION BY-PRODUCT DIBROMOACETIC ACID.

A. S. Murr and J. M. Goldman. *RTD, NHEERL, ORD, USEPA, Research Triangle Park, NC.* Sponsor: A. Cummings.

The drinking water disinfection by-product dibromoacetic acid (DBA) has been shown to adversely affect reproductive function in both male and female rats, albeit at exposure levels well in excess of those normally present in finished water. The present study was designed to explore the reproductive effects in non-pregnant female rats of DBA administered in the drinking water over more extended exposures. Regularly cycling S-D females were provided with 0, 50, 150 or 300 ppm DBA (pH adjusted 5.5 - 6) beginning at 11 wks of age and continued for 20 wks. Estrous cycles were tracked during alternating 2 wk periods throughout the study. In rats continuing to exhibit 4-day cycles, small quantities of tail blood were taken on the day of estrus for estradiol (E2) measurements during the 3rd, 11th and 19th wks of exposure. All animals were killed during wk 20 (diestrous day, 7 3/4 mo.of age). Organ wts were taken, and serum stored for E2, estrone (E1) and corticosterone assays. Calculated intake for 50, 150 and 300 ppm DBA over the 20 wks averaged 5, 16 and 34 mg/kg body wt, respectively. No treatment-related effects on cyclicity were observed over the course of exposure, and by wk 20 50-60% of rats in each group were still cycling regularly. Serum E2 levels in 4d cycling rats on the 3rd & 11th wks, however, showed an increasing dose-related trend, which was consistent with previous reported changes (Goldman/Murr, Repro. Toxicol., in press). For E1 at sac, all DBA dose groups were significantly elevated over controls. In normally cycling rats at 20 wks, there were no significant changes in ovarian, uterine, pituitary or adrenal wts, although liver showed a dose-related increase. In females that exhibited a persistent vaginal cornification, ovarian wts decreased with dose. The data indicate that while DBA elevated circulating levels of estradiol and estrone, the alteration was without effect on estrous cyclicity in this moderately estrogen-sensitive strain of rat. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1066 A FIVE GENERATION REPRODUCTIVE TOXICITY ASSESSMENT OF *P*-NONYLPHENOL (NP) IN CD SPRAGUE-DAWLEY RATS.

J. R. Latendresse[2], C. C. Weis[1], P. W. Mellick[2], R. R. Newbold[3] and B. Delclos[1]. *[1]NCTR, Jefferson, AR, [2]Pathology Associates, Jefferson, AR and [3]NIEHS, Research Triangle Park, NC.*

We are conducting a series of multigeneration studies under identical conditions with agents that have as one of their potential mechanisms of action interaction with estrogen receptors. NP, one of the test agents, is widely used as an intermediate in the manufacture of surfactants and polymer stabilizers. It has raised concern as a possible endocrine active toxicant because of its weak affinity for the estrogen receptor and reported reproductive effects in mammalian test systems and in aquatic organisms. In a dose range finding study, renal toxicity was found to be a limiting factor in dose selection. In the present multigeneration study, the parental generation was exposed to NP (25, 200, and 750 ppm) in a soy- and alfalfa-free diet (Purina 5K96) from 28 days prior to mating until necropsy at PND 140, the age at which all generations were terminated. The F_1 and F_2 generations were exposed continuously from conception, F_3 from conception until wean, and F_4 was not exposed to NP. Under the conditions of the study, clear adverse effects on the multiple reproductive endpoints assessed or on general toxicity were not observed in male or female rats. Microscopic evaluation revealed treatment effects only in the male kidney at the 750 ppm dose. Mineralization in male kidneys was observed only in the 750 ppm group in the F_0 through F_2 generations, with the strongest response in the F_1 and F_2 generations where exposure occurred from conception through termination of the study. An apparent treatment-related increase in renal medullary cysts (minimal severity) occurred in the 750 ppm group in the F_1 and F_2 generations, but the toxicological significance of this effect is doubtful given the appearance of a variable incidence of lesions of similar severity in the control groups of some generations. This study confirmed and extended the data indicating the renal toxicity of NP and did not support the reproductive toxicity of NP under the conditions used in this study.

1067 THE ARYL HYDROCARBON RECEPTOR (AHR) MAY ALTER ESTROGEN PATHWAYS IN THE MOUSE OVARY.

K. R. Barnett[1], W. Fritz[2], T. Lin[2], R. E. Peterson[2] and J. Flaws[1]. *[1]Epidemiology and Preventive Medicine, University of Maryland, Baltimore, Baltimore, MD and [2]University of Wisconsin, Madison, WI.*

The AhR is a ligand-activated transcription factor which functions in mediating the toxicity of various environmental toxicants. Although reproductive studies with AhR deficient (AhRKO) mice have shown defects in the female reproductive system, little is known about the role of the AhR in the mouse ovary. Previous studies in our laboratory have shown that AhRKO mice have a decreased number of antral follicles compared to wild-type (WT) mice. This decrease is not due to follicle death. Instead, it is a result of delayed follicular growth to the antral stage. Since estrogens are required for normal growth and maturation of ovarian follicles, one possible mechanism for this delayed follicular growth may be that the levels of estradiol (E2) or its receptors are altered in AhRKO mice compared to WT mice. Thus, these studies tested the hypothesis that AhRKO mice have lower levels of estradiol and its receptors (ERα & ERβ) than WT mice. To test this hypothesis, blood samples and ovaries were collected from AhRKO and WT mice at postnatal day (PD) 90. Blood samples were subjected to enzyme-linked immunoassay (ELISA) for the measurement of circulating estradiol levels. The ovaries were prepared for immunohistochemistry (IHC) and reverse transcriptase-polymerase chain reaction (rt-PCR) analysis to measure levels of ERα and ERβ. The results indicate that AhRKO mice had significantly lower levels of estradiol compared to WT mice (WT=42±3pg/ml, n=3; AhRKO=23±4pg/ml, n=4, $p \leq 0.014$). In addition, AhRKO ovaries expressed lower levels of ERα mRNA and ERβ mRNA than WT ovaries. IHC experiments with ERα and ERβ antibodies, however, showed that AhRKO follicles had lower levels of ERαand higher levels of ERβ compared to WT follicles. These data suggest that the delayed follicular growth in AhRKO mice may be due to a disruption in estradiol and/or ER levels and that the AhR may be required for normal hormone responsiveness in the ovary. Supported by NIH 38955.

1068 LACK OF INVOLVEMENT OF THE ARYL HYDROCARBON RECEPTOR IN 4-VINYLCYCLOHEXENE DIEPOXIDE-INDUCED OVARIAN FOLLICLE LOSS IN C57BL/6 MICE.

S. M. Bourguet[1], H. L. Brooks[1], M. R. McReynolds[1], I. G. Sipes[2, 3] and P. B. Hoyer[1, 3]. *[1]Physiology, University of Arizona, Tucson, AZ, [2]Pharmacology, University of Arizona, Tucson, AZ and [3]Southwest Environmental Health Sciences Center, Tucson, AZ.*

Repeated dosing of rats and mice with the occupational chemical, 4-vinylcyclohexene diepoxide(VCD) selectively destroys ovarian small pre-antral follicles *via* acceleration of the natural process of atresia(apoptosis). Previous data support that the aryl hydrocarbon receptor(AhR) is involved in VCD-induced small follicle loss in Fischer 344 rats. The AhR antagonist, alpha-naphthoflavone(ANF), protected against VCD-induced follicle loss, and AhR mRNA expression was stimulated by VCD in small follicles. The current study was designed to determine whether the AhR is also involved in VCD-induced follicle loss in mice. C57BL/6 female mice(age 28d) were injected daily (i.p., 15d) with sesame oil(vehicle control), VCD(80mg/kg), ANF(80mg/kg), or ANF+VCD. Following the final dose, ovaries were excised and processed for histological evaluation. Ovaries were sectioned and either stained with hematoxylin and eosin and evaluated for classification and counting of follicles, or prepared for immunostaining using confocal microscopy. Ovaries from another group of animals were dissociated, sorted into separate follicle fractions, and mRNA encoding the AhR was measured using real-time PCR. Compared with controls, primordial follicle numbers were decreased ($p<0.05$) by VCD dosing (Con, 67.8±11.3; VCD, 21.8±4.9). ANF failed to protect against VCD-induced follicle loss (ANF+VCD, 19.8±6.0). ANF alone had no effect

(72.0±6.0). Confocal microscopy in untreated mice localized AhR staining in nuclei of granulosa cells in all follicle types. There were no significant differences in levels of mRNA encoding the AhR in small follicles from ovaries of VCD-treated, versus control animals. These findings suggest that, unlike in rats, in C57BL/6 mice, VCD-induced follicle loss does not involve the AhR. The reason for these mechanistic species differences in VCD-induced follicle loss will be the subject of future investigations. (PBH, ES08979, ES09246; Center Grant ES06694)

1069 DEVELOPMENTAL EXPOSURE TO A COMPLEX MIXTURE OF ENVIRONMENTAL TOXICANTS INTERACTS WITH POSTNATAL GENISTEIN TO INDUCE CHANGES IN REPRODUCTIVE DEVELOPMENT OF FEMALE SPRAGUE DAWLEY RATS.

W. G. Foster[1], M. G. Wade[2], C. L. Hughes[3] and E. V. YoungLai[1]. *[1]OBS/GYN, McMaster University, Hamilton, ON, Canada, [2]Health Canada, Ottawa, ON, Canada and [3]Duke University, Durham, NC.*

Estimates of safe exposure levels are based primarily on the results of animal studies employing single test chemicals, epidemiological studies, and estimates of potential human exposure. However, the consequences of chemical interactions to human reproductive and endocrine function remain unknown. The potential for interaction with hormonally active dietary constituents is also unknown. Therefore the objective of this study was to determine the effects of *in utero* exposure to a complex mixture of persistent environmental toxicants that have been quantified in human reproductive tissues. Pregnant Sprague-Dawley rats were exposed daily to a complex mixture at 1, 10, and 100 times the minimum risk level (MRL) or tolerable daily intake (TDI) as determined by the US-EPA on gestations days 9-16. The mixture used contained organochlorines (TCDD, PCBs, methoxychlor, DDT, DDE, dieldrin, endosulfan, hexachlorobenzene, hexachlorocyclohexane, mirex and heptachlor) and the metals lead and cadmium. Following delivery half the pups of each treatment group were injected subcutaneously with 10 ug genistein/g BW/day on postnatal days 2-8. Randomly selected pups from each litter were necropsied on days 21, 60, 120 and 200 days of age. Treatments had no effect on litter size, sex ratio, birth weight, reproductive organ weights or reproductive endocrine outcome measures. Treatment with the mixture had no effect on sexual maturation in either sex whereas the mixture/genistein combination induced a significant delay in vaginal opening but had no effect on preputial separation. These data suggest that developmental exposure to environmental toxicants representative of contemporary human tissue residues is unlikely to induce additive or synergistic effects on the reproductive tract. However, our data suggest that exposure complex mixtures of toxicants can interact with dietary components to induce adverse effects on reproductive development in the female.

1070 THE INHIBITORY EFFECT OF CIGARETTE SMOKE AND ITS REACTIVE OXYGEN SPECIES ON HAMSTER OOCYTE CUMULUS COMPLEX PICKUP.

C. Gieseke[1, 2], R. Pederson[2] and P. Talbot[2, 1]. *[1]Environmental Toxicology Graduate Program, University of California, Riverside, Riverside, CA and [2]Cell Biology and Neuroscience, University of California, Riverside, Riverside, CA.* Sponsor: J. Arey.

The hamster oviduct and oocyte cumulus complex (OCC) are targets of cigarette smoke. Pickup of the OCC by the ciliated oviduct is inhibited in hamsters during acute *in vitro* exposures of oviducts or OCCs to mainstream and sidestream whole, gas, and particulate smoke solutions. Smoke solutions inhibit OCC pickup by increasing adhesion between the OCC and oviduct and altering ciliary beating. Cigarette smoke generates many reactive oxygen species including superoxide anion and hydrogen peroxide, which are known to alter ciliary beat frequency (CBF) in numerous ciliated epithelia. The purpose of this study was to determine if superoxide anion or hydrogen peroxide is responsible for the observed adverse effects on OCC pickup rate (OPR), adhesion, and oviductal CBF. Chromogenic probes were used to detect the presence of superoxide anion and hydrogen peroxide in the smoke solutions. The antioxidants, superoxide dismutase (SOD) and catalase, were used to confirm specificity of the probes and to indirectly assess the role of superoxide anion and hydrogen peroxide on OCC pickup. When oviducts were exposed to each type of mainstream and sidestream smoke solution plus either 299 University/ml SOD or 2350 University/ml catalase, most adverse effects on OPR and adhesion were eliminated. However, catalase did not eliminate any of the adverse effects due to sidestream whole exposure, and SOD exaggerated the effect of sidestream whole on CBF. When OCCs were exposed to each type of smoke solution plus SOD or catalase, SOD, but not catalase, eliminated the adverse effect of MSW on OPR and adhesion. Both SOD and catalase partially eliminated the effect of SSW on OPR and adhesion. These results show that superoxide anion and hydrogen peroxide generated by cigarette smoke are at least partially, if not fully, responsible for inhibiting OCC pickup. Experiments are in progress that will clearly define the role that each of these reactive oxygen species plays in adversely affecting OPR, adhesion, and CBF.

1071 RESPONSE OF CULTURED MOUSE OVARIAN SURFACE EPITHELIUM (OSE) CELLS TO METHOXYCHLOR (MXC) AND BIS-HYDROXYMETHOXYCHLOR (HPTE).

D. A. Symonds, K. P. Miller, C. Borgeest and J. A. Flaws. *Program in Toxicology, University of Maryland, Baltimore, MD.*

The purpose of this study was to develop a method to isolate and culture mouse OSE cells and use this model to test the hypothesis that the pesticide MXC and its metabolite HPTE affect the growth of the OSE. Since little is known about the metabolic capacity of the OSE, this work also determined whether the OSE contained cytochrome P450 enzymes thought to be involved in MXC metabolism (3A4 and 2C19). To isolate OSE cells, mouse ovaries were incubated in supplemented Medium 199 containing collagenase (500 units; Crude Type XI) at 37° C for 1 hour and vortexed for 2-4 minutes. Aliquots containing 200-500 cells/100µL were inoculated into 96 well plates, and incubated in 5% CO2 at 37°C for 3 days to allow for cell attachment. After attachment, cells were incubated with vehicle (DMSO), MXC (1, 10, 100µg/ml), or HPTE (0.1 or 1µg/ml) for 7 days. Cell proliferation was measured with the CyQUANT assay. As a control, the remaining ovary was histologically examined for residual OSE, and cultured OSE cells were evaluated for epithelial markers by immunohistochemistry (IHC) with a cytokeratin cocktail (AE1/AE3). The presence of 2C19 and 3A4 in OSE was assessed by IHC using ovaries from adult mice dosed for 20 days with either vehicle or 32 mg/kg/day MXC. The results indicate that OSE cells can be isolated and cultured for at least 7 days. The culture procedure yielded an average of 2-6 x 10^3 cells/mm^3, achieving 60% denudement of the OSE, and cytokeratin positivity of harvested cells. The results also indicate that low doses of MXC and HPTE may stimulate proliferation of OSE *in vitro* (control = 466±39, MXC (1µg/ml) = 706±176, HPTE (1µg/ml) = 861±86 total fluorescent units per 100 seeded cells; $p < 0.05$ for control versus MXC- or HPTE-treated cells). Further, the IHC analysis indicates that the mouse OSE contains 2C19 and 3A4. Collectively, these data indicate that OSE cells can be isolated and cultured for toxicity studies, MXC and HPTE may cause proliferation of the OSE, and the OSE may play a role in toxicant metabolism.

1072 METHOXYCHLOR ALTERS THE EXPRESSION OF ANTIOXIDANT ENZYMES IN ANTRAL FOLLICLES OF THE MOUSE OVARY.

R. K. Gupta, K. P. Miller and J. A. Flaws. *Program in Toxicology, University of Maryland, Baltimore, MD.*

The mammalian ovary contains antral follicles, which are responsible for synthesis and secretion of hormones that regulate estrous cyclicity and fertility. Although the organochlorine pesticide methoxychlor (MXC) is known to cause atresia (follicle death *via* apoptosis) of antral follicles, little is known about the mechanisms by which MXC induces antral follicle atresia. Since oxidative stress is known to cause apoptosis in non-reproductive and reproductive tissues, the purpose of this current study was to test the hypothesis that MXC induces oxidative stress by altering the expression of antioxidant enzymes in antral follicles. Using *in vitro* follicle culture, antral follicles were isolated from 39-day-old CD1 mice and cultured in supplemented media in the presence of follicle-stimulating hormone and serum. Follicles were then treated with vehicle control (DMSO), positive control (3µM H_2O_2), or MXC (100µg/ml) for 1, 3, 6, 12, 24, and 48 hrs. Follicles were then snap-frozen and subjected to rt-PCR for measurement of the levels of superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). The results indicate that SOD, GPX, and CAT are highly expressed in isolated mouse antral follicles. At 1-24 hrs MXC treatment did not alter the expression of SOD, GPX, or CAT in antral follicles as compared to vehicle control. At 48 hrs, however, MXC treatment increased the expression of CAT and reduced the expression of GPX in antral follicles as compared to vehicle control. Further, the effects of MXC on CAT and GPX expression were similar to that of H_2O_2 (positive control), but the effects of MXC on SOD expression were distinct from those of H_2O_2. Collectively, these data indicate that antioxidant enzymes like SOD, GPX, and CAT are present in mouse antral follicles and that MXC alters expression of CAT and GPX in antral follicles. Therefore, it is possible that MXC may cause atresia by inducing oxidative stress through CAT and GPX signaling pathways. (Supported by NIH HD 38955)

1073 ROLE OF GAP JUNCTIONS IN THE MODIFICATION ON RAT UTERINE CONTRACTION BY 2, 2′–DICHLOROBIPHENYL.

D. Chung and R. Loch-Caruso. *Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

Previously, we reported that uterine strips from gestation day 10 pregnant rats suspended in isometric muscle baths and exposed to 2, 2′–dichlorobiphenyl (2, 2′–DCB) exhibited increased oscillatory frequency, decreased contraction ampli-

tude and desynchronization of contractions. The present study examined the hypothesis that inhibition of myometrial gap junctions leads to 2, 2′–DCB–induced modification of uterine contraction since gap junctions coordinate myometrial cell contractions, thereby increasing the force of contraction. When uterine strips from gestation day 10 pregnant rats were suspended in isometric muscle baths and exposed to a known gap junction inhibitor (50 μM β–glycerrhetinic acid), the average peak amplitude per contraction and the synchronization of contractions decreased in a manner similar to that observed in 2, 2′–DCB–treated rat uterus, consistent with the hypothesis that modification of uterine contraction by 2, 2′–DCB might be through the inhibition of gap junctions. To determine whether myometrial gap junctions are inhibited by 2, 2′–DCB, we microinjected fluorescent Lucifer Yellow into myometrial cells isolated from gestation day 10 rats and assessed the percentage of dye transfer to the adjacent cells from an injected cell. The percentage of dye transfer decreased in a concentration-dependent manner to 70, 37, 18 and 18% after a 1–h treatment with 25, 50, 75 or 100 μM 2, 2′–DCB. This study suggests that 2, 2′–DCB modifies uterine contraction by a mechanism that functionally inhibits gap junction communication between myometrial cells.

1074 REPRODUCTIVE TOXICOLOGY STUDIES ON GT 56-252, A NOVEL ORALLY AVAILABLE IRON CHELATOR.

J. K. Marquis[1], R. Dagher[1], W. Gaoua[2], O. Foulon[2] and R. Forster[2]. *[1]Genzyme Corporation, Boston, MA and [2]CIT, Evreux, France.*

GT 56-252 is a novel orally available iron chelator, under development for the primary treatment of iron overload resulting from transfusion therapy in beta-thalassemia and sickle cell disease. A complete set of reproductive toxicology studies was performed with this candidate drug by oral administration in order to support the planned clinical development. A study for effects on male and female fertility and early fetal development was performed in the rat, and included additional groups of iron-loaded control and high-dose rats. This was done in order to distinguish between the pharmacological and toxic actions of the test material and to simulate the human therapeutic situation. Hematology and blood biochemistry investigations were included in this study to monitor any disturbances of iron metabolism. Standard studies for effects on embryo-fetal development were performed in the rat and rabbit. In the pre- and post natal development study performed in the rat, additional groups of iron-loaded control and high-dose animals and clinical pathology monitoring were again included in the study design; iron loading of the females was performed prior to mating. Drug levels were evaluated in samples of milk from the treated dams on Day 17 pp. Iron loading was achieved by repeated intraperitoneal injection with an iron dextran saline solution, followed by a 2 week period to permit physiological redistribution of iron within the body. In the fertility study transient general toxicity was observed at the high dose-level selected, accompanied by some expected alterations in iron and haematology parameters, but no effects on male or female fertility were observed. No harmful effects were detected on embryo-fetal development in the rat or rabbit. In the pre- and post natal study the only effect observed on the pups was lower body weight in the high dose group immediately following birth; these values returned to normal limits by the time of weaning. These studies show that treatment with GT56-252 does not exert any adverse effects on reproductive functions.

1075 MULTI-GENERATION REPRODUCTIVE TOXICITY STUDY OF IMPLANTED DEPLETED URANIUM IN RATS: RESULTS FROM THE 1ST GENERATION.

A. R. Thitoff, A. E. Jung, E. W. Johnson, S. L. Lohrke, S. A. Stutler, K. R. Still and D. P. Arfsten. *Naval Health Research Center Toxicology Detachment, Wright-Patterson AFB, OH.*

The risk for penetration injuries to battlefield personnel caused by depleted uranium (DU) projectiles has increased substantially since the 1970s. Removal of DU projectiles from the body may not always be possible because of their location in the body or their small size. Embedded DU solubilizes in the body over time and can translocate to various organs and tissues including the reproductive organs and developing fetus. DU is a toxic heavy metal and is possibly mutagenic, therefore, embedded DU fragments could have a negative impact on reproductive organ function and/or reproductive success. To test this hypothesis, 8 week male and female Sprague-Dawley rats (P1 generation) were implanted with 0, 4, 8, 12, or 20 DU 1 x 2 mm pellets approximating 0.03%, 0.06%, 0.10%, and 0.15% by weight of a 500 g rat, respectively. The animals were then cross-mated in various combinations at 30 days post-implantation. Parameters measured were reproductive success over a 7-day mating period, gestation weight gain, # of live-born pups, average pup weight at PND1, # of pups surviving to PND4, and pup weight gain through PND20. Collection of urine and feces from P1 animals at 25 days post-implantation showed that DU was being excreted in the urine of DU-implanted animals. No evidence of toxicity was apparent for P1 DU-implanted animals at 30 days post-implantation. Our preliminary findings suggest no large differences between study groups for live birth indices, PND4 and PND20 *via*bility indices, and average pup weight gain from PND4 to PND20. PND4 F1 pups born to DU-implanted animals did not contain any detectable levels of uranium (limit of detection: 0.5 microg/mg tissue). Investigations are on-going to determine the levels of DU detectable in PND4 pup tissues, specifically kidney, liver, GI tract, bone, brain, thymus, and ovaries/testes. P1 animals will also be mated at 120 days post-implantation and data for the listed parameters will be gathered.

1076 IMPACT OF MATERNAL TOXICITY ON PRE- AND POSTNATAL DEVELOPMENT OF RATS.

I. D. Waalkens-Berendsen, M. M. Tegelenbosch-Schouten, A. Dijkstra and A. P. Wolterbeek. *Target Organ Toxicology, TNO Nutrition and Food Research, Zeist, Netherlands.* Sponsor: V. Feron.

Developmental toxicity might be secondary to maternal toxicity, raising great difficulties for risk assessors involved in evaluation of drugs and chemicals to estimate or quantify the contribution of maternal toxicity to effects observed on pre- and/or postnatal development of the offspring. Maternal toxicity mainly manifest itself by decreased food consumption and body weight(gain), organ system toxicity and changes in hematology and clinical chemistry. In the absence of a toxicological active substance, maternal toxicity can be mimicked by changes in body weight(gain) induced by food restriction. In a prenatal developmental toxicity study, Wistar rats were fed *ad libitum* or a limited amount of food (approx.85 and 70% of *ad libitum*) from gestation day (GD) 0-21. On GD 21, fetuses were collected by Caesarian section and processed for skeletal (bone and cartilage) and visceral examination. Body weights of the dams of the food restricted groups were significantly decreased during the entire gestation period. Except for some skeletal retardations, no significant effects were observed on visceral and skeletal findings. The most prominent effects on fetal development were observed on fetal and placental weights. In a postnatal developmental study, rats were food restricted (approx. 85 and 70% of *ad libitum*)during the entire gestation and lactation periods. Body weights of the dams were significantly decreased during the entire study. On postnatal day 1 (PND1) no effects on pup weights were observed, but pup weight gain was significantly decreased during the lactation period. No or only minor effects were observed on physical and sensory examinations (surface and air righting, pinna unfolding, hair growth, tooth eruption, nipple retention, eye opening, auditory canal opening, auditory response, pupil reflex) and sexual development (anogenital distance, vaginal opening, preputial separation). These data suggest that maternal toxicity, as reflected by decreased body weight, influences pre- and postnatal developmental only to a limited extent.

1077 DEVELOPING CHEMILUMINESCENT ASSAYS FOR MEASURING ENDOCRINE DISRUPTION IN MAMMALS.

C. Morris[1], E. Wood[2], K. Roberts[1, 3] and S. Woodhead[1]. *[1]Molecular Light Technology Research, Cardiff, CF14 5DL, United Kingdom, [2]SafePharm Laboratories Ltd., PO Box 45, Derby, DE1 2BT, United Kingdom and [3]Cardiff University, School of Biosciences, PO Box 911,, Cardiff, CF10 3US, United Kingdom.* Sponsor: A. Smith.

The aim of this study was to investigate if a novel chemiluminescent technique, the hybridisation protection assay (HPA), could be used to quantify gene expression changes in the uterus during treatment with estrogenic compounds. The HPA consists of three steps; the probe labelled with acridinium ester (AE) is hybridised to its target, then addition of an alkaline reagent causes preferential hydrolysis of unhybridised probe, followed by initiation of the chemiluminescent reaction. The AE molecule reacts rapidly with hydrogen peroxide under alkaline conditions to produce light at 430nm. The intensity of emitted light is directly proportional to the amount of target present and as little as 0.1 fmol of mRNA transcript can be accurately quantified by this method. Five groups of sexually immature female Sprague-Dawley rats (n=10) were treated with 1, 0.5, 0.1, and 0.01 mg/kg of Diethylstilbestrol (DES) by oral gavage on three consecutive days and a vehicle control group was included. The uterus weights of each group were recorded: mean uterus weights in the control group were 80 ± 23mg compared to 224 ± 74mg in the lowest dose group. The HPA was used to measure gene expression levels of rat complement C3 gene and beta actin. The levels of uterine C3 mRNA were below detectable limits in the control group and increased in all four groups treated with DES (to a maximum level of 0.78 ± 0.16 fmol/mg in the lowest dose group). Beta actin mRNA was also quantified and the mean concentration was 2.4 fmol/mg RNA. However, actin expression was significantly lower in the control group (1.36 ± 0.18 fmol/mg, $p<0.0001$) suggesting that its expression may also be related to uterine weight. In conclusion, the HPA can be used to measure the changes in uterine gene expression and these results confirm that expression of C3 mRNA parallels uterine weight changes in response to DES.

1078 28-DAY ORAL AND REPRODUCTIVE/DEVELOPMENTAL SCREENING TOXICITY STUDIES OF 1, 2-ETHANEDIAMINE, N-{3-(TRIMETHOXYSILYL)PROPYL}- IN RATS.

W. H. SIDDIQUI[1], L. S. Meeker[1], T. R. Barfknecht[2], S. D. Crofoot[1] and K. P. Plotzke[1]. *[1]Dow Corning Corporation, Midland, MI and [2]Celanese International Corporation, Dallas, TX.*

The potential toxicity of 1, 2-ethanediamine, N-{3-(trimethoxysilyl) propyl}-, a High Production Volume (HPV) chemical, was evaluated in a combined repeated dose toxicity study with a reproductive/developmental toxicity screening test. The study design utilized the enhanced version of the protocol in accordance with the OECD and EPA test guidelines Nos. 422/426 and 870.3650, respectively. Developmental neurotoxicity endpoints including Functional Observational Battery (FOB) and Motor Activity were performed on male and female rats. The test substance was gavaged daily in corn oil to 10 male and 10 female rats at 0, 25, 125 or 500 mg/kg/day. Clinical signs exhibited by several high dose animals included perioral soiling, increased nasal sounds, labored respiration, or soft vocalization. A dose-related resistance to dosing, salivation, wetness around the mouth at 5-30 minutes following dosing were also observed. There were no effects on body weight or food consumption among the groups. No treatment-related changes were observed in any FOB or motor activity parameters. There were no alterations in hematology, serum chemistry, or macroscopic examination of organs/tissues for any of the animals. Also, no effects were noted in mean organ weights or organ to body weight ratios. Histopathological examination of all gross lesions, tissues and organs for control and high dose animals demonstrated no microscopic findings. No effects were observed in any of the reproductive parameters evaluated. Two high dose and one low dose reproductive group females that did not produce litters had positive evidence of copulation. Six of the eight surviving high dose reproductive group females produced litters that were similar in all respects to control litters. Based on the results of this study, the NOAEL for 1, 2-Ethanediamine, N-{3-(trimethoxysilyl)propyl}- in rats *via* the oral route was considered to be 500 mg/kg/day.(Supported by the Silicones Environmental, Health and Safety Council).

1079 Q-SWITCH LASER AND TATTOO PIGMENTS, A CHEMICAL ANALYSIS OF LASER INDUCED DECOMPOSITION COMPOUNDS.

W. Baumler[1], R. Vasold[2], B. Koenig[2] and M. Landthaler[1]. *[1]Department of Dermatology, Regensburg, Germany and [2]Institute of organic chemistry, Regensburg, Germany.* Sponsor: P. Howard.

In the western world there are more than 50 millions of people with tattoos. Tattoo colorants are applied like medical drugs since the pigments are injected into the skin even colouring the lymph nodes. Most of the tattoos colorants have been never produced by the chemical industry to be used in humans but to stain consumer goods. A high number of people request for removing their tattoos due to an improved self-image or social stigmatization. Since several years laser light at very high intensities is applied to the skin in order to destroy these tattoo pigments. Based on a recent analysis of tattoo pigments two widely-used azo compounds (pigment red 22, pigment red 9) were irradiated in suspension using a laser pulse energy of 15 mJ at a pulse duration of 8 ns. After that the suspension was analyzed by using quantitative high performance liquid chromatography and mass spectrometry. A significant increase of decomposition products 2-methyl-5-nitroaniline and 4-nitrotoluene was found for pigment red 22. The irradiation of pigment 9 led to an increase of 2, 5-dichloraniline and 1, 4-dichlorobenzene These products are proven to be toxic or carcinogenic. Moreover, the results show that the tattoo colorants are already contaminated with these products prior to laser irradiation. In view of a high number of patients undergoing laser treatment of tattoos, it is high time and an important goal to perform a risk assessment in humans regarding laser induced decomposition products.

1080 OCHRATOXIN A: LACK OF FORMATION OF COVALENT DNA ADDUCTS.

W. Dekant[1], H. Zepnik[1], P. Wanek[1], E. Eder[1], K. Dingley[3], H. Ihmels[2], W. Voelkel[1] and A. Mally[1]. *[1]Department of Toxicology, University of Wuerzburg, Wuerzburg, Germany, [2]Department of Organic Chemistry, University of Wuerzburg, Wuerzburg, Germany and [3]Lawrence Livermore National Laboratory, Livermore, CA.*

The mycotoxin Ochratoxin A (OTA) is a potent nephrotoxin and renal carcinogen in rodents. However, the mechanism of OTA induced tumor formation is unknown and conflicting results have been obtained regarding the potential of OTA to bind to DNA. OTA is poorly metabolized and no reactive intermediates capable of interacting with DNA have been detected *in vitro* or *in vivo*. Recently a hydroquinone/quinone redox couple and a carbon-bonded OTA-deoxyguanosine (OTA-dG) adduct formed by electrochemical oxidation and photoreaction of OTA have been reported and suggested to be involved in OTA carcinogenicity. This study was designed to characterize the role of DNA-binding and to determine if formation of these derivatives occurs *in vivo* and in relevant activation systems *in vitro*. Horseradish peroxidase activation of OTA and its dechlorinated analogue ochratoxin B (OTB) yielded ochratoxin A - hydroquinone (OTHQ), but the postulated OTA-dG adduct was not detectable using LC-MS. In support of this, no OTA-related DNA adducts were observed by 32P-postlabelling. *In vivo*, only traces of OTHQ were found in urine of male F344 rats treated with high doses of OTA (2mg/kg bw) for two weeks, suggesting that this metabolite is not formed to a relevant extent. In agreement with the *in vitro* data, OTA-dG was not observed in liver and kidney DNA extracted from treated animals. In addition, DNA-binding of OTA and OTB was assessed in male rats given a single dose of 14C-OTA or 14C-OTB using accelerator mass spectrometry (AMS), a highly sensitive method for quantifying extremely low concentrations of radiocarbon. The 14C-content in liver and kidney DNA from treated animals was not significantly different from controls, indicating that OTA does not form covalent DNA adducts in high yields. In summary, the results presented here demonstrate that DNA binding of OTA is not detectable with sensitive analytical methods and is unlikely represent a mechanism for OTA induced tumor formation.

1081 MITOCHONDRIAL AUTOPHAGY IS ASSOCIATED WITH A HIGH RATE OF MTDNA TURNOVER IN REMODELING RAT HEPATOCYTES.

Y. Kai, S. Rodriguez-Enriquez and J. J. Lemasters. *Cell & Developmental Biology, UNC-CH, Chapel Hill, NC.*

BACKGROUND. Stellate and other cells differentiate in culture in a manner similar to that occurring in pathophysiological states. Accordingly, hepatocyte de-differentiation or remodeling *in vitro* may be pathophysiologically relevant. Here, our AIM was to determine the relationship between mitochondrial autophagy and mitochondrial DNA (mtDNA) turnover during remodeling of cultured hepatocytes. METHODS. Isolated rat hepatocytes were cultured in Waymouth's medium with 10% fetal calf serum, 100 nM insulin and 100 nM dexamethasone for 1 to 3 days. Cytochrome c oxidase activity in cell extracts was determined polarigraphically. Total DNA was extracted, separated by agarose gel electrophoresis, and transferred onto membranes to be analyzed by Southern blot. Probes for mtDNA and 18 S rRNA gene were simultaneously used to estimate and normalize mtDNA content per cell. RESULTS. In agreement with our previous study showing a 51% decrease of mitochondrial number by the process of autophagy between 1 and three days of culture, respectively, cytochrome c oxidase activity also declined by 44%. By contrast, mtDNA per hepatocyte remained constant from the first to the third day of culture. Two days of treatment with ethidium bromide (0.5 ug/ml, an inhibitor of mtDNA replication) caused a 41 % decrease of mtDNA per hepatocyte from the first to the third culture day. CONCLUSIONS. During remodeling, cultured hepatocytes lose a large proportion of their mitochondria, but adaptive synthesis of mtDNA compensates for mtDNA loss during this autophagic remodeling. Thus, the content of the mitochondrial genome is preserved even as mitochondrial number and enzyme activity are lost during culturing.Thus, the content of the mitochondrial genome is preserved even as mitochondrial number and enzyme activity are lost during culturing, which indicates independent regulation of mitochondrial mass and mtDNA content.

1082 DIETARY FAT RICH IN ω-6 POLYUNSATURATED FATTY ACIDS MODULATES OXIDATIVE DNA DAMAGE AND REPAIR IN MOUSE LIVER.

C. Powell[1, 2], P. Ross[2], O. Kosyk[2], B. Bradford[2] and I. Rusyn[1, 2]. *[1]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC and [2]Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC.*

Dietary fats such as corn oil, which are rich in ω-6 polyunsaturated fatty acids (PUFA), have been shown to increase the rate of growth of established tumors and promote metastasis in animal models. In contrast, fish oil, a rich source of ω-3 eicosapentaenoic acid, has been shown to be protective. The ω-6 PUFAs found in dietary oils are known to induce the production of free radicals that could serve as a possible source of DNA damage leading to carcinogenesis. Here, we hypothesize that dietary fats rich in ω-6 PUFAs play an important role in carcinogenesis *via* modulation of oxidative DNA damage and its repair. C57BL/6J male mice were administered corn or fish oil acutely (by gavage) or in a diet (20.5% calories from fat) for up to 3 weeks. A highly sensitive slot blot assay was used to quantitate apurinic/apyrimidinic (AP) sites in DNA extracted from liver tissues. An increase in AP sites was observed only for corn oil treated mice. Total RNA was extracted from liver tissue and expression of base excision DNA repair (BER) genes was analyzed by a multi-probe RNase protection assay. Increases in expression of BER genes re-

sponsible for removal of oxidized DNA lesions were evident after a single dose of corn oil with 2 fold increases in 8-oxoguanine DNA glycosylase 1 (Ogg1) and AP endonuclease1 (APE) and a 3 fold increase in N-methylpurine DNA glycosylase (MPG). Fish oil had no effect. Similar up-regulation of BER genes was detected in dietary feeding studies with corn oil. In addition to DNA damage and repair, cell proliferation of liver parenchymal cells was enhanced by almost 2 fold based on BrdU uptake. Collectively, corn oil but not fish oil was shown to generate abasic sites, lead to selective induction of BER genes, and increased cell proliferation. These studies are important for better understanding of the molecular mechanisms that link high-fat diet and carcinogenesis.

1083 MECHANISMS FOR THE INDUCTION OF DNA DAMAGE (COMET) FOLLOWING 2-BUTOXYETHANOL EXPOSURE.

S. M. Corthals, L. M. Kamendulis and J. E. Klaunig. *Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.*

Previous studies showed that chronic exposure to 2-butoxyethanol induced an increase in liver hemangiosarcomas in B6C3F1 mice. We have proposed that liver hemangiosarcomas result from oxidative stress arising from Kupffer cell activation, secondary to hemolysis from selective metabolites of 2-butoxyethanol. The present study examined the effect of 2-butoxyethanol and activated mouse macrophages on mouse endothelial cell DNA damage as measured by the Comet assay. Treatment with 2-butoxyethanol (1, 5, and 10mM) failed to induce an increase in endothelial cell Comet over control after 24 hours of continuous treatment. In our proposed hypothesis, Kupffer cell activation is a key event in the induction of endothelial cell neoplasia. To further address this, the effects of hydrogen peroxide (an oxidant resulting from macrophage activation) and LPS activated macrophages on endothelial cell Comet were examined. Hydrogen peroxide (50, 75, and 100μM) resulted in increased Comet after 1, 2, and 4 hours (5—2, 3—6, and approximately 2—3 fold over control). Co-culture of endothelial cells with activated macrophages resulted in increased Comet in endothelial cells after 4 hours (2 fold over control). These results show that 2-butoxyethanol does not directly damage endothelial cell DNA. These studies also showed a correlation between the number of macrophages co-incubated with endothelial cells and the amount of endothelial cell Comet detected. Activated macrophages or hydrogen peroxide produced a dose dependent increase in endothelial cell DNA damage. This resulting damage may provide the mutational and/or cell proliferation events necessary for the neoplastic development (hemangiosarcoma) seen following chronic 2-butoxyethanol exposure.

1084 ATM-DEPENDENT, DNA DAMAGE-INDUCED G1 CHECKPOINT FUNCTION REGULATES GENE EXPRESSION IN HUMAN FIBROBLASTS.

T. Zhou[1, 3], D. A. Simpson[1, 3], Y. Zhou[1, 3] and W. K. Kaufmann[1, 2, 3]. *[1]Pathology & Lab. Medicine, University of North Carolina, Chapel Hill, NC, [2]Center for Environmental Health and Susceptibility, University of North Carolina, Chapel Hill, NC and [3]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.*

Cells from ataxia telangiectasia (AT) patients are radiation-hypersensitive and display defects in the G1 and G2 checkpoints in response to DNA damage due to inactivating mutations in ATM (ataxia telangiectasia mutated). We are testing the role of ATM in transcriptional responses to DNA damage that may relate to the checkpoint defects and hypersensitivity to ionizing radiation (IR). A telomerized human fibroblast (F1-TERT) and a telomerized AT fibroblast line (AT-TERT) were used in the study. Although no difference in DNA damage was measured after 0.5 to 4.5 Gy IR by using comet assay, clonogenic assay showed the AT cells to be radiation-hypersensitive. A 1.5 Gy dose of IR that inactivated colony formation by 45% in F1-TERT and 90% in AT -TERT was studied for its effects on G1 and G2 checkpoint functions using quantitative flow cytometric assays and for its effects on the patterns of gene expression using a 16K oligoDNA microarray. F1-TERT exhibited 95% G1 arrest and 97% G2 delay after 1.5 Gy IR, while AT-TERT displayed only 15% G1 arrest and 41% G2 delay. The patterns of changes in gene expression at 2, 6 and 24 hr after IR in F1-TERT cells indicated that the predominant response corresponded to the ATM- and p53-dependent G1 arrest. Several p53 target genes, including p21Waf1, TP53INP1 and BTG2, were significantly up-regulated, while several genes whose expression is regulated by E2F family transcription factors were significantly down-regulated including Cdc6 and all MCM family members. Several genes that participate in DNA metabolism and checkpoint functions including MSH, RFC4, topoII-alpha, Bub1, and BARD1 were also repressed by IR in normal fibroblasts but not AT fibroblasts. All the results indicate that ATM-dependent gene regulation in response to IR-induced DNA damage can be stuied globally. Supported by PHS grants ES11391 and ES10126

1085 PHENOBARBITAL AND PREGENOLONE 16α-CARBONITRILE INDUCE HEPATIC EXPRESSION OF MDM2 INDEPENDENT OF P53.

D. M. Nelson, V. Bhaskaran, W. R. Foster, B. Gemzik and L. D. Lehman-McKeeman. *Discovery Toxicology, Bristol-Myers Squibb, Princeton, NJ.*

Mdm2 negatively regulates wild-type p53 activity by directly binding to p53 and mediating its ubiquitination and subsequent proteosomal degradation. Mdm2 is also directly oncogenic because it transforms cells when over expressed. In the present study, the hepatic gene expression patterns induced by phenobarbital (PB; 100 mg/kg) and pregenolone 16α-carbonitrile (PCN, 100 mg/kg) were evaluated in male and female Sprague-Dawley rats using Affymetrix Rat Genome U34A gene arrays. In addition to changes in the hepatic expression of well-characterized drug metabolizing enzymes associated with CAR and PXR activation, an increase in Mdm2 mRNA was observed with both compounds and detected consistently after single or repeat dosing (5 days). The results of the gene array analyses were confirmed by real time PCR, which indicated that PB increased Mdm2 mRNA by 1.4- and 3.9-fold in male rat liver and 3.1- and 2-fold in female rat liver at 6 hr and 5 days, respectively. Similar results were noted in PCN-treated male rats, whereas the largest induction of Mdm2 mRNA was noted in PCN-treated female rats, reaching an 11-fold increase after 5 days of dosing. The results of real-time PCR analyses were further confirmed by Western blotting of liver homogenates, which again showed the highest increase in Mdm2 protein in PCN-treated female rats. Mdm2 expression is regulated at least in part in a p53-dependent manner. However, gene array analyses did not reveal changes in other p53-dependent genes. To determine whether the effects of PB and PCN on Mdm2 expression were related to p53 activation, homozygous p53 knockout mice were adminstered PB and PCN (100 mg/kg for 5 days) and hepatic expression of Mdm2 was measured by real time PCR. Mdm2 mRNA levels were similarly increased in wild type C57/Bl6 and the p53 knockout mice. These results indicate that PB and PCN increase hepatic mRNA and protein levels of Mdm2 through mechanisms independent of p53 and suggest that CAR and PXR activation directly regulate Mdm2 expression in rodent liver.

1086 MOLECULAR EPIDEMIOLOGY OF ESOPHAGEAL CANCER USING THE IARC TP53 HUMAN TUMOR DATABASE.

R. K. Elespuru[1] and S. M. Jenning[2]. *[1]Genetic Technology Lab., USFDA Federal Laboratories at White Oak, Silver Spring, MD and [2]School of Engineering, Columbia University, New York, NY.* Sponsor: J. MacGregor.

Understanding specific causes of cancer is invaluable in the design of strategies for risk analysis of new products, and for developing preventive health measures for minimizing disease incidence. Mutations in the p53 gene are the most commonly occurring mutations in human cancer. A database of such mutations was initiated by Hollstein and Curtis and is maintained by Olivier and Hainaut at IARC. As the database grows and searching capabilities improve, new insights into cancer etiology may be found. We began our analysis with esophageal squamous cell carcinoma (ESCC), a cancer with known regional hot spots and life style associations, and a relatively large database at IARC. In our analysis, based on approximately 30 published studies reporting on more than 1000 mutations in IARC release R8 (June 2003), we stratified by pathology, gender, smoking status, and geographical location (comparing France, Japan, China, Iran and Brazil). We analyzed mutational sequence profile, mutant codon location, and DNA strand bias in mutations from males. An enhancement in AT to TA transversions was observed related to cigarette smoking, significant at a p value of .003. This result is in contrast to the mutation most associated with smoking related lung cancer, GC to TA. France, Japan and China exhibited the enhancement individually, while Brazil did not; studies on smoking were not reported in the data sets from Iran. The AT to TA sequence change exhibited substantial strand bias, consistent with an adenine lesion repaired on the transcribed strand. As observed by others, biomarker mutations occur in Iran (GC to AT) and China (G to T at codon 176, although this result has been criticized). GC to AT transitions occur with equal frequencies at CpG and non-CpG sites in most of the data sets, indicating that spontaneous deamination at CpG sites appears not to be a major pathway of mutation generation in this cancer.

1087 EFFECT OF TRICHLOROACETIC ACID IN MALE B6C3F1 MOUSE HEPATOCYTES.

D. J. Smith, L. M. Kamendulis and J. E. Klaunig. *Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.*

Trichloracetic acid (TCA) is an organic acid formed in the chlorination of drinking water as well as through the metabolism of selected chloroethylene compounds. TCA is a known hepatocarcinogen in mice, but not in rats, and acts through an apparent non-DNA reactive mode of action. TCA has also been shown to induce peroxisome proliferation, DNA synthesis, and decrease apoptosis in mouse liver following a short term *in vivo* exposure. The present study examined the effect of TCA

on DNA synthesis, peroxisomal beta-oxidation (PBOX), and apoptosis in male B6C3F1 mouse primary hepatocytes. Hepatocytes were cultured for 24 hours with TCA (0—100.0 mM TCA), and toxicity measured. A dose dependent increase in cell death, as measured by trypan blue exclusion, was seen with TCA. Subsequent examination of DNA synthesis and PBOX used five sub lethal doses (0.5, 1.0, 2.5, 5.0, and 10.0 mM) of TCA. DNA synthesis was examined in hepatocytes dosed for 24 hours with TCA. TCA caused a decrease in DNA synthesis from control at 0.5 mM TCA (0.65 fold of control), and a dose related increase in DNA synthesis from 1 to 10 mM. Peroxisomal beta-oxidation was measured in hepatocytes dosed for 24 and 48 hours with TCA (0—10 mM). After 24 hours, TCA produced a dose related increase in PBOX activity. TCA treatment for 48 hours produced a similar dose related increases in PBOX. These studies are consistent to what has been previously reported *in vivo* and thus establish a model by which further investigations on the carcinogenic mode of action of TCA can be examined in rodent and human liver.

1088 QUANTITATION OF CARCINOGENESIS-INITIATING EFFECTS OF 2-ACETYLAMINO-FLUORENE AND THEIR NO-OBSERVED-EFFECT LEVELS IN RAT LIVER.

M. J. Iatropoulos, A. M. Jeffrey, J. D. Duan and G. M. Williams. *Pathology, New York Medical College, Valhalla, NY.*

In order to explore for potential thresholds in liver carcinogenicity, we conducted a series of studies utilizing a carcinogenesis initiation/promotion model in rat liver with the DNA-reactive carcinogen 2-acetylaminofluorene (AAF) (Toxicol. Sciences. 45, 152, 1998; Toxicol. Pathol. 28, 388, 2000). In two current studies, AAF was administered in exact intragastric doses during the initiation segment (IS) to male F344 rats for 12 or 16 weeks. The cumulative exposures at the end of initiation in these experiments were 0.094, 0.124, 0.94, 1.24, 9.4, 28.2, 94.1, 188.2 and 282.2 mg/kg. The findings at the end of the IS can be summarized as follows: (i) formation of AAF DNA adducts was nonlinear, with a no-observed-effect level (NOEL) at a cumulative exposure of 0.94 mg/kg over 12 weeks and with a plateau at higher exposures (282.2 mg/kg); (ii) cytotoxicity (necrosis) showed a NOEL at a cumulative exposure of 28.2 mg/kg over 12 weeks; (iii) for compensatory hepatocellular proliferation, the NOEL was at a cumulative exposure of 28.2 mg/kg over 12 weeks and it was supralinear at high exposures (282.2 mg/kg); (iv) formation of preneoplastic hepatocellular altered foci (HAF) had a NOEL at a cumulative exposure of 28.2 mg/kg over 12 weeks and was supralinear at high exposures (282.2 mg/kg); (v) the earliest parameter to be affected with very low exposure was the formation of DNA adducts, followed by increased cell proliferation. The finding of NOELs for critical events of initiation of hepatocarcinogenesis supports our finding that there is a NOEL for phenobarbital-promotable liver tumors initiated by AAF (Toxicol. Sciences. 45, 152, 1998). We conclude that there is a practical threshold for AAF-induced liver cancer in rats.

1089 DICHLOROACETIC AND TRICHLOROACETIC ACID INDUCED ALTERATION IN THE METHYLATION OF TUMOR SUPPRESSOR GENES AND IN THE ACETYLATION OF HISTONE H3 IN MOUSE LIVER AND TUMORS.

L. Li, L. Tao, P. M. Kramer and M. A. Pereira. *Department of Pathology, Medical College of Ohio, Toledo, OH.*

Dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are mouse liver carcinogens that alter the methylation of DNA and genes. Mouse liver tumors promoted by DCA or TCA contained DNA hypomethylation. Furthermore, DCA and TCA induced DNA hypomethylation in mouse liver that was prevented by methionine. DNA hypomethylation releases histone deacetylase from DNA resulting in increased acetylation of histones. Hypermethylation of tumor suppressor genes, i.e. the estrogen receptor- alpha (ER-α) and p16 genes has also been associated with hepatocarcinogenesis. Female B6C3F1 mice were administered 3.2gm/l DCA in their drinking water with/without 8.0gm/kg methionine in the diet for 4 and 8 weeks. The acetylation of histone 3 (H3) was increased by DCA. Methionine prevented the increased acetylation of H3. These findings support the hypothesis that DNA hypomethylation induced by DCA promotes liver carcinogenesis by increasing histone acetylation. Hypermethylation in the regulatory region of genes usually results in the silencing of the genes. DNA was isolated from mouse liver tumors induced by DCA and TCA, treated with bisulfite, PCR amplification for the ER-α or p16 genes and sequenced to determine the methylation status of 17 and 30 CpG sites in the regulatory region of the genes. The percentage of the CpG sites that were methylated in the ER-α gene was 3.9 ± 1.9% in normal liver tissue, while in DCA and TCA-promoted tumors the percentages was increased to 42.3 ± 10.9% and 23.5 ± 7.9%. The extent to which the CpG sites in the p16 gene were methylated in normal liver ranged from 0-1 site, while DCA and TCA-promoted tumors contained 2-3 methylated CpG sites. Hence, DCA and TCA increased the methylation of the two tumor suppressor genes in liver tumors. This research was supported by a grant from the USEPA's Science to Achieve Results (STAR) program Grant number R82808301.

1090 PHENOBARBITAL (PB) INDUCES INITIAL HYPOMETHYLATION OF THE PROMOTER REGION OF HA-RAS, BUT NOT LINE-1 ELEMENTS, IN THE LIVER OF B6C3F1 MICE.

A. Carnell and J. I. Goodman. *Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

While mutagenesis is often considered to be important in carcinogenesis, it is clear that epigenetic mechanisms play a key role. In particular, DNA methylation as a regulator of gene expression is a central focus. We hypothesize that susceptibility to carcinogenesis is related inversely to the capacity to maintain normal DNA methylation patterns. Ha-*ras* an oncogene implicated in tumorigenesis, and the LINE-1 DNA element, a retrotransposable element capable of disrupting genomic stability when expressed, serve as two distinct gene regions whose expression may be influenced by changes in the methylation status of their 5' promoter regions. PB, a nongenotoxic rodent carcinogen, induces global hypomethylation in liver; however, few specific genes have been examined. Liver tumor-sensitive male B6C3F1 mice were administered PB, 0.05% (w/w), in drinking water for 2-wk. Hepatic DNA was isolated and the methylation of the promoter regions of Ha-*ras* and LINE-1 was assessed using the enzymatic regional methylation assay (ERMA). This involves bisulfite modification of DNA, PCR amplification with primers containing GATC sites (dam methylase recognition sequence) which serves as an internal control to normalize DNA quantity, incubation with dam methylase and [^{14}C-methyl]S-adenosyl-L-methionine (SAM) followed by incubation with [^{3}H-methyl] SAM and SssI methylase, which methylates C's 5' to G's, to quantify DNA methylation status. Hypomethylation is evidenced by a decreased $^{3}H/^{14}C$ ratio as compared to control. The mean Ha-*ras* and LINE-1 $^{3}H/^{14}C$ ratios for controls were 30.2±0.2 and 50.8±7.8 while the mean treated ratio was 24.1±0.4 and 53.3±4.4, respectively. The results were confirmed by bisulfite sequencing. PB caused a decrease in 5-methylcytosine content of the promoter region of Ha-*ras*, but not LINE-1 elements. This leads to two conclusions: a) PB-induced hypomethylation is initially selective, and b) hypomethylation of Ha-*ras*, increasing potential for expression, may be an early event during PB-promotion of mouse liver tumorigenesis.

1091 SPECIES DIFFERENCES IN THE INHIBITION OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION (GJIC) BY DIETHANOLAMINE.

L. M. Kamendulis, D. J. Smith and J. E. Klaunig. *Division of Toxicology, Indiana University School of Medicine, Indianapolis, IN.*

Diethanolamine increased the incidence and multiplicity of liver tumors in the mouse following chronic dermal exposure. Tumor induction by diethanolamine appears to be through a non DNA reactive mode of action and has been suggested to involve inhibition of cellular choline uptake. Choline deficiency is known to produce tumors in rodents, therefore, one might postulate that diethanolamine, through depletion of choline, may result in the development of tumors in rodents. Previously, we have reported that diethanolamine as well as choline depletion produces a dose-related induction of DNA synthesis in primary cultured mouse and rat hepatocytes, while no increase was seen in primary human hepatocytes. Furthermore, the DNA synthesis induced by diethanolamine was blocked by choline supplementation. These results support the hypothesis that diethanolamine-induced neoplasia results from choline depletion. Another hallmark of non DNA reactive carcinogen exposure is the inhibition of gap junctional intercellular communication (GJIC). The present studies examined the effect of diethanolamine and choline depletion on GJIC in primary cultured mouse, rat and human hepatocytes. Mouse and rat hepatocytes cultured with 0 - 500 µg/ml diethanolamine for 1, 4 and 24 hours showed a dose-related decrease in GJIC at all time points examined. Incubation of mouse and rat hepatocytes with medium containing reduced choline concentrations (1/100 of normal medium; 0.0898 vs. 8.98 mg/L) resulted in a similar decrease in GJIC at 1, 4 and 24 hours. Supplementation with medium containing 10-fold higher choline concentration restored GJIC in diethanolamine treated hepatocytes to control levels. No inhibition of GJIC was seen in human hepatocytes following diethanolamine treatment or choline depletion for 24 hours. These results showing a species selective inhibition of GJIC by diethanolamine that is reversed by choline supplementation provides further support that the mechanism for diethanolamine-induced neoplasia involves cellular choline depletion.

1092 PURIFICATION OF THE BIOACTIVE INGREDIENT IN PSYLLIUM THAT UP-REGULATES GAP JUNCTIONAL COMMUNICATION IN RAS-TRANSFECTED RAT LIVER EPITHELIAL CELLS.

Y. Nakamura[1], N. Yoshikawa[1], K. Sato[1], K. Ohtsuki[1], C. Chang[2], B. L. Upham[2] and J. E. Trosko[2]. *[1]Food Science, Kyoto Pref. University, Kyoto, Japan and [2]NFSTC, Michigan State University, Lansing, MI.*

Psyllium is a common source of soluble fiber derived from the husk of the plant *Plantago ovata*, which is used for colon care with its moderate laxative effect. We previously determined that psyllium powder restores gap junctional intercellular communication (GJIC) in a tumorigenic WB-F344 rat liver epithelial cell line transfected with the *H-ras* oncogene (WB*ras*), but not on WB cell lines transfected with the *src*, *neu* and *myc* oncogenes. The mechanism is due to increased levels of connexin43 protein and reduced levels of the Ras protein resulting in reversing the tumorigenic phenotype, namely anchorage independent growth, of this cell line. In the current study, we used purification techniques to isolate the bioactive fraction of psyllium that employed non-toxic solvents, which are amendable to safe food preparation. The effective dose of the crude psyllium powder that up-regulated GJIC in WB*ras* was 1.5 mg/ml. A simple ethanol extraction increased the activity of the extract to an effective dose of 50-200 ug/ml, suggesting that the active component is most likely not a fiber. This ethanol extract was further fractionated by adsorption chromatography using an HP-20 column. A step gradient ranging from 100% water to 100% ethanol was used to elute the bioactive component. The highest activity eluted at 90% ethanol, with an effective dose of 22 ug/ml. This is a purification-fold increase of 68 times that of the original crude powder. Additional purification was achieved using silica-gel adsorption column chromatography. The column (70-230mesh) was pre-eluted with hexane followed by elution with 70% ethylacetate. The active eluted fraction had an effective dose of 1.3 ug/ml, which is an 1154X purification-fold over the crude fraction. The final yield was 93ppm. In conclusion, adsorption chromatography can effectively increase the purity of the bioactive fraction of psyllium that decreases the tumorigenic phenotype of WB*ras* cells by up-regulating GJIC.

1093 PPARβ AMELIORATES CHEMICALLY-INDUCED LIVER TOXICITY.

W. Shan[1], M. T. Bility[1], C. J. Nicol[2], M. J. Kennett[1], J. M. Ward[2], F. J. Gonzales[2] and J. M. Peters[1]. *[1]Veterinary Science, Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University, University Park, PA and [2]Lab. of Metabolism, National Cancer Institute, Bethesda, MD.*

Peroxisome proliferator-activated receptor β (PPARβ) has attracted central attention concerning its multiple effects in lipid metabolism, development and carcinogenesis, but its role in hepatic function remains elusive. Preliminary studies examining the role of PPARβ in colon cancer suggested that PPARβ protects the liver from environmental chemical agents and prevents toxicity and carcinogenesis. To begin to test this hypothesis, wild-type and PPARβ-null mice were exposed to azoxymethane (AOM) and carbon tetrachloride (CCl4) and markers of liver toxicity were examined. PPARβ-null mice showed extensive bile-duct hyperplasia and severe liver lesions in response to AOM treatment, and this was not observed in similarly treated wild-type mice. PPARβ-null mice also exhibited abnormal lipid deposition around liver lobules as a result of CCl4, and this was not found in CCl4-treated wild-type mice. Consistent with histological analyses, higher levels of serum GPT were found in both AOM and CCl4-treated PPARβ-null mice as compared to wild-type mice. While expression patterns of cytochrome P450s were similar between both genotypes treated with AOM or CCl4, CYP2B10 was differentially expressed between genotypes. Combined, these observations suggest that PPARβ functions to attenuate liver toxicity induced by diverse environmental chemicals.

1094 BIOCHEMICAL, HISTOLOGICAL AND MOLECULAR CHARACTERIZATION OF THE INFLUENCE OF INITIATION ON CLOFIBRIC ACID-INDUCED HEPATOCARCINOGENESIS IN THE RAT.

C. Michel[1], C. Desdouets[2], V. Guilpin[1], M. Slaoui[1], R. A. Roberts[1] and E. Boitier[1]. *[1]Drug Safety Evaluation, Aventis Pharmacology, Vitry sur Seine, France and [2]INSERM U370, Paris, France.*

Clofibric acid (CA) is a non-genotoxic hepatocarcinogen in rodents that causes altered hepatocellular foci and /or hepatocellular neoplasms. The rate of appearance of foci and tumors can be accelerated by initiation using DNA-damaging agents such as diethylnitrosamine (DEN), although how initiation acts on tumor promotion and progression remains unknown. The aim of this study was to evaluate the impact of DEN on the promotion of CA-induced hepatocarcinogenesis. Male F344 rats were given a single injection of DEN (30 mg/kg). Thirteen days later, animals were fed diets containing 5000ppm CA for up to 12 months. Clinical pathology, gross macroscopic evaluation and gene profiling (Affymetrix microarrays) in normal liver tissues and liver tumors were performed. Mildly elevated mean alkaline phosphatase, secondary to CA-induced liver toxicity, was observed in rats treated with DEN+CA or CA alone. Tumors were macroscopically visible in rats treated with DEN+CA at 9 months as compared to a latency of 12 months in rats administered CA only. Gene expression profiles of these tumors demonstrated similar patterns, such as the regulation of genes involved in apoptosis (apoptosis-inducing factor, Caspase11). In order to evaluate the similarity of gene expression profiles in the normal liver tissue from the various groups, comparisons were performed using clustering methods. This showed that gene expression profiles from DEN and control groups clustered together; likewise those from normal tissue taken from DEN+CA and CA-treated animals were highly similar. Taken together, these results suggest that, even though DEN initiation accelerates tumor onset, it did not induce large changes in gene expression profiles of tumors and of normal tissues from treated animals. The next steps will be to focus on the evolution of DEN-initiated cells by comparing gene expression profiles of microdissected foci from DEN+CA rat livers with those obtained from CA rat livers.

1095 LACK OF BOTH HEPATIC PORPHYRIA AND TUMORS IN CYP1A2(-/-) MICE EXPOSED TO PCBs AND IRON.

A. G. Smith[1], B. Clothier[1], R. Davies[1], R. E. Edwards[1], T. P. Dalton[2], D. W. Nebert[2] and P. Greaves[1]. *[1]MRC Toxicology Unit, University of Leicester, Leicester, United Kingdom and [2]Environmental Health, University of Cincinnati, Cincinnati, OH.*

The mechanism of how environmental polyhalogenated aromatic chemicals cause liver tumors in rodents has never been elucidated, although of some pertinence to risk assessment. In rats, all chemicals of this type that have been investigated have shown marked sexual dimorphism towards females—both in propensity to induce liver tumors and development of porphyria. Evidence suggests a role for iron metabolism in these processes. In C57BL/10ScSn or C57BL/6 Cyp1a2(+/+) mice, porphyria and liver tumor incidence caused by hexachlorobenzene, polychlorinated and polybrominated biphenyls are markedly stimulated by iron overload. However, porphyria does not occur in C57BL/6 Cyp1a2(-/-) mice exposed to hexachlorobenzene or TCDD, even after additional treatment with iron. In this study, male Cyp1a2(+/+) and Cyp1a2(-/-) mice (5 per group) were administered the PCB mixture Aroclor 1254 in the diet (0.01%) for 7 weeks, with or without a prior sc dose of iron-dextran (800 mg Fe/kg). Hepatic porphyria was observed only in Cyp1a2(+/+) mice (mean value 12 nmol of uroporphyrin/g liver) and especially those also given iron (mean value 219 nmol/g). All other groups, including Cyp1a2(-/-) mice given iron and Aroclor, had mean values <1 nmol/g with no significant difference from untreated animals. When similar groups of male Cyp1a2(+/+) and Cyp1a2(-/-) mice (10 per group) were exposed to Aroclor for up to 57 weeks, survival time was decreased in the Aroclor/iron (+/+) mice but not in the null mice with correspondingly larger size livers (mean livers sizes of 21 and 16% of body weight respectively). All Aroclor or Aroclor/iron Cyp1a2(+/+) mice had large clear-cell or eosinophilic liver foci and/or adenomas; none was observed in the Cyp1a2(-/-) mice—although hepatocellular damage appeared as evident as the wild type. In conclusion, these findings support the hypothesis of a link between porphyria and liver tumor formation caused by polyhalogenated aromatic chemicals in rodents.

1096 ISOLATION AND ENRICHMENT OF PRENEOPLASTIC HEPATOCYTES DURING MALIGNANT CELL TRANSFORMATION.

A. Ashley, M. Lohitnavy, Y. Lu, L. Chubb, R. Billings, J. Campain and R. Yang. *Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO.*

We modified Ito's medium-term liver foci bioassay by incorporating time-course sacrifices to study pharmacokinetics/pharmacodynamics related to the initiation-promotion process. In our earlier clonal growth modeling studies on a series of chlorobenzenes, the probable existence of two cell types in glutathione-S-trasferase Π (GSTP) foci became apparent. As suggested by other researchers, these two cell types are either susceptible or resistant to mitoinhibitory effects as a response to external chemical insults, and those resistant to mitoinhibitory effect would have a growth advantage serving as basis for malignant transformation. Thus, as preneoplastic GSTP foci form, isolation of GSTP positive (GSTP+) cells from liver across time allows capturing changing characteristics of an emerging malignant population. This study describes an attempt to isolate primary GSTP+ hepatocytes using Ito's bioassay protocol with diethylnitrosamine (DEN) as an initiating agent and a 2/3 partial hepatectomy with concomitant dosing of hexachlorobenzene (HCB;

28.4 mg/kg/day) with and without coexposure to PCB 126 (9.8 μg/kg/day) as promoting agents. Male F344 rats were sacrificed at 20, 24, 28, 47 and 56 days, and 3, 6, and 9 months following DEN injection. By day 56, a significant increase in liver weight was observed in rats coexposed to HCB and PCB 126. Either primary hepatocytes or liver sections were taken to analyze formation of GSTP+ foci. By treating cell suspension and/or culture with ethacrynic acid (EA) (Stenius et al., Carcinogenesis 15:1561-1566, 1994), GSTP+ hepatocytes were enriched by inducing toxicity to non-GSTP+ cells. In so doing, progressively more malignant cell populations can be subjected to cell cycle kinetic studies, genomic analyses, and clonal growth modeling to aid our insight into the carcinogenic process. (Supported by NIOSH/CDC grant 1 RO1 OH07556 and NIEHS Training Grant 1 T32 ES 07321)

1097 MIPAFOX-INHIBITED ACHE YIELDS A DOUBLY AGED ACTIVE SITE.

T. J. Kropp and R. J. Richardson. *Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

Inhibition of AChE by organophosphorus (OP) compounds involves organophosphylation of the active site serine. Restoration of activity is prevented by aging of the inhibited enzyme. Aging usually involves loss of a ligand from the organophosphyl moiety to create a negative charge, e.g., AChE inhibited by diisopropylphosphorofluoridate (DFP) ages by net loss of an isopropyl group. In contrast, AChE phosphoramidated by tabun ages by scission of the P N bond to lose dimethylamine. The present study was undertaken to determine the mechanism of aging of MIP-inhibited AChE using DFP-inhibited AChE as a reference. The k_i, k_3, and k_4 for MIP-inhibited human recombinant AChE were 1.47 ± 0.047 $M^{-1}min^{-1}$, 78 ± 1.442 min^{-1}, and 0.0192 ± 0.00131 min^{-1}, respectively, and for DFP-inhibited enzyme were 404.5 ± 21.5 $M^{-1}min^{-1}$, 15 ± 0.132 min^{-1}, and 0.0213 ± 0.00156 min^{-1}, respectively. Using peptide mass mapping with surface enhanced laser desorption/ionization mass spectrometry, *m/z* peaks corresponding to active site peptides and their intact or modified adducts were examined in control and treated samples at 0, 1, 2, 12, 24, and 36 h after inhibition. Time dependent mass shifts representing organophosphylation of the active site peptide and subsequent ligand loss from the organophosphyl group were found for MIP- and DFP inhibited AChE. Loss of a single ligand was not found for MIP-inhibited AChE. Instead, a peak corresponding to net loss of two isopropylamine ligands (doubly aged, yielding a simple inorganic phosphate on the active site peptide) was found at time points corresponding to the kinetically aged MIP-inhibited AChE. The presence of this doubly aged moiety was confirmed by immunoprecipitation with an anti phosphoserine antibody. Analysis of DFP inhibited AChE revealed a singly aged phosphylated active site as expected. The results suggest that MIP inhibited AChE might be stabilized in its mono-aged state, allowing for a second aging reaction. No singly aged intermediate was found, suggesting that the second aging reaction proceeds at a relatively rapid rate. (Supported in part by ARO grant DAAD19-02-1-0388).

1098 EFFECTS OF CHRONIC DERMAL EXPOSURE TO METHYL PARATHION ON GLUTAMATE RECEPTOR SUBTYPES IN THE RAT BRAIN.

T. Ma, T. Sun, R. C. Baker, R. E. Kramer and I. K. Ho. *Pharmacology & Toxicology, University of Mississippi Medical Center, Jackson, MS.*

Toxicity to organophosphorus insecticides is mediated primarily through inhibition of acetylcholinesterase, which results in abnormally high cholinergic activity. However, organophosphorus compounds can influence other neurotransmitter systems, and actions at noncholinergic synapses may also contribute to organophasphate toxicity. For example, the excitatory amino acid plays a prominent role in the maintenance of organophosphate-induced seizures and in the subsequent neuropathology especially through an overactivation of glutamate receptor subtypes. In present study, we investigated the effects of chronic dermal exposure to sublethal doses of methyl parathion on glutamate receptor subtypes. Adult female rats (5 animals/treatment group) received 0, 0.1 or 1.0 mg/kg/day methyl parathion for 95 days. Receptor binding was analyzed be autoradiographic method. Methyl parathion treatments significantly increased [3H]MK-801 binding to the N-methyl-D-aspartate (NMDA) receptor subtype in several brain regions including striatum as well as inter- and superior-colliculus. No significant change in [3H]AMPA binding to the AMPA receptor subtype was found in the two methyl parathion-treated groups. The results indicated that NMDA receptor is, but not AMPA, a target for the organophosphorus insecticide. Toxicological implication of methyl parathion-induced alteration of NMDA receptor needs to further study. (Supported by CDC grant RO6/CCR419466)

1099 MOTOR FUNCTIONS BUT NOT ACQUISITION AND RETENTION OF ACTIVE AVOIDANCE RESPONSE ARE IMPAIRED IN METHYL PARATHION-TOLERANT RATS.

T. Sun[1], I. A. Paul[2] and I. Ho[1]. *[1]Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS and [2]Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS.*

Previous work in this lab indicated that repeated exposure to methyl parathion (MP) caused prolonged inhibition of acetylcholinesterase (AChE) activity, and down-regulation of muscarinic receptors in the brain regions, which showed the neurochemical evidence of development of tolerance to MP. These changes were accompanied by a slower body weight gain and progressive deterioration of motor function in MP-treated Sprague-Dawley rats. MP produced substantial effects on several different aspects of motor function, including spontaneous locomotor function, rearing and sniffing and neuromuscular coordination. A lack of tolerance to the motor suppressant effects of MP was observed throughout the exposure with the proceeding in reduction of muscarinic receptors. But the neuronchemical changes did not cause impairment in the performance of acquisition and retention in active avoidance responding. Our studies indicate that prolonged inhibition of AChE activity and down-regulation of central nervous muscarinic receptors produced selected behavioral effects. The motor functions appear more sensitive to the chronic MP exposure comparing to cognitive behaviors. It is consistent with our previous finding that the most extensive down-regulation of muscarinic receptors was found in the striatum (-30%), the area believed to involve in the regulation of motor function.

1100 EFFECTS OF ESTROGEN ON THE NEUROTOXICITY OF DELTAMETHRIN IN THE RAT CORTICAL BRAIN SYNAPTOSOMES.

N. Shi, L. Chen, J. Dong, T. Li and D. Chen. *Department of Health Toxicology, Tongji Medical College Huazhong University of Science and Technology, Wuhan, Hubei, China.* Sponsor: Z. Lai.

Deltamethrin is a pyrethroid insecticide that shows neurotoxicity for the animals. It is still unclear how deltamethrin affect central neural system in the animals. In the present study, cortical brain synaptosomes from ovariectomized rats were incubated with deltamethrin in absent or present of estradiol and/or tamoxifen in order to investigate the effects of deltamethrin on cortical brain synaptosomes and to see if estradiol has neuroprotective effects against deltamethrin. Our results show that 2×10^{-5} mol/L of deltamethrin induced significant increases in Asp and Glu release from the cerebral cortical synaptosomes at the depolarizing state evoked by 50 mmol/L KCl. However, these effects could be partial blocked by estradiol at the level of 10^{-8} and 10^{-11} mol/L, when the cerebral cortical synaptosomes were pre-incubated with estradiol for 1-hour before co-incubation with deltamethrin. Furthermore, the activities of ATPase of cortical synaptosomes were inhibited by 2×10^{-5} mol/L of deltamethrin, and these effects could be blocked by 10^{-5} mol/L of estradiol. No effects of tamoxifen either on the function of estradiol or on the effects of deltamethrin were found. Taken together, our findings confirm that the neurotoxicity of deltamethrin is related to the increases of Asp and Glu release and inhibition of ATPase, and estradiol can against the effects of deltamethrin on cerebral cortical synaptosomes. Tamoxifen did not alter the effects of estradiol against deltamethrin, suggesting that the effects of estradiol against deltamethrin might not be mediated through estrogen receptor. These results indicate a nongenomic mechanism of estradiol on its neuroprotective effects.

1101 MODULATION OF DOPAMINE METABOLISM BY SEVERAL METABOLITES OF THE HERBICIDE ATRAZINE IN RAT STRIATAL SLICES.

N. M. Filipov[1], M. Tsunoda[1,2] and S. C. Sistrunk[1]. *[1]CEHS, Basic Sciences, Mississippi State University, Mississippi State, MS and [2]Public Health, Fukushima Medical University, Fukushima, Japan.*

Environmental factors, such as pesticide exposure, have been postulated to contribute to the etiology of Parkinson's Disease. Recently, it was demonstrated that (*i*) the herbicide atrazine (ATR) modulates dopamine (DA) metabolism in PC-12 cells and (*ii*) ATR exposure results in a dose-dependent decrease of tissue DA in rat striatal slices. Some of the major metabolites of ATR in the environment and in mammals are: hydroxyatrazine (HA), deethylchlorotriazine (DE), deisopropylchlorotriazine (DIP) and diaminochlorotriazine (DACT). The objective of the current study was to evaluate the ability of these ATR metabolites to modulate DA metabolism in rat striatal slices. Rat striata were incubated with up to 500 μM of each ATR metabolite in a metabolic shaker bath for 4 h. Tissue and media levels of DA and its metabolites 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA)) were determined by HPLC-ECD. Lactate dehydrogenase (LDH) release into the culture media was used as an index of non-specific cytotoxicity. Exposure

to all metabolites at the 100 and 250 µM doses, increased media and tissue DOPAC levels and, to a lesser extent, media HVA levels. DE and DIP were the most effective ATR metabolites in increasing DOPAC levels. However, in contrast to the previously observed dose-dependent decrease in tissue DA levels by ATR, none of its metabolites tested in this study affected tissue DA levels significantly. The effects of the ATR metabolites on DA metabolism were observed in the absence of increased LDH release. Thus, it could be concluded that (*i*) all studied metabolites, contrary to ATR itself, are largely ineffective in decreasing tissue DA, (*ii*) these metabolites are nevertheless capable of modulating DA metabolism and (*iii*) of the four metabolites tested, DE and DIP were most effective whereas DACT, although showing similar trends, was least effective. Supported in part by: P20 RR017661 (NIH).

1102 NEUROTROPHIN EXPRESSION IN THE SPINAL CORD OF CHICKENS DURING ACTIVE NERVE FIBER DEGENERATION FOLLOWING EXPOSURE TO ORGANOPHOSPHATE COMPOUNDS.

M. J. Pomeroy, D. Parran, M. Ehrich and B. Jortner. *Virginia Tech, Blacksburg, VA.*

Exposure to certain organophosphates (OP) has been shown to induce a delayed neuropathy (OPIDN) characterized by Wallerian-like degeneration in certain animals, including humans. The hen serves as the major animal model for this neuropathy as it demonstrates clinical signs within days of OP dosing. We hypothesized that fiber degeneration induced by OP administration results in an upregulation of neurotrophin expression, particularly nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) in susceptible regions of the nervous system. Hens were exposed to TOTP (500 mg/kg), a neuropathy-inducing OP. By day 9, all hens demonstrated clinical signs of OPIDN ranging from clumsiness to postural alterations. On the day of sacrifice (day 14), all hens demonstrated ataxia and two birds had obvious difficulty standing. Hens were sacrificed by pentobarbital overdose. Microscopic study of the nerve of the biventer cervicis muscle confirmed the presence of a neuropathy. Cervical spinal cord tissue from each hen was used for enzyme-linked immunosorbant assays (ELISAs) in order to determine the presence of NGF, BDNF, and NT-3. Commercially available rat-derived NGF and mouse-derived NT-3 antibodies and mouse-derived BDNF antibody were suitable for these studies. Relative to controls, we detected a near doubling of NGF and BDNF levels, and an increase in NT-3 of almost 25% in the spinal cord of OP treated hens 14 days post-dosing. This study suggests levels of specific neurotrophic factors are increased in hens with OPIDN.

1103 CHLORPYRIFOS ALTERS FUNCTIONAL INTEGRITY AND STRUCTURE OF AN *IN VITRO* BBB MODEL.

D. Parran, G. Magnin, W. Li, B. S. Jortner and M. Ehrich. *Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.*

The blood-brain barrier (BBB) is a structural and functional interface between the circulatory system and the brain. Organophosphorous (OP) compounds such as chlorpyrifos (CPF) may cross the BBB and disrupt BBB integrity and function. To determine possible mechanisms of action for CPF toxicity, we used an *in vitro* BBB model where bovine microvascular endothelial cells (BMEC) and neonatal rat astrocytes were co-cultured. We hypothesized that CPF is metabolized by the BBB to CPF-oxon leading to an inhibition of esterase activity and a disruption of the BBB. The co-culturing of BMECs and astrocytes resulted in tight junction formation as evident by electron microscopy, electrical resistance and western blot analysis of two tight junction-associated proteins (ZO-1 and e-Cadherin). We observed a time dependent increase in ZO-1 and e-Cadherin expression as well as electrical resistance during BBB formation, which was maximal after 9 to 13 days of co-culturing. The CPF concentration and production of its metabolites were monitored by HPLC following 24 hr exposure to CPF. We found that the BBB metabolized CPF, with the metabolite 2, 3, 6-trichloro-2-pyridinol being the major product. The detected quantity of CPF and its metabolites on the luminal and abluminal side of the BBB represented 1% or less of the initial quantity added to the incubation medium, suggesting that not all of the CPF (900 - 8, 600, 000 nM) crossed the BBB. At all tested concentrations (100 -10, 000 nM) CPF inhibited both carboxylesterase (CaE) and cholinesterase (ChE) activity by 43% to 100%, while CPF-oxon totally inhibited CaE and ChE activity. There was also a concentration-dependent decrease in electrical resistance. A decrease in electrical resistance was observed at CPF concentrations as low as 1 nM with complete inhibition at 1000 nM. These data suggest that low concentrations of CPF and its metabolites are trapped within the BBB. These metabolites, including CPF-oxon, are contributing to the inhibition of CaE and ChE activity and the alteration of BBB integrity and structure.

1104 CONCURRENT CHRONIC STRESS AND CHLORPYRIFOS ATERED SWIMMING BEHAVIOR MORE THAN CHRONIC STRESS ALONE.

T. Pung, K. Knight, G. Magnin, K. Fuhrman, J. Hinckley and M. Ehrich. *Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.*

Effects of concurrent chronic stress and chlorpyrifos (CPF) on swimming behavior, enzymes of acetylcholine synthesis and metabolism in brain, and blood chemistry were studied in Long Evans rats. Groups of rats (n=7) were handled 5 days/week, restrained 1 hour/day for 5 days/week, swum 30 minutes for 1 day/week, or restrained 4 days with swimming 1 day/week, for 28 days. On day 24, each group was gavaged either with corn oil or CPF 160 mg/kg SC 4 hours after restraint. Blood samples were collected on day 28 for acteylcholinesterase (AChE) activity and clinical pathology. Brains were dissected into hippocampus (HP) and cerebral cortex (CC) for levels of AChE, carboxylesterase (Cbxy), and choline acetyltransferase (ChAT). On day 28, CPF-treated rats that were swum and rats restrained and swum were less able to tolerate being in the water than rats treated with corn oil with average swim times of 27.1 and 28.9 minutes, respectively. Stressed rats given corn oil swum an average of 29.4 and 30 minutes, respectively. CPF inhibited AChE activity in blood, CC and HP, but stress did not affect AChE activity. CPF inhibited Cbxy activity in CC and HP. Restraint with swim stress also inhibited cortical Cbxy (40.05 2.21 compared to ranges of 53.49-56.54 nmole phenyl valerate hydrolyzed/min/mg protein in other groups). Neither chronic stress nor CPF affected ChAT activity in CC and HP. The levels of plasma total protein (6.71 0.08 g/dL), albumin (3.44 0.03 g/dL), and sodium (139.48 0.44 mEq/L) were higher in restrained and swum rats than in handled rats and restrained rats; however, potassium levels (7.01 0.26 mEq/L) were lower. CPF also decreased the levels of total protein and potassium but did not show an interaction with stress. Rats, therefore, responded differently to different types of stress.

1105 DOSE RESPONSE OF NEUROPATHY TARGET ESTERASE INHIBITORS ON ATP PRODUCTION AT COMPLEX I AND II IN HUMAN NEUROBLASTOMA CELLS IN SITU.

K. M. Knight[1], C. Massicotte[2] and M. Ehrich[1]. *[1]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA and [2]Matthew J. Ryan Veterinary Hospital, University of Pennsylvania, Philadelphia, VA.*

Organophosphate (OP) neurotoxicants that inhibit neuropathy target esterase (NTE) can cause delayed neuropathy (OPIDN) in susceptible animals. Altered energy production in target cells is a possible contributing mechanism and was investigated in human neuroblastoma SH-SY5Y cells. For these studies, mitochondrial ATP production was examined 30 min after exposure to 0.1 - 1 mM of the neuropathic OP, phenyl saligenin phosphate (PSP) and 0.1 - 5 mM phenyl methyl sulfonyl fluoride (PMSF), an inhibitor of NTE unable to induce OPIDN. After depletion of residual ATP, digitonin permeabilized cells were incubated at 37 C/5% CO2 in buffer supplemented with ADP and either glutamate and malate (Complex I substrates) or succinate (Complex II substrate). After 30 min, cells were lysed by addition of 8% trichloroacetate and neutralized with a MOPS/potassium hydroxide buffer. ATP was quantified using a luciferase enzyme assay (Sigma Chemical Co., St. Louis, MO). Results demonstrated an inhibition of ATP production at Complex I that was concentration dependent (0.1-1 mM for PSP; 1-5 mM for PMSF), with highest concentrations providing 88% and 74% less ATP than control cells, respectively. PSP also suppressed ATP production at Complex II in a concentration-dependent manner, with 46% inhibition at 1 mM. Inhibition of ATP production by the Complex I inhibitor rotenone was augmented in the presence of PSP but not PMSF. These results suggest that NTE inhibitors affect cellular energetics of SH-SY5Y cells in situ in a concentration-dependent manner, with PSP more potent than PMSF.

1106 ORAL BIOAVAILABILITY AND NERVOUS TISSUE DISTRIBUTION OF CYHALOTHRIN IN RATS.

M. R. Martinez-Larranaga, M. Martinez, M. A. Martinez, M. J. Diaz, M. T. Frejo and A. Anadon. *Toxicology & Pharmacology, Faculty of Veterinary Medicine, Complutense University, Madrid, Spain.*

The primary site of action of the pyrethroid insecticides is the nervous system. Type II pyrethroids, such as cyhalothrin, are characterized by blocking nerve conduction and by causing convulsions and paralysis. The nerve-blocking action of Type II pyrethroids has been associated with the release of neurotransmitters. Because the toxicokinetic data should be considered for the risk assessment of this drug, the objective of the present study was to determine the kinetic behaviour of cyhalothrin after oral administration. Male Wistar rats were treated orally with cyhalothrin (dissolved in corn oil) (single dose of 20 mg/kg) and killed at different time period after

treatment. Plasma and nervous tissue samples (hypothalamus, cerebellum, midbrain, spinal cord and ciatic nerve) were isolated, homogenized and extracted in acetonitrile to determine cyhalothrin levels by HPLC-UV. The cyhalothrin plasma and tissue profile could be adequately described by a two-compartment open model. The plasma disposition of cyhalothrin was characterized by a rapid absorption and distribution phases and a slow elimination phase ($t_{1/2}a$ = 0.89 hr, $t_{1/2}\alpha$ = 1.87 hr, $t_{1/2}\beta$ = 9.90 hr). The oral bioavailability was 71%. The tissue elimination half-lives and the ratios of tissue/plasma area under the concentration-time curves indicated a tendency of cyhalothrin to accumulate in nervous tissues. It is anticipated that knowledge of the kinetics of cyhalothrin would lead to a better understanding of its neurotoxicity.

_____ Cmax(μg/ml);Tmax(h);$t_{1/2}\beta$(h);AUCtissue/AUCplasma

_____ Hypothalamus: 25.56 μg/ml; 2.35 h; 34.65 h; 1.89 Cerebelum: 18.44 μg/ml; 2.75 h; 15.06 h; 1.11 Midbrain: 18.48 μg/ml; 2.15 h; 25.67 h; 1.12 Spinal cord: 12.31 μg/ml; 3.19 h; 23.10 h; 0.90 Sciatic nerve: 20.08 μg/ml; 3.67 h; 19.80 h; 2.17

1107 DECREASE OF 5-HT LEVELS AFTER FIPRONIL TREATMENT.

A. Anadon, R. Pita, Y. Garcia-Uzcategui, M. J. Diaz and M. R. Martinez-Larranaga. *Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Complutense University, Madrid, Spain.*

Fipronil is the first phenylpyrazole insecticide introduced for pest control. It is highly effective broad-spectrum insecticide with many applications in agriculture, public health and animal health, and good efficacy against many resistant strains. While fipronil is known to be a highly effective blocker of insect GABA receptor- and glutamate receptor-chloride channels, its neurotoxicity on mammals has been less characterized. Because of a variety of putative biochemical and physiological target sites may contribute to fipronil toxicity, the objective of the present study was to investigate neurochemical effects following the oral administration of fipronil (5 and 10 mg/kg for 6 days) in male Wistar rats (n =6/group). Animals were sacrificed 24 hours following drug administration and their brains were rapidly removed. The hypothalamus, hippocampus and striatum were dissected and analyzed for content of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxy-3-indole acetic acid (5-HIAA) using a HPLC method with electrochemical detection. Fipronil produced a serotonin depleting effect in a dose-dependent maner. Fipronil (10 mg/kg for 6 days) decreased 5-HT levels in hypothalamus (29%, $P<0.01$), hippocampus (40%, $P<0.01$) and striatum (45%, $P<0.001$) and decreased 5-HIAA levels in hypothalamus (26%, $P<0.05$), hippocampus (34%, $P<0.01$) and striatum (45%, $P<0.001$) respect to corn oil controls. The data presented herein suggests that a lower activity of serotonergic system exists in the action of fipronil.

1108 INHIBITION OF CHOLINESTERASE AND CARBOXYLESTERASE FOLLOWING *IN VIVO* EXPOSURE OF RATS TO MIXTURES OF PARATHION AND CHLORPYRIFOS.

E. Meek, R. Carr, H. Chambers, J. Kamykowski and J. Chambers. *Mississippi State University, Mississippi State, MS.*

The patterns of inhibition of brain and serum cholinesterase (ChE) and serum carboxylesterases (CbxE) were determined after oral administration of two (organophosphorus) insecticides singly or in simultaneous or sequential mixtures. The effective dose levels that would inhibit 10% of brain ChE at the time of peak inhibition were determined to be 1.0 mg/kg parathion (P) and 5.0 mg/kg chlorpyrifos (C) at 10 and 12 hr, respectively. Sequential exposures were performed with the first insecticide administered followed by the second insecticide at the peak inhibition time for the first. Tissue samples were collected at 2, 4, 8, 10, 12, and 24 hr. Brain and serum ChE levels were determined by continuous spectrophotometric assay, and serum CbxE inhibition was determined by discontinuous spectrophotometric methods. Inhibition of brain ChE for C was 4% at 4 hr, 7% at 8 hr, and 11% at 12 hr, and 5% at 24 hr; for P was 7% at 8 hr, 12% at 10 hr, and 0% by 24 hr. Simultaneous exposure prolonged inhibition from 2% at 8 hr to 21% at 12 hr. Sequential exposure (P followed by C) indicated greater-than-additive effects; inhibition ranged from 21% at 4 hours to 20% at 24 hr with peak inhibition at 8 hours (36%). C followed by P produced inhibition at earlier time points, compared to the single compounds; 15% at 2 hr, peak at 4 hr (25%), and 0% by 24 hr. Inhibition of serum ChE levels decreased at successive time points, and simultaneous and sequential exposures showed no increase in inhibition. Serum CbxE was greatly inhibited by P (from 81% at 2 hr to 28% at 24 hr) and C (from 93% at 2 hr to 50% an 24 hr). Inhibition of serum CbxE levels decreased at successive time points following simultaneous and sequential exposures. There is evidence of some interactions following sequential exposure to binary mixtures of these two insecticides administered at relatively low dosages with differences in patterns of response occurring with different order of administration of the two compounds. (Supported by American Chemistry Council CRAM 2a-99).

1109 PHARMACOKINETIC DIFFERENCES MAY EXPLAIN THE AGE-RELATED SENSITIVITY OF DELTAMETHRIN, A PYRETHROID INSECTICIDE, IN RATS.

W. Haines[1, 2], R. S. Marshall[2], D. L. Hunter[2] and S. Padilla[2, 1]. *[1]Toxicology, UNC- CH, Chapel Hill, NC and [2]NHEERL, USEPA, Research Triangle Park, NC.*

This study was designed to examine the age-related sensitivity to the pyrethroid insecticide, deltamethrin [(S)-?-cyano-3-phenoxybenzyl (1R, 3R)-3-(2, 2-dibromomovinyl)2, 2-dimethylcyclopropanecarboxylate], in Long Evans, hooded, male rats. Deltamethrin has been shown to be more acutely toxic to weanling (21 days) rats (Sheets et al., Toxicol Appl. Pharmacology, 1994) than adults. Our hypothesis is that weanling rats are more sensitive than adults to deltamethrin due to pharmacokinetic differences. Therefore, we conducted time course studies in both weanling (PND 21) and adult (PND 90) male rats to determine the intensity of and time of peak toxic signs [i.e., salivation and choreoathetosis (jerky, involuntary movements)] as well as deltamethrin concentrations in the brain. Rats (n = 5 per age and treatment) were gavaged with either corn oil or 30 mg/kg deltamethrin for the adults and 4 mg/kg deltamethrin for weanlings. The toxic signs observed in the weanlings were as severe as the adults. Peak brain concentrations of deltamethrin were comparable in both the adult and weanling rats. These findings indicate that weanling animals given a dosage which is 13% of the adult deltamethrin dosage show toxic signs and brain concentrations of deltamethrin comparable to the adult. This abstract of a proposed presentation does not imply EPA policy.

1110 TWO-GENERATION DIETARY REPRODUCTIVE TOXICITY STUDY WITH CHLORPYRIFOS-METHYL IN CD RATS: INHIBITION OF ACETYLCHOLINESTERASE.

B. Marable[1], K. E. Stebbins[1], A. B. Liberacki[1], S. Marty[1], R. Billington[2] and E. W. Carney[1]. *[1]The Dow Chemical Company, Midland, MI and [2]Dow Agrosciences, Oxon, United Kingdom.*

As part of a two-generation study to assess the reproductive function and development of rats exposed to chlorpyrifos-methyl (CPM), acetylcholinesterase (AChE) activities were measured in the brains, red blood cells (RBC), and hearts of adults and pups (post-natal day (PND) 1, 4, and 22; n = 5/sex/group). Male and female CD rats were exposed to 0, 1.0, 3.0, or 10.0 mg/kg/day CPM in their diets for 10 weeks prior to breeding, during gestation, and lactation for two generations. No treatment effects were seen on any of the reproductive or developmental parameters examined in either generation. Gross effects were limited to increased adrenal gland weights in P1 animals (high-dose males and females), and P2 animals (high-dose males and females; mid-dose females), which were correlated with vacuolization of the zona fasciculata of the adrenal glands. The no-observed-effect level (NOEL) for reproductive toxicity was 10 mg/kg/day and the no-observed-adverse effect level (NOAEL) for parental toxicity, based on adrenal histopathology in mid-dose females, was 1.0 mg/kg/day. In adults, dose-dependent inhibition of RBC AChE occurred at all doses levels, in both sexes, in both generations. In high-dose adults, brain and heart AChE activities were significantly lower (by 13.3 and 23.3%, respectively) than controls. Similar differences in adult heart AChE activities were not seen in the P2 generation. In high-dose pups (PND 1 and 4), RBC AChE activities were lower (by 21.3%) than controls, but these differences disappeared by PND 22 in both generations. No effects on pup brain or heart AChE activities were seen in any dose group at any time during the study. These data indicate that inhibition of RBC AChE occurs at much lower (10-fold) doses in adults exposed to CPM in their diets than pups exposed during gestation and lactation. Overall, pup brain and heart AChE activities were unaffected by exposure to CPM at any of the doses examined.

1111 PROPERTIES AND FIPRONIL MODULATION OF INSECT GLUTAMATE-GATED CHLORIDE CHANNELS.

X. Zhao[1], J. Z. Yeh[1], V. L. Salgado[2] and T. Narahashi[1]. *[1]Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL and [2]Global Biology Insecticides, Bayer CropScience, Monheim, Germany.*

Glutamate-gated chloride channels (GluCls), found only in invertebrates but not in mammals, are an important target for some insecticides and may play a critical role in their selective toxicities between mammals and insects. The present study was to further characterize the properties of GluCls and to explore the mechanisms of action of fipronil, a phenylpyrazole insecticide, on the receptors in cockroach thoracic ganglion neurons using the whole-cell patch clamp technique. Two types of

GluCls, desensitizing and non-desensitizing GluCls, were elicited by glutamate, and they could coexist with and function independently of the GABA receptor chloride channels. The non-desensitizing GluCls exhibited a higher sensitivity than the desensitizing GluCls to picrotoxinin, a GABA receptor antagonist. Fipronil inhibited the desensitizing and non-desensitizing GluCls with IC_{50}s of 801 nM and 10 nM, respectively. Kinetic analysis revealed that fipronil preferentially blocked open channels. The recovery of the desensitizing current from fipronil block required channel openings, whereas the recovery of non-desensitizing current from block was independent of channel openings but occurred in a time-dependent manner. The high potency of fipronil for inhibition of the non-desensitizing current was due to a slow unblocking rate constant. In addition, the non-desensitizing GluCls pre-occupied by picrotoxinin became less sensitive to fipronil block. It is concluded that GluCls are a critical target for fipronil forming the basis for the selective toxicity between mammals and insects. Supported by NIH grant NS14143.

1112 TIME AND CONCENTRATION DEPENDENT ACCUMULATION OF [^{3}H]-DELTAMETHRIN IN *XENOPUS* OOCYTES.

J. A. Harrill[1], C. A. Meacham[2], T. J. Shafer[2] and K. M. Crofton[2]. *[1]Curriculum in Toxicology, University of N. Carolina, Chapel Hill, NC and [2]Neurotoxicology Division, NHEERL, ORD, USEPA, Research Triangle Park, NC.*

The primary target of pyrethroids is the voltage sensitive sodium channel, a channel protein that can be expressed in oocytes from *Xenopus laevis*. A common assumption when utilizing this experimental platform is that media concentration is a predictive marker of tissue concentration. This assumption may not hold true for lipophilic xenobiotics. [^{3}H]-deltamethrin (DLT) was used to test the hypothesis that media concentration is a good surrogate for tissue concentration. Accumulation of [^{3}H]-DLT (97+%; 0.001-10 μM) in oocytes exposed for 20 minutes was determined using liquid scintillation counting. The time course (1-180 min) of tissue accumulation of [^{3}H]-DLT (1.0 μM (0.50ppm) in media) was also determined. Results demonstrate that DLT accumulates in oocytes as a function of time (~0.010 ppm at 1 min: ~1.04 ppm at 180 min). The relationship between media and oocyte concentration is non-linear. Below 0.5 μM DLT in the media, media and oocyte concentrations are approximately equivalent. Above 0.5 μM, media concentration under-predicts oocyte concentrations by up to 5-fold. No apparent asymptotes were found in the ranges tested for either variable. These data suggest that media concentration may not accurately predict tissue concentrations in *in vitro* studies of deltamethrin. *(This abstract does not necessarily reflect USEPA policy).*

1113 CHRONIC DIETARY EXPOSURE WITH INTERMITTENT SPIKE DOSES OF CHLORPYRIFOS FAILS TO ALTER SOMATOSENSORY EVOKED POTENTIALS, COMPOUND NERVE ACTION POTENTIALS, OR NERVE CONDUCTION VELOCITY IN RATS.

J. E. Graff, R. S. Marshall, D. L. Hunter and D. W. Herr. *Neurotoxicology, USEPA, Research Triangle Park, NC.*

Human exposure to pesticides is often characterized by chronic low level exposure with intermittent spiked higher exposures. Cholinergic transmission is involved in sensory modulation in the cortex and cerebellum, and therefore may be altered following chlorpyrifos (CPF) exposure. This study examined the effects of chronic (1 year) dietary exposure (0, 1, or 5 mg/kg/day) to CPF in weight maintained (350g) male Long Evans rats (100-110 days old at study initiation) on somatosensory evoked potentials (SEPs). Peripheral compound nerve action potentials (CNAPs) and nerve conduction velocity (NCV) were also examined to differentiate possible changes in the central vs peripheral nervous system. The CPF doses were chosen to produce minimal and approximately 50% inhibition of brain cholinesterase activity, respectively. In addition to dietary exposure, half of the rats received an oral bolus of 45 mg/kg CPF (in corn oil) every other month (n = 16-17 rats/treatment). In order to examine only irreversible effects, the animals were tested 2.5 months after final exposure. Rats were implanted with screw electrodes over the cortex and cerebellum. One week later, unanesthetized animals were restrained and their tails inserted into a stimulation/recording device. Biphasic stimulation at 1, 2 and 3 mA was delivered *via* two pairs of stimulation electrodes (stainless-steel 25 ga needles) placed in the tail. Another pair of electrodes recorded the CNAP and NCV. The evoked potentials showed the expected intensity-dependent changes in all treatment groups, indicating that the animals' responses were under stimulus control. Chronic dietary CPF (alone or in combination with spike doses) did not produce any irreversible changes in cortical or cerebellar SEPs, CNAPs or NCVs in adult animals. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

1114 BLOOD LEAD IS A PREDICTOR OF HOMOCYSTEINE LEVELS IN A POPULATION-BASED STUDY OF OLDER ADULTS.

J. H. Schafer[1], T. A. Glass[2] and B. S. Schwartz[1, 2]. *[1]Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD and [2]Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Clinical and epidemiologic research suggests that homocysteine increases the risk of cardiovascular disease and cognitive dysfunction. Lead is also associated with these two outcomes. We evaluated relations between whole blood lead and serum homocysteine in an analysis of first visit data among subjects in the Baltimore Memory Study, a longitudinal study of 50-70 year old randomly- selected residents in Baltimore City. Households in the study area were randomly selected (Department of Planning database), and were telephoned for recruitment. Of the community-based subjects on whom eligibility could be determined, 60.8% were scheduled for an enrollment visit; and 1, 140 were enrolled and tested. Subjects had a mean (SD) age of 59.3 (6.0) years, and were 65.8% female, 53.5% white, and 41.6% African American. Mean (SD) blood lead and homocysteine levels were 3.5 (2.2) μg/dL and 9.9 (3.9) μmol/L, respectively. Homocysteine levels varied by gender (mean [SD] 9.3 [3.6] in women and 11.2 [4.2] in men) but not by race/ethnicity. In unadjusted analysis, blood lead and homocysteine were moderately correlated (Pearson's $r = 0.27$, $p < 0.01$). After adjustment for age, gender, race/ethnicity, education, tobacco and alcohol consumption, and body mass index using multiple linear regression, results revealed that homocysteine levels increased 3.5 μmol/L per 10 μg/dL increase in blood lead ($p < 0.01$). The relations of lead with homocysteine levels did not differ in subgroups by age, gender, or race/ethnicity. To our knowledge, these are the first data to reveal an association between blood lead and homocysteine. The results suggest that homocysteine could be a mechanism that underlies the effects of lead on the cardiovascular and central nervous systems. We believe that inhibition by lead of cystathionine β-synthase, a heme-containing enzyme in the trans-sulfuration pathway of methionine metabolism that is regulated by redox changes, should be evaluated as a possible explanation.

1115 IMMUNOGLOBULINS TO AUTOANTIGENS OF NERVOUS AND REPRODUCTIVE SYSTEMS IN MALES OCCUPATIONALLY EXPOSED TO LEAD.

H. A. El-Fawal[1], A. De Feo[1] and M. Shamy[2]. *[1]Neurotoxicology Laboratory, Mercy College, Dobbs Ferry, NY and [2]Institute of Public Health, University of Alexandria, Alexandria, Egypt.* Sponsor: M. Ehrich.

Lead is toxic to nervous and reproductive systems. Toxicity, in part, may be due to its disruption of vascular beds, including the blood-brain (BBB), blood-nerve (BNB) and blood-testicular barriers (BTB). In addition to neurodegeneration, the compromise of these barriers may allow interaction between system-specific autoantigens and the immune system as indicated by serum autoantibodies. In this study, demographically and socioeconomically matched male workers at a lead (Pb) battery factory (n=30; age=42+6) or a frozen food plant (reference group R; n=30; age=41+7) were given a neurological exam and blood was collected. Blood Pb (PbB, μg/dl) determination and enzyme-linked immunosorbent assay (ELISA) for autoantibodies to neurofilament (NF) proteins, myelin basic protein (MBP) and anti-sperm antibodies (ASA) were performed. PbB was 24 ±7, and 10 ±3 for the Pb group and R, respectively. Dorsal column or spinothalamic tract deficits were absent in R, however, 40% of those exposed to Pb had some signs of dorsal column (vibration and stereognosis) or spinothalamic (pain) deficits. Motor deficits, hand tremors or hyporeflexia, were rare and confined to the Pb group. Autoantibodies (IgG) to one, or more, of the NF triplet proteins, particularly NF-68, were detected in the Pb group, and only in 2 of the males in the R group. These IgG levels correlated significantly ($r=0.5$; $p=0.001$)with the clinical score and PbB. Only 6 of those exposed to Pb had IgG to MBP. Confirmation of these results was determined by Western immunoblot of random serum samples. ASA, IgA or IgG, were found in 40% of the Pb workers, but not in R. These correlated significantly with PbB ($r=0.3$; $p=0.05$). Autoantibodies to nervous system proteins have been reported to contribute to neuropathogenesis, while ASA contribute to immunological infertility. This study demonstrates that Ig to system-specific autoantigens may be generated as markers of insult resulting from exposure to agents that compromise barriers such as the BBB or BTB.

1116 LOW LEVEL LEAD EXPOSURE ALTERS THE MESOCORTICOLIMBIC EXCITATORY/INHIBITORY AMINO ACID NEUROTRANSMITTER BALANCE.

Y. Gedeon and A. L. Jadhav. *Texas Southern University, Houston, TX.*

Environmentally relevant level of lead (Pb) exposure produces deleterious cognitive effects attributed to Pb-induced mesocorticolimbic neurotransmitter disruption. Previous report indicates that time plays a critical, region-specific role in Pb's effects

on the mesocorticolimbic dopaminergic system (Gedeon et al. 2001). Monoamine and amino acid neurotransmitter interaction plays a critical role in cognitive function. This study further examined whether these Pb-induced dopaminergic changes were accompanied with alterations in the temporal profile of aspartate (ASP), glutamate (GLU), serine (SER), glutamine (GLN), glycine (GLY), taurine (TAU), alanine (ALA) and γ-amino butyric acid (GABA) levels in the nucleus accumbens (NAC), hippocampus (HIP), and the frontal cortex (FC). 23-day old male Long-Evans rats were exposed to 0 and 50 ppm Pb for 180 days. At each 30-day interval of the 180-day exposure period, sets of animals from the control (n = 16-20) and Pb-exposed (n = 16-20) groups were sacrificed by cervical decapitation; blood Pb (BPb) and regional brain amino acids (i.e ASP, GLU, SER, GLN, GLY, TAU, ALA and GABA) were analyzed by atomic absorption spectroscopy and high performance liquid chromatography respectively. The exposure protocol produced BPb < 2 g/dl in the control group and BPb > 9 g/dl in the Pb-exposed group. Pb exposure changed the excitatory/inhibitory amino acid neurotransmitter balance in the mesocorticolimbic system producing transient hypoexcitatory/hyperinhibitory states in the NAC, the HIP and the FC. These findings suggest that low level Pb's effects on the mesocorticolimbic aminoacidergic system may play a role in Pb-induced cognitive impairments. Supported by ATSDR Cooperative Agreement # U50/ATU 398948.

1117 INORGANIC LEAD (PB) EXPOSURE ACTIVATES STRIATAL CFOS EXPRESSION AT LOWER BLOOD LEVELS AND INHIBITS AMPHETAMINE-INDUCED STRIATAL CFOS EXPRESSION AT HIGHER BLOOD LEVELS IN THE RAT.

M. W. Lewis and D. K. Pitts. *Pharmaceutical Sciences, Wayne State University, Detroit, MI.* Sponsor: G. Corcoran.

This study examined the impact of inorganic lead (Pb) on dopamine (DA) neurotransmission in the basal ganglia. In the striatum, D-amphetamine (AMP)-induced activation of the immediate early gene (IEG), cFOS, is mediated by the DA D1 receptor. The effect of Pb on AMP-induced cFOS activation was examined, and the number of neurons showing cFOS immunoreactivity in the striatum was compared among 3 treatment groups. Weanling rats were exposed to 0, 50, or 250 ppm Pb acetate (con, Pb50 or Pb250) *via* drinking water for 3 weeks. On the day of the experiment, all the subjects were challenged with AMP (4mg/kg, i.p.) or vehicle (normal saline, i.p.), and 2 hours later were sacrificed for either immunohistological analysis or determination of blood and brain Pb concentrations. The untreated con group showed negligible cFOS expression in the absence of AMP challenge. AMP challenge in the untreated con group significantly increased cFOS expression by approximately 35 fold over con. However, in the Pb50 group, the vehicle challenged rats also showed a statistically significant increase in cFOS expression of approximately 7 fold over con. This strongly suggests that the Pb50 exposure induced cFOS expression. AMP induced a statistically significant increase in cFOS in the Pb50 group, which was not significantly different from the AMP-induced increase in the untreated con group. However, in the Pb250 group, neither vehicle nor AMP challenge significantly increased cFOS above the con. These results indicate that 50 ppm Pb exposure induced a low, but statistically significantly level of cFOS gene activation, and was without effect on the AMP-induced cFOS activation. However, 250 ppm Pb exposure inhibited the amphetamine-induced activation of cFOS in the striatum by about 90%. Therefore, Pb is capable of both activating cFOS expression at low levels of exposure (mean blood Pb level: 21.6+/-1.9 μg/dl), and inhibiting AMP-induced cFOS expression at higher levels of exposure (mean blood Pb level: 47.4+/-2.6μg/dl).

1118 INHIBITORY EFFECT OF LEAD ON PKC ISOFORMS AND NF-KAPPA B *IN VIVO* AND *IN VITRO*.

S. xu, B. Rajanna and C. Shan. *Biological Sciences, Alcorn State University, Alcorn state, MS.*

In this study We examined the effect of lead on PKC isoforms associated with NF-kB, nNOS both *in vivo* and *in vitro*. *In vivo* 0.1% lead acetate in distilled water (DDW) was given to female rats from gestation through the 21st day old of the pups. Control rats were drinking DDW. Pups were sacrificed on postnatal days (PND) 1, 5, 7, 10, 15, 21, 30 and 45. Brain stem(BS), cerebellum (CB), hippocampus (HC) and front cortex(FC) of rat brain parts were collected. PKC isoforms-a, b, g, e and m, IKK-a, NF—kB and nNOS were examined by immunoprecipitation and western blotting. *In vitro* neurons were isolated from fetal rats at 14-16 days gestation, and planted in 6 wells plates for 10 days for use. Neurons were exposed to 10-10-10-7 M lead for 14 h. Cell extraction was immunoprecipitated and run western blotting. NO production was assayed by Griess reagent. Cell *via*bility was assayed by MTT. DNA damage was observed using acridine orange (AO) stain. Our results indicated *in vivo* that on PND 1, PKC-g both in cytosolic and membrane fraction was decreased in HC and FC by lead. IKK-a, NF—kB and nNOS across all the regions, and particularly in HC and FC, were substantially suppressed; On PND 10, all of the above PKC isoforms were inhibited in BS, CB, HC and FC membrane fraction as well as IKK-a, NF—kB and nNOS; On PND 45 the above PKC isoforms as well as IKK-a, NF—kB and nNOS but not PKC-e in lead treated group were restored to normal. *In vitro* NO production was dose dependently decreased. Only 26.4% neurons survived after lead treatment as confirmed by MTT. Fluorescent intensity was enhanced in lead treated neurons. PKC isoforms-a, b, g, l except d, IKK-a, NF—kB and nNOS were dose-dependently inhibited. It was concluded that translocation of most PKC isoforms was suppressed in HC and FC by lead *in vivo*, particularly g and e, whose roles were pertinent to memory formation and long term potential. Concurrently, PKC isoforms expressions were dose-dependently decreased *in vitro*. The inhibition of nNOS was due to NF-kB-IKK a suppression.Lead directly caused DNA damage and mitochondria dysfunction *in vitro*.

1119 DIFFERENT MECHANISMS MEDIATE UPTAKE OF LEAD IN A RAT GLIAL CELL LINE.

J. P. Bressler[2, 3], D. Bannon[1, 2], L. Olivi[3], J. Cheong[4, 2, 3] and K. Kim[2, 5, 3]. *[1]Center for Health Promotion and Preventive Medicine, US Army, APG, Aberdeen, MD, [2]Environmental Health Sciences, Johns Hopkins University, Baltimore, MD, [3]Neurotoxicology, Kennedy Krieger Institute, Baltimore, MD, [4]School of Pharmacy, Sahmyook University, Seoul, South Korea and [5]Department of Preventive Medicine, Soonchunhyan University, Seoul, South Korea.*

The mechanism by which lead (Pb) enters glial cells was examined in order to characterize specific pathways for transport. Pb uptake was saturable at pH 5.5 and 7.4, although quantitative differences existed in the Michaelis-Menten constants. At pH 7.4, the Vmax and Km was 2700 fmoles/mg protein/min and 13.4 μM Pb, respectively, whereas the Vmax and Km was 329 fmoles/mg and 8.2 μM Pb in the buffer at pH 5.5, respectively. Extracellular iron inhibited uptake in a buffer at pH 5.5 but not at pH 7.4. Cells treated with the iron chelator deferoxamine displayed higher levels of the iron transporter divalent metal transporter 1 (DMT1) mRNA and protein, and consistent with increased DMT1 expression, displayed greater uptake of Pb in the buffer at pH 5.5 but not at pH 7.4. Alternatively at pH 7.4, the transport of Pb was blocked by the anion transporter inhibitor 4, 4-diisothiocyanatodihydrostilbene-2, 2-disulfonic acid (DIDS), which bound to cell surface proteins at concentrations that were similar to those that blocked Pb uptake. DIDS did not inhibit uptake in the buffer at pH 5.5. Greater uptake of Pb was observed in a buffer containing sodium bicarbonate, which was abrogated in the presence of DIDS. In summary, glial cells display two distinct pH sensitive transport mechanisms for Pb.

1120 BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)POLYMORPHISM ASSOCIATIONS WITH BEHAVIORAL MEASURES OF MEMORY IN MERCURY (Hg)-EXPOSED HUMANS.

D. Echeverria[2, 1], J. S. Woods[1, 2], N. J. Heyer[2], A. C. Bittner[2] and F. M. Farin[1]. *[1]Environmental Health, University of Washington, Seattle, WA and [2]Battelle Centers for Public Health Research and Evaluation, Seattle, WA.*

Associations were hypothesized between BDNF polymorphisms and memory deficit, an adverse mercury-related health effect. A population of exposed subjects (n=480) provide buccal cells and were administered the Behavioral Evaluation for Epidemiologic Studies (BEES) Test Battery. We evaluated DNA samples for the presence of a previously identified SNP at nucleotide 196 (G/A), which produces a valine to methionine substitution at codon 66 (V66M). Genotyping results identified 68% WT, 28% Het, 4% Mut within this population. Discriminant and multiple regression analyses examed predictive associations between polymorphism status and seven summary behavioral factor scores; 1) Cognitive Reasoning (Trails A/B, Symbol Digit Rate, and Pattern Discrimination Rate), 2) Switching Attention (Letter, Picture, and Direction Comparison), 3) Finger Speed (Finger Tap: L, R, Alt.), 4) Hand Speed (Move from Simple and Choice RT), 5) Tracking (Tracking and Lift from SRT), 6) Memory (Digit and Spatial Span), and 7) Vigilance. Discriminant weightings of 0.118, -.003, and -2.329 for wild type, het, and mutant status are consistent with the magnitude of relative degradation reported with losses in episodic memory. The standardized correlation between performance on memory and BDNF (β=.91***) was pronounced in the presence of the log of mercury (β=.10**), vocabulary, that served as a surrogate for premorbid intelligence(β=.12**), and age (β=.07**) (p<.05=**). Separate regression results tempered the association between memory and the BDNF polymorphism (β=.39***). The second Switching factor was affected to a lesser extent (p<.04 directionally). Concurrent analyses also indicated associations between self-reported symptoms for poor attention and memory in the presence of the BDNF polymorphism. Our observations support the hypothesis that mercury-related memory deficits are exacerbated among subjects with the BDNF polymorphism. Supported by ES04696 and ES07033.

1121 EVIDENCE FOR DIRECT MODULATION OF GLUTAMATE (AMPA) RECEPTOR CHANNEL PROPERTIES BY METHYLMERCURY.

T. Vaithianathan, V. Suppiramaniam and P. Dey. *Pharmacal Sciences, Auburn University, Auburn, AL.*

Persistent psychomotor disturbances and cognitive deficits are hallmarks of many heavy metal neurotoxicants, however the molecular mechanisms underlying these effects are poorly understood. Accumulating evidence from animal and cell culture model systems implicates derangements in AMPA subtype ionotropic glutamanergic receptor activity in learning and memory disorders. Studies investigating the ability of the organometal neurotoxicant methylmercury to directly alter ion channels have been complicated by the complex interactions of the compound with multiple cell surface receptors observed using traditional patch-clamp technique. We have utilized purified AMPA receptors reconstituted in tip-dip lipid bilayers to demonstrate the direct modulatory effects of methylmercury on AMPA receptor channels. In a related set of studies, hippocampal synaptoneurosomes were isolated and incorporated into bilayers in order to model the organometals action on synaptic AMPA receptors. Methylmercury concentrations ranging from 75 ηM to 75 μM were infused into receptor-reconstituted lipid bilayers. In purified AMPA receptors preparations, methylmercury decreased the AMPA receptor properties in a dose-dependent fashion. At the highest methylmercury concentration, a 50-60% reduction in the open probability of AMPA activated channels was observed. These studies indicate that methylmercury may directly modulate AMPA receptor channel kinetics, contributing to deficits in learning and memory observed following exposure to the neurotoxicant. Supported by a Auburn University Biogrant [V.S.] and Graduate School Funding [P.M.D].

1122 EFFECTS OF METHYLMERCURY ON $GABA_A$ RECEPTOR-MEDIATED CURRENTS (I_{GABA}) IN RAT CORTICAL CELLS IN CULTURE.

C. Herden[2], Y. Yuan[1, 2] and B. Atchison[1, 2]. [1]*Department Pharmacology/Toxicology, Mich State University, East Lansing, MI and* [2]*Neuroscience Program, Mich. State University, East Lansing, MI.*

The cerebellum is particularly sensitive to acute and chronic exposure to methylmercury (MeHg). Inhibitory GABAergic synaptic transmission appears to be especially sensitive to the effects of MeHg, as it is blocked more quickly than is excitatory synaptic transmission in isolated brain slices. Pharmacological and electrophysiological properties of $GABA_A$ receptors vary depending on the phenotype or subunit composition of the $GABA_A$ receptor. Previous studies in our lab using the cerebellar slice preparation have demonstrated that GABAergic synaptic transmission in cerebellar granule cells, which express a unique α_6 subunit-containing $GABA_A$ receptor, is more sensitive to the block by MeHg than is inhibitory synaptic transmission in neighboring Purkinje cells, which express the α_1 subunit-containing $GABA_A$ receptor. Thus, differential $GABA_A$ receptor expression may underlie the differential effects of MeHg observed in these cerebellar neurons. As an initial step in testing this hypothesis, we investigated the effects of MeHg on primary cultures of rat cortical cells, which contain the same α_1-containing $GABA_A$ receptor phenotype as do Purkinje cells. I_{GABA} were obtained by means of the whole-cell voltage-clamp technique using a symmetrical chloride concentration. In cortical cells cultured for 10 - 15 days, MeHg (0.1 - 10 μM) blocked I_{GABA} in a time- and concentration-dependent manner. Time to block of I_{GABA} by MeHg was not dependent upon GABA (10 μM - 1 mM) concentration in these cells, suggesting that the block by MeHg is non-competitive. Moreover, stepping the voltage from -80 mV to +60 mV in the presence of MeHg produced a linear I-V relationship, suggesting that block of I_{GABA} by MeHg in cortical cells is voltage-independent. Thus in cells expressing the α_1 subunit of the $GABA_A$ receptor, MeHg causes a rapid and complete block of current. Supported by NIH grant ES11662.

1123 ALTERATIONS BY METHYLMERCURY (MEHG) OF PRESYNATIC TERMINAL CA^{2+} CONCENTRATION APPEAR TO BE RESPONSIBLE FOR MEHG-INDUCED INITIAL STIMULATION OF SPONTANEOUS INHIBITORY POSTSYNAPTIC CURRENTS.

Y. Yuan and W. D. Atchison. *Pharmacology/Toxicology, Michigan State University, East Lansing, MI.*

Bath application of methylmercury (MeHg) caused an initial stimulation prior to suppression of the frequency and amplitude of $GABA_A$ receptor-mediated spontaneous inhibitory postsynaptic currents (sIPSCs) in Purkinje cells of rat cerebellar slices, suggesting that both pre- and postsynaptic mechanisms are involved. The goal of the present study was to determine if the initial increases by MeHg in frequency and amplitude of sIPSCs in Purkinje cells are related the changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in the presynaptic terminal and postsynaptic sites. sIPSCs and temporal and spatial changes in $[Ca^{2+}]_i$ in presynaptic fibers (parallel- and climbing-fibers), dendrites and somata of Purkinje cells in cerebellar slices were examined by combination of fast confocal laser-scanning microscopy and whole-cell patch-clamp recording techniques in the absence and presence of MeHg. In slices preloaded with the Ca^{2+}-selective indicator Fluo-4, bath application of 10 - 100 μM MeHg induced prominent increases in fluorescence signals in the entire molecular layer (including parallel- and climbing-fibers, dendrites and the subplasmalemmal shell of Purkinje cells) and granule cells of cerebellar slices. However, no significant changes in fluorescent signals were observed in the soma region of Purkinje cells. Coincidental recordings of sIPSCs revealed a MeHg-induced increase in sIPSC frequency. The time to onset of change in fluo-4 fluorescent signals appeared to be correlated to the time to onset of increases in frequency of sIPSCs recorded from the soma of Purkinje cells. Thus, these results suggest that MeHg-induced increases in the presynaptic $[Ca^{2+}]_i$ appear to be responsible for the initial increases in frequency of sIPSCs caused by MeHg in cerebellar Purkinje cells. Supported by NIH grants R01ES03299 and R01ES11662

1124 CALBINDIN D-28K TRANSFECTED HUMAN EMBRYONIC KIDNEY (HEK 293) CELL LINE IS RESTANT TO METHYLMERCURY-INDUCED ALTERATIONS OF CALCIUM HOMEOSTASIS.

J. R. Edwards and W. D. Atchison. *Michigan State University, East Lansing, MI.*

Methylmercury (MeHg) acts in a time- and concentration-dependent manner to cause disruption of Ca^{2+} homeostasis in a variety of neuronal cell types. Cerebellar granule cells are one of the more sensitive cell types to the cytotoxic effects of MeHg as well as disruption of Ca^{2+} homeostasis. In comparison, Purkinje neurons are relatively less sensitive to disruption of Ca^{2+} homeostasis and subsequent cytotoxicity caused by MeHg. One potential determinant of this selective sensitivity to MeHg cytotoxicity, and ability to disrupt Ca^{2+} homeostasis, is the expression of the Ca^{2+} binding protein, calbindin D-28k (CB). Purkinje cells abundantly express CB while granule cells do not. CB is thought to prevent cell death and buffer against elevations of $[Ca^{2+}]_i$. In this study, CB-transfected, vector-transfected and non-transfected HEK cells were loaded with the calcium indicator, fura-2, then exposed to MeHg (1, 2.5 and 5 μM) and resulting changes in fura-2 fluorescence were recorded. Qualitatively, vector-transfected and non-transfected HEK cells displayed an abrupt spike in fura-2 fluorescence followed by a return to baseline in the 1 and 2.5 μM MeHg treatment groups. CB-transfected cells did not exhibit these abrupt changes in fura-2 fluorescence at 1 or 2.5 μM MeHg. However, at 5 μM MeHg cells in all treatment groups responded in a similar manner with a sustained increase in fura-2 fluorescence. Thus there appears to be a differential response of HEK 293 cells to low (1 and 2.5 μM) and high (5 μM) concentrations of MeHg. In addition, CB may be involved in attenuating MeHg-induced disruption of Ca^{2+} homeostasis at 1 and 2.5 μM, but not at 5 μM MeHg in HEK 293 cells. Supported by NIH grant ES03299.

1125 LACK OF EXPRESSION OF CALBINDIN-D 28K CORRELATES WITH INCREASED SENSITIVITY TO METHYLMERCURY CYTOTOXICITY IN GUINEA PIG MYENTERIC PLEXUS NEURONS.

A. Rodriguez, J. R. Edwards and B. Atchison. *Department Pharmacology/Toxicology, Mich State University, East Lansing, MI.*

In isolated cerebellar granule cells in culture methylmercury (MeHg) causes an elevation of $[Ca^{2+}]_i$ which is associated with a delayed-onset cell death. Granule cells do not express the Ca^{2+}binding protein, calbindin-D 28k (CB); adjacent Purkinje neurons do express CB and are more resistant to MeHg-induced cytotoxicity and increased $[Ca^{2+}]_i$. CB also protects neurons from other forms of Ca^{2+}-mediated cell death. Thus CB may be an important cellular constituent in protecting neurons against MeHg-induced cytotoxicity. As granule and Purkinje cells have other differences including cell size, we investigated the cytoprotective role of CB against MeHg using an alternate model. Guinea pig myenteric plexus neurons are either CB-immunopositive or CB-immunonegative; myenteric plexus neurons immunonegative for CB are selectively immunopositive for nitric oxide synthase (NOS). We hypothesize that myenteric plexus neurons immunopositive for NOS (CB-negative) will be more sensitive to the cytotoxic effects of MeHg as compared to CB-positive myenteric plexus neurons. To examine this, myenteric plexus neurons were grown in culture, and exposed to 0.5, 1, 2 or 5 μM MeHg for 2 hr; *via*bility was assayed 24 hr later. Cells were then immunostained for the presence of NOS or CB. Consistent with our previous studies, the proportion of *via*ble CB-positive myenteric plexus neurons increased with increasing concentrations of MeHg. Little or no cell death occurred in control, or for NOS-positive cells at 0.5 or 1 μM MeHg. However, a significant proportion of NOS-positive cells died in the 2 μM MeHg treatment group, and no *via*ble NOS-positive cells remained in

the 5 μM MeHg treatment group. Compared to CB-positive myenteric plexus neurons, NOS-positive neurons (CB-negative)were more sensitive to the cytotoxic effects of MeHg. This study further supports the potential role of CB in attenuating MeHg cytotoxicity. Supported by NIH grant ES03299.

1126 TRANSFECTION OF PC12 CELLS WITH CALBINDIN-D 28K INCREASES RESISTANCE TO METHYLMERCURY-INDUCED DISRUPTION OF INTRACELLULAR CALCIUM HOMEOSTASIS.

J. R. Gomulka[2, 1], J. R. Edwards[1] and B. Atchison[1]. [1]*Department Pharmacology/Toxicology, Mich State University, East Lansing, MI and* [2]*College of Veterinary Medicine, Mich. State University, East Lansing, MI.*

Methylmercury (MeHg) causes a preferential loss of cerebellar granule cells— cells which do not express the Ca^{2+} binding protein, calbindin-D 28k (CB). This contrasts to adjacent Purkinje neurons which abundantly express CB and are much more resistant to MeHg cytotoxicity. An early, prominent effect of MeHg especially notable in granule cells is elevation of $[Ca^{2+}]_i$; this leads to a delayed onset cell death. CB is thought to prevent cell death and buffer against elevations of $[Ca^{2+}]_i$. CB-transfected pheochromocytoma (PC12) cells have greater resistance to cytotoxicity of glutamate exposure and serum withdrawal than do nontransfected cells. Also, CB-transfected cells exhibited less Ca^{2+} entry through voltage-gated Ca^{2+} channels than do wild-type cells. Thus, the purpose of this study was to determine if expression of CB imparts resistance to the dysregulation of Ca^{2+} homeostasis caused by MeHg exposure. We also sought to determine if CB increases MeHg resistance *via* actions on Ca^{2+} channels. MeHg was applied continuously by perfusion to PC12 and PC18 cells, a sub-clone of the PC12 cells lacking voltage-gated Ca^{2+} channels. Cells were loaded with fura-2 and resulting changes in fura-2 fluorescence were recorded using microfluorometric imaging techniques. For both cell types, there was a typical time- and concentration-dependent biphasic increase in fura-2 fluorescence following MeHg exposure. CB-transfected PC12 cells had a delayed time-to-onset of MeHg-mediated increase in fura-2 fluorescence at 0.5, 1, 2 and 5 μM MeHg compared to non-transfected cells. In contrast, CB-transfected PC18 cells did not have a significantly delayed time-to-onset of fura-2 fluorescence compared to non-transfected PC18 cells at 1 μM MeHg. These data indicate a potential role of CB to protect against MeHg-mediated disruption of Ca^{2+} homeostasis. Furthermore, CB may interact directly with Ca^{2+} channels to delay the actions of MeHg. Supported by NIH grant ES03299 and 5T35RR017491.

1127 NEUROBEHAVIORAL AND MITOCHONDRIAL MEMBRANE POTENTIAL CHANGES IN CEREBELLAR GRANULE CELLS OF MICE EXPOSED TO METHYLMERCURY.

S. Bellum, K. A. Thuett and L. C. Abbott. *CVM, VAPH, Texas A&M University, College Station, TX.* Sponsor: E. Tiffany-Castiglioni.

Methylmercury (MeHg) is a potent environmental neurotoxicant, which is known to cause degeneration of neurons including cerebellar granule cells. Recent studies using cultured cerebellar granule cells exposed to MeHg indicate calcium ion homeostasis is altered leading to increased intracellular calcium concentrations and eventual cell death. However, key gaps still exist in our knowledge concerning the specific mechanisms that bring about granule cell death following acute *in vivo* MeHg exposure. Damage to mitochondria in general results in decreased ATP production and release of cytotoxic/cell death signals. We examined mitochondrial function by comparing mitochondrial membrane potential (MMP) of cerebellar granule cells taken from control, 1.0 mg/kg and 5.0 mg/kg MeHg-treated male and female C57BL/6J mice. In addition, motor coordination using rotarod and spontaneous locomotion and rearing activity in open field were completed. Cerebellar granule cells were acutely isolated using dissociation medium containing protease and plated onto poly-D-lysine coated coverslips. The cells were then loaded with TMRM, which is a lipophilic cation that accumulates in mitochondria in proportion to the membrane potential. Upon sufficient accumulation, the dye exhibits a red shift in absorption and fluorescence emission spectra. Fluorescent images of granule cells from all three treatment groups were acquired and analyzed. The MMP observed in the granule cells from 1.0 mg and 5.0 mg MeHg-treated mice were significantly lower than from controls. While the performance on the rotarod was not statistically significant among control, 1.0 mg/kg and 5.0 mg/kg MeHg-treated mice, the MeHg-treated mice showed a tendency to stay on the rotarod for a shorter time than the control mice. Performance on the open field is being analyzed. These observations suggest that *in vivo* MeHg exposure diminishes mitochondrial function in cerebellar granule cells, which ultimately could lead to death and/or dysfunction of these neurons.

1128 EFFECTS OF METHYLMERCURY ON EPH AND EPHRIN PROTEINS IN EMBRYONAL CARCINOMA CELL DERIVED NEURONS.

D. T. Wilson[1], M. A. Polunas[1], R. Zhou[1, 2], H. E. Lowndes[1, 3] and K. R. Reuhl[1, 3]. [1]*JGPT, Rutgers University, Piscataway, NJ,* [2]*Chem. Biol., Rutgers University, Piscataway, NJ and* [3]*Pharmacology and Toxicol., Rutgers University, Piscataway, NJ.*

Repulsive interactions between neuronal cells and their surroundings mediated by EPH tyrosine kinase receptors and their ephrin ligands guide and shape several morphogenetic events, including topographical mapping of CNS pathways. Methylmercury (MeHg) induces morphological changes during brain development suggestive of neuronal guidance errors. The effects of MeHg on EPH and ephrin mRNA expression in embryonal carcinoma (EC) cells and EC-derived neurons were previously investigated. The present report describes protein levels of these molecules in the EC cell culture system following MeHg exposure. Five days after induction by retinoic acid, EC-derived neurons were exposed to 0.0, 0.5, 1.5, or 3.0 uM MeHg for 24 hours and analyzed for Eph and ephrin levels using immunoblotting and immunofluorescence. Consistent with increased mRNA levels shown previously, the protein levels of EphA4 and ephrins-A3, A5, B1, and B2 were up-regulated by 0.5 and 1.5 uM MeHg. Despite elevated levels of Eph and ephrin mRNA at 3.0 uM, protein levels were comparable to control or slightly decreased. This apparently bi-phasic pattern was most evident for EphA4 and ephrins-A5, B1, and B2. These results indicate that MeHg affects the protein synthetic machinery directly, independent of changes in transcription. MeHg also affects Ephs and ephrins selectively as GAPDH and actin protein levels were unaffected by MeHg. The results support our hypothesis that Ephs and ephrins are involved in the disrupted morphogenesis seen in MeHg-exposed rodents. (Supported by NIH/EPA ES11256 and NIH ES05022).

1129 PROTECTIVE EFFECT OF GLUTATHIONE PEROXIDASE OVEREXPRESSION IN METHYLMERCURY NEUROTOXICITY.

M. Polunas[1, 2], O. Prokopenko[3], O. Mirochnitchenko[3, 1], M. Philbert[4] and K. Reuhl[1, 2]. [1]*JGPT, Rutgers/UMDNJ, Piscataway, NJ,* [2]*Department Pharmacology/Toxicology, Rutgers University, Piscataway, NJ,* [3]*Department Biochem., UMDNJ, Piscataway, NJ and* [4]*Department Environment Health Sciences, University Mich., Ann Arbor, MI.*

The precise mechanisms underlying methylmercury neurotoxicity remain obscure. Several lines of evidence implicate reactive oxygen species (ROS) as playing a key role in the mitochondrial dysfunction seen in MeHg poisoning. Previous *in vivo* and *in vitro* studies have shown that exogenous antioxidants protect against both ROS production and loss of mitochondrial membrane potential. The intracellular antioxidant enzyme glutathione peroxidase (GPx1) is localized both in the cytosol and mitochondrial matrix and efficiently utilizes lipid peroxides as well as H_2O_2 for substrates. Primary neocortical neuron cultures derived from transgenic mice (C57BL/6XCBA/J) overexpressing human GPx1 (GPx1 activity 0.29 and 0.12 University/mg protein for neurons derived from GPx1 and wild-type (wt) mice, respectively) were used to examine the potential of endogenous antioxidant systems to ameliorate MeHg-induced mitochondrial toxicity. MitoTracker Green (MTG), which passively diffuses across the plasma membrane and preferentially accumulates in active mitochondria due to their negative membrane potential, was used as an index of mitochondrial membrane integrity/loss of membrane potential. Day 10 wt or transgenic GPx1 neurons were pre-incubated with MTG for 30 min and exposed to 0, 1.5, or 2.5 μM MeHg for 60 min. Both concentrations of MeHg produced substantial dimming and diffusion of the bright, punctate MTG staining seen in live wt and GPx1 vehicle-treated controls, this loss reflecting relaxation of the mitochondrial membrane gradient. This correlated with a dose-dependent loss of β-III positive neurites and perikaryal microtubules. In contrast, GPx1 overproducing neurons were largely protected against MeHg-induced loss of MTG and exhibited fewer toxicity-induced cytoskeletal changes, suggesting MeHg-induced ROS production to be a mechanism contributing to the mitochondrial and morphological changes observed in MeHg neurotoxicity. (ES07148, ES11256, ES08846)

1130 INCREASE IN INTRACELLULAR REACTIVE OXYGEN SPECIES FORMATION INDUCED BY METHYLMERCURY IN CULTURED ASTROCYTES: A CONFOCAL MICROSCOPIC STUDY.

G. Shanker, L. A. Mutkus, Q. Wu and M. Aschner. *Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC.*

Oxidative stress has been implicated in a variety of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. There is a strong association between metal-induced oxidative stress and the accumulation of high levels of reactive species, in-

cluding reactive oxygen species (ROS), as well as unbound metal ions. ROS are highly oxidizing and damaging to cellular redox-sensitive proteins, enzymes, and DNA. The present study was carried out to test the hypothesis that ROS formation is an early event in methylmercury (MeHg)-induced neurotoxicity. MeHg-dependent increase in ROS formation was assessed with confocal microscopy in neonatal rat cortical astrocyte cultures with 2 redox sensitive fluorescent probes, 5, 6-chloromethyl-2', 7'-dichloro-dihydrofluorescein diacetate (CM-H2DCFDA; reacts with intracellular ROS), and reduced chloromethyl-X-rosamine (CM-H2XRos; a mitochondria specific probe). Astrocytes were treated with 10 uM MeHg for 30 minutes, followed by addition of the probe/s and image collection at 25 minutes. The results showed significant MeHg-dependent increases in astrocytic fluorescence intensity ($p<0.05$) of both probes indicative of increased intracellular oxidized state. Additional time-series experiments in the presence of both dyes revealed that the initial fluorescence changes in response to MeHg treatment were associated with the mitotracker dye (CM-H2XRos), suggesting that mitochondria most likely represent early primary sites of ROS formation. The studies establish the early generation of ROS in MeHg treated cells and support a key role for astrocytes as a source for ROS and the ensuing demise of neurons. This work was supported by PHS grant NIEHS 07331.

1131 GENE EXPRESSION ANALYSIS OF THE MICE CEREBELLAR CELLS *IN VITRO* EXPOSED TO METHYL MERCURIC CHLORIDE.

E. S. Calderon-Aranda[1, 3], A. E. Jedlicka[2], A. S. Scott[2] and E. K. Silbergeld[1]. *[1]EHS, Johns Hopkins University, Baltimore, MD, [2]MMI, Johns Hopkins University, Baltimore, MD and [3]Seccion de Toxicologia, Cinvestav, Mexico, DF, Mexico.*

The immature CNS is an extremely sensitive target to methyl mercury (MeHg). In exposed children, effects ranged from subtle developmental delay to severe cerebral palsy. In animal models and cell cultures, low doses of MeHg induced alteration in neuronal migration and proliferation, particularly in cerebellar cortex, and microglia activation. However, the molecular mechanism through which MeHg might affect neuronal migration in the cerebellum remains to be defined. This study examined gene expression in cerebellar organotypic cultures exposed to MeHg to identify molecular targets associated with the mechanism of neuronal alteration induced by Hg. Organotypic cells were obtained from the cerebellum of five day old CD1 pups, and after five days in MEM media, unstimulated or LPS-stimulated cells were exposed to 100nM MeHgCl, for 48h. Unexposed cultures were used as control, both unstimulated or stimulated with LPS. Total RNA was extracted from cultures and after cRNA synthesis, GeneChips (Affymetrix MOE430A) were hybridized and the arrays were analysed by Microarray Suite 5.1 and GeneSpring software. The preliminary results showed that, LPS modified the expression of many different genes. Increasing expression ranged from 1- to 500-fold, compared with expression in control cells. MeHgCl exposure of LPS-stimulated cells resulted in alteration of genes induced with LPS, decreasing approximately 15% and inducing an additional increment around 25% in other. Hg exposure increased expression in around 20% of genes negatively regulated by LPS-stimulation whereas 15% of those genes had an additional decrement. In unstimulated cells MeHgCl induced alteration in the gene expression profile compared with the control, although the changes were more discrete than Hg in LPS-stimulated cells. The information obtained from this work will help to define the mechanisms involved in Hg toxicity in the cerebellum. This work was supported by Cure Autism Now and Heinz Family Foundations.

1132 MICE OLFACTORY BULB NEURONAL DEATH AFTER V2O5 INHALATION.

L. COLIN-BARENQUE[2], M. AVILA-COSTA[2], V. DELGADO[1], I. SANCHEZ[1], I. LOPEZ[1], F. PASOS[1] and T. I. FORTOUL[1]. *[1]BIOLOGIA CELULAR Y TISULAR, UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, MEXICO CITY, Mexico and [2]NEUROCIENCIAS, FES IZTACALA, MEXICO, Mexico.*

Vanadium (V) inhalation exerts harmful effects, and its toxicity had been related with an increase of reactive oxygen species. Since there is no information about ultrastructural changes in granule cells of the olfactory bulb we decided to find out if the inhalation of this element induces changes in this structure. CD-1 male mice were exposed to vanadium pentoxide (V2O5) 0.02 M one hour twice a week for 12 weeks, and sacrificed each week. Olfactory bulbs were obtained and processed for transmission electron microscopy. Necrosis and apoptosis were observed, as well as, electrondense mitochondrion, dilated Golgi apparatus and cytoplasm vacuolation. Also increased lipofucsin granules at all exposure times were present. Oxidative stress could be the cause of the alterations observed but further analysis is needed to dilucidate the mechanisms by which V2O5 is inducing either apoptosis or necrosis. PAPIIT IN-210301

1133 DIFFERENTIAL RESPONSES TO CADMIUM IN AN *IN VIVO* AND *IN VITRO* MODEL OF NEUROTOXICITY.

C. Bolin, D. Cox and F. Cardozo-Pelaez. *Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT.* Sponsor: A. Holian.

Cadmium is a ubiquitous environmental toxin found in industrial pollutants, drinking water, food contaminants, and is one of the major components in cigarette smoke. The accumulation of cadmium and its associated toxicity has been demonstrated in many organs such as kidney, liver, lung, testis, and brain. The specific mechanisms by which cadmium toxicity operates are unclear, however several reports have associated the effects of cadmium with an increase in reactive oxygen species (ROS); this is supported by evidence of elevated levels in markers of oxidative stress to cell biomolecules *in vivo* and *in vitro*. Cadmium cannot directly form ROS and it is therefore hypothesized that indirect action through the alteration in activity of important antioxidants and DNA repair mechanisms may be leading to the apparent accumulation of oxidative damage. The aim of this study was to determine the role of cadmium exposure in the activity of the base excision repair enzyme 8-oxoguanine-DNA glycosylase (OGG1) responsible for the removal of the oxidized DNA base product 8-hydroxy-2-deoxyguanosine (oxo^8dG) *in vivo* and *in vitro*. We found that adult male SVJ/129 mice exposed to $CdCl_2$ in their drinking water for a period of twenty days do not exhibit a reduction in the activity of OGG1 in discrete brain regions, although there was an increase in the levels of reduced glutathione and alterations in the activity of the superoxide dismutase enzymes. In contrast, the PC12 catecholaminergic cell line exhibited a significant reduction in OGG1 activity after incubation with $CdCl_2$. These conflicting findings suggest a differential response to cadmium *in vivo* and *in vitro* that needs to be addressed to elucidate its toxic effects.

1134 ARSENIC LEVELS AND GLUTATHIONE REDUCTASE ACTIVITY IN CD1 MICE BRAIN.

M. E. Gonsebatt[1], J. H. Limon[1], V. Rodriguez[2], M. M. Giordano[2] and L. M. Del Razo[3]. *[1]Medical Genomics and Environmental Toxicology, Instituto de Investigaciones Biomedicas, Mexico, DF, Mexico, [2]Instituto de Neurobiologia, Queretaro, Queretaro, Mexico and [3]Toxicology Section, CINVESTAV, IPN, Mexico, DF, Mexico.*

Arsenic crosses the blood-brain barrier and accumulates in the brain where it has been shown to exert neurotoxic effects. In animals models as rats and mice effects on the basal ganglia and increased content of striatal dopamine have been reported respectively. Nevertheless, there is scarce information regarding the metabolism of arsenic in this tissue, especially with respect to the presence of methylated metabolites and the effects of the arsenicals on the activity of detoxifiying brain enzymes. To investigate the presence of methylated arsenic metabolites in brain tissue and to determine the activity and expression of glutahione reductase (GR), CD1 mice were treated orally with 0, 2.5, 5 and 10 mg/Kg of sodium arsenite daily during 10 days. Animals were killed by decapitation; the brain was removed and frozen in liquid nitrogen. The presence of inorganic arsenic (iAs), the monomethylated (MMA) and dimethylated As metabolites (DMA) was determined in liver and brain tissue by hydride generation-atomic absorption spectroscopy The presence of GR was ascertained by immunoblotting with a polyclonal antibody and the activity of the enzyme was determined by the NADPH+H assay. Treated animals showed significantly-increased amounts of the inorganic and methylated arsenic forms. Although the total amount of arsenic detected in brain mice treated with 5 or 10 mg/kg was similar (402.5 ± 17.7 and 396.5 ± 33.9 ng/g respectively) the proportion of DMA doubled the amount of MMA in the mice treated with 10 mg/kg. Interestingly, a significant inhibition of the expression and activity of GR was observed only at the highest concentration used.

1135 THE TRIMETHYLTIN MODEL OF HIPPOCAMPAL INJURY: GENE ARRAY ANALYSIS REVEALS EARLY AND DIVERSE CHANGES IN GENE EXPRESSION ASSOCIATED WITH NEURONAL INJURY AND GLIAL ACTIVATION.

A. R. Little and J. P. O'Callaghan. *TMBB, CDC-NIOSH, Morgantown, WV.*

Damage to the CNS results in a complex series of molecular and cellular events involving the injured cells and their supportive environment. Understanding the earliest molecular changes associated with CNS injury offers the potential for developing neuroprotection strategies. The use of injury models with known cellular targets and a known time-course of damage facilitates this process. One such model is the hippocampal neurotoxicant, trimethyltin (TMT) which cause extensive loss of hippocampal neurons and an accompanying microglial and astroglial activation. Previously, we have shown that TMT causes a marked and early increase in the chemokine, Ccl2 (JE/MCP-1), coincident with microglial activation. Here were used the Affymetrix gene chip to obtain a more comprehensive portrait of gene expression changes during the earliest phases of TMT-induced damage. Changes seen on the chip were confirmed by Real Time PCR. After a single systemic dose of TMT (8.0 mg/kg) we confirmed an increase in expression of Ccl2 at two days post

dosing which was not accompanied by an increase in its two receptors, CCR2 and CCR5. Increases in mRNA expression for STAT5a, GSK3alpha and several proteasome-related enzymes were observed at 2 and 5 days post TMT. Immuno-blot analysis for phospho-GSK3 alpha and beta showed dramatic increases in p-GSK 3 (alpha & beta) by day 5. These data suggest an involvement of the protein degradation pathway during early stages of neuronal degeneration. Cellular localization of STAT5a induction remains to be clarified but induction of GSK3 implicates activation of the Wnt pathway, for which the upstream effectors have been associated with gliosis. These results link protein degradation and cellular differentiation events to the earliest stages of neuronal injury.

1136 HIPPOCAMPAL DENDRITIC SPINES LOSS AND MEMORY DETERIORATION IN MICE EXPOSED TO VANADIUM (V2O5) INHALATION.

G. Nino-Cabrera[2], M. Avila-Costa[1], L. Colin-Barenque[1], P. Bizarro-Nevares[2], S. Acevedo-Nava[2], I. Sanchez-Cervantes[2], I. Lopez[2], A. Gonzalez-Villalva[2], F. Pasos[2] and T. I. Fortoul[2]. *[1]Neuroscience, UNAM campus Iztacala, Tlalnepantla, Edo. Mex., Mexico and [2]Facultad de Medicina, UNAM, MEXICO, D.F., Mexico.*

Vanadium exposure exerts damage in different organs, but there is no information about damage in nervous system. It would be possible that if V produces alterations in nervous tissue, there will be alterations on its function. The mechanisms by which memories are acquired and stored in the mammalian brain are assumed to involve modifications in synaptic plasticity that implicates dendritic spines. We hypothesized that if vanadium produce alterations in neuronal structures of the hippocampus the memory could be alterated. CD-1 male mice were trained in spatial memory task and exposed by inhalation to 0.02 M of V2O5, one hour twice a week for 4 weeks, after each inhalations animals were evaluated for the memory task, then were sacrificed after the 2nd, 4th, 6th and 8th inhalation. The rapid Golgi stained sections of hippocampus were examined by counting the number of dendritic spines in 20 ?m of 5 secondary and 5 tertiary dendrites of 20 neurons. Our results showed that there was a significant decrease of dendritic spines after the different exposition times, these data correlate with memory alterations in a dose dependent way. Vanadium toxicity affects neuronal structure in the hippocampus and the formation of new memories. Supported by:PAPIIT IN210301.

1137 ERYTHROPOIETIN PREVENTS TRIMETHYLTIN-INDUCED NEURONAL DEATH AND GLIAL ACTIVATION.

B. Viviani, S. Bartesaghi, E. Corsini, L. Lucchi, C. L. Galli and M. Marinovich. *Department of Pharmacological Sciences, University of Milan, Milan, Italy.*

Erythropoietin (EPO) is a glycoprotein originally identified as the principal regulator of erythroids progenitor cells. Nevertheless, EPO expression is upregulated in both glia and neurons following ischemic brain injury to promote neuronal survival. Trimethyltin (TMT), a neurotoxicant characterized by neuronal degeneration and reactive gliosis, was used in this study to identify the molecular mechanisms involved in EPO-induced neuroprotection. Increasing concentrations of EPO (1-100 University/ml) dose-dependently decreased TMT-induced death in primary hippocampal neurons. Treatment of hippocampal neurons with 10 University/ml EPO resulted in an increased expression and release of brain-derived neurotrofic factor (BDNF), an important neuronal survival factor, suggesting the involvement of this cytokine. Consistently, exposure of hippocampal neurons to BDNF prevented TMT-induced neuronal death, while BDNF neutralizing antibody inhibited EPO-induced neuroprotection. We have previosly demonstrated that TMT increases glial TNF-alfa production and release due to neuronal apoptosis, furtherly exacerbating neuronal damage. We thus evaluated whether EPO neuroprotection could be explained also by a reduction of TMT-induced TNF-alfa release by glial cells. The addition of EPO (10 University/ml) reduced the amount of TNF-alfa produced significantly. EPO did not affect glia-derived TNF produced by exposure to neuronal homogenate or to LPS, suggesting that TNF reduction is a consequence of the inhibited neuronal death rather than of an anti-inflammatory action directly exerted on glial cells. These findings suggest that EPO attenuates both glial activation and TMT-induced injury by increasing BDNF production, and provide new insights on the role of beneficial cytokines in the progression of a neurotoxic insult.

1138 DOPAMINERGIC CELL DEATH AND VANDIUM INHALATION.

M. Avila-Costa[1], L. Colin-Barenque[1], E. Montiel-Flores[1], P. Aley[1], I. Sanchez[2], L. Irma[2], A. Gutierrez[2], J. Ordones[2], S. Acevedo-Nava[2], A. Gonzalez-Villalva[2], G. Nino-Cabrera[2], P. Bizarro[2], J. Espinosa-Villanueva[2], F. Pasos[2], P. Mussali-Galante[2], V. Delgado[2] and T. I. Fortoul[2]. *[1]Neuroscience, UNAM campus Iztacala, Tlalnepantla, Edo. Mex., Mexico and [2]Facultad de Medicina, UNAM, Mexico, DF, Mexico.*

Vanadium (V), a transition metal has increased in the air as a component of suspended particles originated from fuel combustion. In this report a model of inhaled V in mice was implemented to identify the effect that V exerts in the substantia nigra neurons, structure with high dopamine concentrations and scarce antioxidants burden. CD-1 male mice inhaled one hour twice a week 0.02 M V205; animals were sacrificed from 1 to 8 weeks of inhalation, perfused and processed for tyroxine hidroxylase (TH) inmunocytochemistry. The analysis consisted in counting the number of TH immunoreactive neurons in the substatia nigra compacta. Our results showed that there was a significant reduction of TH-positive neurons which decrease in a time dependent manner. Changes induced by V in the substantia nigra might have functional repercussions in motor and learning behaviors, and the fashion by which V is exerting its effect should be acknowledged. Supported in part by DGAPA-UNAM 210301 and P.O.S Institute.

1139 INHALATION OF URANIUM OXIDE: PHYSIOLOGICAL EFFECTS ON RATS.

J. Lewis[1], J. Karlsson[1], G. Bench[3], O. Myers[1], W. Barrington[1], E. Barr[2] and F. Hahn[2]. *[1]Community Environmental Health Program, University of New Mexico, Albuquerque, NM, [2]Lovelace Respiratory Research Institute, Albuquerque, NM and [3]Lawrence Livermore National Laboratory, Livermore, CA.*

Depleted uranium (DU) has been implicated as a potential factor contributing to Gulf War illness. Upon impact, DU-containing armor penetrators burn and release uranium dust particles of respirable size. Inhaled metals can be directly transported into the brain *via* the olfactory system, and inflammation in nasal epithelium may increase brain metal uptake further. We therefore investigated the deposition of uranium in brain and kidney as well as neuroinflammatory response in olfactory bulb. Rats were exposed to either: a) insoluble UO2, b) soluble UO3, c) 50% UO2 + 50% UO3, d) DU oxide (DUOx), e) TaO2 (negative control) or f) air (control) for 15 min in a nose-only inhalation chamber. The metal concentration in aerosols ranged from 300-600 mg/m3 and particle size was 1.5-2.4 µm. Nasal inflammation was induced in a subset of animals by endotoxin instillation in both nostrils 48 hours prior to air, DUOx or UO2 + UO3 exposure as above. Animals were sacrificed on day of exposure (0 day) or 30 days post-exposure for tissue analysis of uranium content. Astroglial response in olfactory bulbs at he same timepoints was evaluated using glial fibrillary acidic protein (GFAP) immunoreactivity. Of the 30 rats exposed to UO3, 12 females and 3 male rats died within 12 days after exposure and were found to have acute renal tubular necrosis and uremic pneumonia. There were no early deaths in other experimental groups. Uranium levels in kidneys as well as brain olfactory system were below the detectable level at 0 and 30 days in all experimental groups. Nevertheless, GFAP intensity in olfactory bulb glomeruli was significantly elevated in UO3 exposed animals at 0 and 30 days compared to air-exposed control rats. There was a trend for DUOx to increase GFAP response at 0 days. Thus, uranium oxide can, even at levels too low for detection in brain, lead to neuroinflammation. Effects of uranium on other tissue types and brain regions is currently under investigation.

1140 ACUTE EXPOSURE TO URANIUM (University) DECREASES POTASSIUM-STIMULATED HIPPOCAMPAL GLUTAMATE RELEASE.

S. M. Lasley and K. R. Vietti. *Biomedical & Therapeutic Sciences, University of Illinois College of Medicine, Peoria, IL.*

Gulf War veterans who retained fragments of depleted uranium (DU) shrapnel have exhibited lowered performance on neurocognitive tests, and rats chronically exposed to DU have displayed apparent decreases in neuronal excitability. The goal of this study was to determine if acute exposure to University *in vitro* disrupts synaptic processes underlying K+-stimulated glutamate release. A crude hippocampal P2 fraction was prepared from 1-2 month old Sprague-Dawley rats, and 0.8 mg synaptosomal protein loaded onto glass fiber filters. Synaptosomes were superfused with a phosphate-free 25 mM HEPES buffer saturated with O2/CO2 (95:5) and maintained at pH 7.4. Release was stimulated with brief perfusion of high K+-HEPES containing a glutamate reuptake blocker. Concentrations of University oxide (University(VI), 0.1 uM to 1.0 mM) were added to perfusion solutions, and 2-min fractions were collected beginning ~45 min after exposure was initiated. Aliquots were derivatized and quantified by binary gradient liquid chromatography with fluorescence detection. In the absence of University, K+ stimulation resulted in a 2-4-fold elevation in glutamate concentration over baseline values. Perfusion of University(VI) diminished endogenous potassium-evoked glutamate release at 0.1 mM (-37%) and 1.0 mM (-70%) compared to control values, resulting in an approximate IC50 of 0.25 mM. The duration of University(VI) exposure is appropriate for investigating its effects on membrane calcium channels involved in exocytosis, but this form of the metal is less potent than other more well studied divalent cations such as Pb+2 or Cd+2. Other more biologically significant forms of University (e.g., uranyl ion, divalent UO2) may exhibit greater potency on transmitter release processes. The intracellular actions of University(VI) on exocytosis could also be important. These initial findings suggest a measurable effect of University(VI) on hippocampal glutamatergic transmission and indicate the need for further investigation. (Supported by USAMRMC grant DAMD17-02-1-0212)

1141 MITOCHONDRIAL MEMBRANE POTENTIAL IN CEREBELLAR GRANULE CELLS OF CALCIUM CHANNEL MUTANT LEANER MICE UNDER VARIOUS CONDITIONS.

K. A. Thuett, S. Bellum and L. C. Abbott. *CVM, VAPH, Texas A&M University, College Station, TX.* Sponsor: E. Tiffany-Castiglioni.

Homozygous leaner (tg^{la}/tg^{la}) mice carry an autosomal recessive mutation in the carboxy-terminus of the pore forming, α1A subunit of P/Q-type voltage-gated calcium ion channels. These channels are highly expressed in cerebellar granule cells. Leaner granule cells begin to die *via* apoptosis on postnatal days (P)10-12, with peak cell death at P20. Altered calcium signaling can damage mitochondria, resulting in the release of cell death signals. To determine whether mitochondrial damage is involved in leaner granule cell death, we examined mitochondrial membrane potentials (MMP) of leaner and age-matched wild type cerebellar granule cells at P20 and P30. Granule cells were acutely isolated, plated on coverslips and loaded with TMRM, a lipophilic cation that accumulates in mitochondria in proportion to the MMP. Fluorescent images of granule cells were acquired and analyzed. At P20, the MMP observed in leaner granule cells was significantly lower than in wild type cells. However, at P30 no difference was observed between leaner and wild type granule cells. We speculate that changes in calcium ion current and intracellular homeostasis alter leaner granule cell mitochondrial integrity, leading to activation of neuronal cell death signaling pathways. At P30, cell death has terminated and surviving cells exhibit no MMP differences. Methylmercury (MeHg) toxicity can alter $[Ca^{2+}]$ in cerebellar granule cells *in vitro*, and death in cells exposed to MeHg occurs *via* apoptosis and necrosis. Since the $[Ca^{2+}]$ and MMP of leaner mice are already altered at P20, we hypothesize that MeHg will have a profound affect on the MMP of leaner mice when compared to wild type, MeHg treated wild type, and non-treated leaner mice. MeHg treatment will occur *via* subcutaneous injection for a total dose of 5.0 mg/kg MeHg. Granule cells will be isolated and MMP measured at P20 and P30 as stated previously. The leaner granule cell death and any MeHg associated changes observed in mice treated with MeHg may be a result of mitochondrial damage due to alterations in calcium signaling.

1142 LEAD EXPOSURE IS A RISK FACTOR FOR OSTEOPOROSIS.

J. E. Puzas, K. O. Hochberg, R. J. O'Keefe, E. M. Schwarz, M. J. Zuscik, J. Campbell and R. N. Rosier. *Department of Orthopaedics, University of Rochester School of Medicine, Rochester, NY.*

Relevance: We have taken a programmatic approach to investigate multiple skeletal systems for the effects of lead (Pb). Prior studies have suggested that Pb adversely affects bone metabolism, but there has been no comprehensive effort to document the extent and severity of this toxicant on the skeleton. We have examined the effects of Pb on bone cell function, growth plate activity, fracture healing and as a clinical factor affecting bone mineral density (BMD). Methods: Isolated osteoblast (OB) and osteoclast (OC) cell cultures were used to document the effect of Pb on TGFbeta, RANK ligand (RL) and osteoprotegerin (OPG) signaling pathways. Isolated growth plate chondrocytes were used to study the effect of Pb on endochondral ossification. Reporter analysis, RT-PCR and Western blots were employed for this work. Histological and biochemical assays were used to evaluate the effect of Pb in a fracture-healing model. Clinical evaluations with DEXA BMD were used to translate our findings into a human population. Results: 1) Pb adversely affects OB and OC function. The mechanism of the effect for OBs is mediated through an inhibition of TGFbeta signaling. The effect on OCs is through a down regulation of RL production and upregulation of OPG production. 2) Pb accelerates early chondrogenesis but blocks maturation of chondrocytes in the growth plate. That is, type II collagen synthesis is stimulated early, but type X collagen synthesis is dramatically delayed later. 3) A similar molecular picture exists for the fracture healing process, *in vivo*. 4) Early DEXA evaluations of Pb exposed humans indicate that they have higher BMDs than control subjects, however, we have discovered a potential artifact in the measurement of BMD by this low-energy x-ray technique that may account for this finding. Discussion: Pb adversely affects bone cells, chondrocytes and fracture callus. It also appears to influence BMD. All of these results, taken together, implicate Pb exposure as a serious risk factor for diseases such as osteoporosis.

1143 SODIUM ARSENITE (ASIII) MODULATORY EFFECT ON THE HEART AND BLOOD LEVELS OF GLUTATHIONE IN APOE(-/-)XLDLR(-/-) MICE.

F. E. Pereira, M. Hassani and H. D. Beall. *Center for Environmental Health Sciences, The university of Montana, Missoula, Missoula, MT.*

Arsenic is a potent toxicant, carcinogen and modulator of the antioxidant defense system and it may exert its toxicity by generating reactive oxygen species. Glutathione (GSH), a 3-amino acid thiol peptide, has been shown to have an important protective role against arsenic-induced oxidative damage. This study attempted to elucidate the impact of sub-acute (14-day) arsenic exposure on the blood and heart levels of GSH in apoE(-/-)xLDLR(-/-) mice. These double knockout mice are atherogenic and have been characterized as a model for investigating effects of arsenic on cardiovascular diseases such as atherosclerosis. Two groups, one saline and the other sodium arsenite (AsIII) treated apoE(-/-)xLDLR(-/-) mice were included in this study, 8 mice each. Each saline-treated mouse received an i.p. injection of 100 µl saline and AsIII-treated mice received 1 mg arsenic per kg body weight *via* injection of adjusted volumes of a prepared arsenic stock solution. The mice were injected on a weekly basis for two weeks. The mice were bled, immediately sacrificed at the end of the second week and the hearts were collected. A GSH assay, measuring total non-protein thiols, was performed on the blood and heart samples of these mice. The assay for the blood samples showed a significant increase in GSH levels in arsenic-treated mice compared to the saline-treated group. Although there is a trend toward an increase in the heart GSH levels of the arsenic-treated group compared to the control group, it is not statistically significant. The adaptive protective effect of GSH against this sub-acute exposure of arsenic is exhibited similarly in the blood and heart of these mice. It seems that due to possible arsenic-induced lipid peroxidation and oxidative stress, which can deplete GSH resources in these tissues shortly after exposure, the GSH compensating mechanisms are activated and increase GSH synthesis significantly in a time wise manner. This work was supported by NIH grant RR-017670.

1144 ARSENIC-INDUCED TRANSFORMATION CAUSES GENERALIZED RESISTANCE TO APOPTOSIS IN CULTURED HUMAN KERATINOCYTES.

J. Pi[1], Y. He[2], C. Bortner[3], J. Huang[2], J. Liu[1], W. Qu[1], J. M. Reece[3], M. Styblo[4], C. F. Chignell[2] and M. P. Waalkes[1]. *[1]ICS, LCC, NCI at NIEHS, Research Triangle Park, NC, [2]LPC, NIEHS, Research Triangle Park, NC, [3]LST, NIEHS, Research Triangle Park, NC and [4]Department of Pediatr, UNC, Chapel Hill, NC.*

Inorganic arsenic (As) is a well-documented human carcinogen that targets the skin, although the underlying carcinogenic mechanism is not well understood. Tumorigenesis is a multistep process in which acquired apoptotic resistance is a common event. In this study, when HaCaT cells, an immortalized, non-tumorigenic human keratinocyte cell line, were transformed by continuous exposure to low level (100 nM) inorganic arsenite [As(III)] for 28 weeks, a generalized resistance to apoptosis was observed. This included resistance to apoptosis induced by ultraviolet A (UVA) radiation, a human skin carcinogen, or a high dose of As. Concurrent with this acquired resistance, the As-transformed cells exhibited morphological changes and increased secretion of matrix metalloproteinase 9, which plays a crucial role in tumor invasion and is often associated with malignant transformation. Since cellular apoptosis is dependent on the balance between proapoptotic and survival pathways, the roles of caspase and protein kinase B (PKB), a key antiapoptotic molecule, in As-induced apoptotic resistance were investigated. Western blot analysis indicated that the As-transformed cells exhibited much less caspase-3 and -7 activation than control cells after UVA or high dose As(III) exposure. In the control cells, UVA or high dose As(III) markedly decreased nuclear phosphorylated PKB (P-PKB) levels prior to the apoptosis, whereas the As-transformed cells exhibited an increased stability of nuclear P-PKB. Pretreatment of the As-transformed cells with LY294002 or wortmanin, which inhibit PKB phosphorylation, completely blocked the acquired apoptotic resistance. These data demonstrate that the acquired apoptotic resistance observed concurrently with As-induced cellular transformation is associated with increased stability of nuclear P-PKB. As-induced acquired resistance to apoptosis may be an important event in skin cancer development by allowing damaged cells to escape normal cell population control.

1145 SUBCYTOTOXIC INORGANIC ARSENITE TARGETS MITOCHONDRIA IN HK-2 HUMAN PROXIMAL TUBULAR CELLS: IMPLICATIONS ON MECHANISM OF CELL DEATH.

M. Peraza, D. E. Carter and A. Gandolfi. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

The kidney is a known target organ for arsenic (As) and is critical for both As biotransformation and elimination. Previous studies have demonstrated that at high doses (ppm) inorganic As (arsenate) is toxic to mitochondria primarily by affecting cellular respiration. However the effect of inorganic As on mitochondria after low level exposures is not known, particularly in the kidney. Thus the mitochondrial toxicity of low level inorganic arsenite was investigated in a human proximal tubular cell line, HK-2. Subcytotoxic concentrations of arsenite (1-10 µM) were found to affect MTT processing by mitochondria in the HK-2 cells. Mitochondrial injury was further assessed by examining the alteration of the mitochondrial membrane potential using MitoTracker Red, a mitochondrion selective dye. In a subset of cells, subcytotoxic arsenite led to mitochondrial membrane depolarization, which

could subsequently lead to permeability transition and apoptosis. Subcytotoxic arsenite also induced translocation of phosphatidylserine to the outer layer of the plasma membrane, indicative of apoptosis. To confirm whether subcytotoxic arsenite induces cellular and/or mitochondrial morphological alterations consistent with apoptotic cell death, HK-2 cells were evaluated with both light and transmission electron microscopy. Classic morphological changes indicative of apoptosis were not observed at either the light microscopic nor the electron microscopic level with subcytotoxic arsenite exposures; however, evidence of necrotic changes in cytoplasmic structure and morphology, particularly in the mitochondria, were apparent. Therefore, based on the externalization of phosphatidylserine, HK-2 cells appear to initiate apoptosis following subcytotoxic arsenite insult, but based on the morphological changes seen, fail to complete apoptosis and undergo necrosis instead. Subcytotoxic arsenite can be sufficiently toxic to mitochondria that they lose their ability to keep the cell on course for apoptotic cell death. (NIH ES 04940, ES 06694)

1146 ARSENIC TRIOXIDE INHIBITS NUCLEAR RECEPTOR SIGNALING BY PHOSPHORYLATION OF RXR.

K. K. Mann[1], A. L. Colosimo[1], H. Lee[2], J. M. Kurie[2] and W. H. Miller[1]. *[1]Molecular Oncology, Lady Davis Institute for Medical Research, McGill University, Montreal, QC, Canada and [2]Thoracic/Head and Neck Medical Oncology, University of Texas/MD Anderson Cancer Center, Houston, TX.*

Arsenic is a wide spread environmental contaminant, but arsenic trioxide (ATO) is also used to treat patients with acute promyelocytic leukemia (APL). We have previously published that ATO inhibits APL cell differentiation induced by retinoic acid, the other common treatment for APL, complicating the development of combination therapy using these two effective agents. Upon further investigation, we have found that ATO inhibits the transcriptional activation in transient transfections not only of retinoic acid receptor, but also several other nuclear receptors, all of which heterodimerize with the retinoid X receptor (RXR). ATO does not inhibit transactivation of the estrogen receptor, which acts as a homodimer. Previously published data show that phosphorylation of RXR can inhibit nuclear receptor signaling. Indeed, we find that ATO phosphorylates RXR in the N-terminal ABC region exclusively on serine residues, as assessed by *in vivo* labeling and phosphoamino acid experiments. We have previously identified JNK as a kinase activated by ATO and therefore, sought to determine if RXR is a target of JNK-mediated phosphorylation. When JNK activation is impaired through pharmacologic inhibition or by genetic deletion of the upstream regulator, SEK1, the ability of ATO to inhibit transactivation of the RXR/vitamin D receptor heterodimer is abrogated. Inhibition of JNK activity also decreases the level of ATO-induced RXR phosphorylation. These data suggest ATO-induced JNK activation leads to RXR phosphorylation and the inhibition of transcription by RXR heterodimers. This suggests that consecutive use of retinoic acid and ATO in the treatment of patients with APL may be preferred. In addition, consideration of this interaction between ATO and nuclear receptor function may give some insight into molecular mechanisms of arsenic-induced carcinogenesis resulting from environmental exposure.

1147 EFFECT OF LOW DOSE AS(III) IN THE DRINKING WATER OF MICE ON TUMOR GROWTH AND ANGIOGENESIS.

M. A. Ihnat, L. Hess, S. Curilla and C. Clark. *OUHSC, Oklahoma City, OK.* Sponsor: R. Kaltreider.

Arsenite (As(III)), a major drinking water contaminant, is associated with many vascular abnormalities in contaminated populations. We have previously shown that As(III) at levels approaching the current US drinking water standard (10 PPB) stimulates angiogenesis and As(III) injected into tumor-bearing mice at levels below those used to treat cancer can actually stimulate tumor growth. We now show that B16-F10 melanoma tumor-bearing mice exposed to 10, 50, and 200 PPB As(III) in their drinking water for 10 weeks prior to tumor implantation show considerably higher tumor growth rates versus mice given nanopure water. Additionally, we show by immunohistochemistry that levels of HIF-1α and two of its regulated proteins, VEGF and PAI-1, are substantially increased in mice receiving 10 and 50 PPB As(III)), but not in mice receiving 200 PPB As (III). Interestingly, tumor blood vessel counts were substantially higher in animals given all doses of As(III). In isolated B16 cells, a 4 hr exposure to high dose (75 and 750 PPB) As(III) stimulated HIF-1α protein levels by immunoblot analysis. In contrast, a 72 hr exposure to low dose (0.75 and 7.5 PPB) As(III) caused comparable HIF-1α protein induction. Using the CAM angiogenesis assay, the VEGFR kinase inhibitor SU5416 (10μM) and an inhibitor of HIF-1α, YC-1 (10μM), abrogated the angiogenic effects of As(III). Finally, the antioxidants tocopherol (100μM), NAC (1mM), DMSO (0.5 percent) and TEMPOL (1mM) all reduced As(III)-mediated vessel formation in the CAM assay. These results indicate that the angiogenic effects of low dose As(III) can enhance tumor growth and the angiogenic stimulation by low dose As(III) likely involves reactive oxygen species (ROS) and HIF signaling.

1148 PANCREATIC OXIDATIVE DAMAGE AND ENDOCRINE FUNCTION IN RATS SUBCHRONICALLY EXPOSED TO ARSENITE.

J. A. Izquierdo-Vega[1], C. A. Soto[2], L. C. Sanchez-Pena[1], L. O. Valenzuela[1], E. A. Garcia-Montalvo[1] and L. M. Del Razo[1]. *[1]Toxicology Section, Cinvestav-IPN, Mexico D.F, Mexico and [2]Biologic Systems Department, UAM-Xochimilco, Mexico D.F., Mexico.*

Chronic exposure to inorganic arsenic (iAs) contributes to oxidative stress in several organs and systems through production of reactive oxygen species. Since the pancreas is a susceptible organ to the oxidative stress and target of the toxic action of iAs exposure, our study evaluated the presence of oxidative stress and endocrine function of pancreas in rats subchronically exposed to iAs. The oral glucose tolerance test (OGTT) was performed in male Wistar rats after 60 days of treatment with sodium arsenite at 1.7mgAs3+/kg/12h. The effect of subchronic iAs administration on blood glucose, serum insulin, lipid peroxidation (LPO), levels of glutathione (GSH) and As species in pancreas were evaluated after 90 days of iAs treatment in other group of male Wistar rats exposed with sodium arsenite at 1.7mgAs3+/kg/12h. The OGTT shown an impaired glucose tolerance. Hyperinsulinemia (2.7±0.9 vs 1.4±0.6 ng/ml of control group) and hyperglycemia (9.3± 0.7 vs 7.1 ±0.8 mmol/l) was observed at the end of iAs treatment. On the other hand the pancreatic LPO and glutathione levels increased significantly in iAs exposed rats. Dimethylated arsenic (DMA) was the main specie present in the pancreas. The results of this study suggest that subchronic iAs exposure causes oxidative damage and pancreatic beta cell dysfunction related to insulin resistance and hyperglycemia. These results may be related with the presence of pre-diabetes status.

1149 HEAT SHOCK PROTEIN 70 AS AN INDICATOR OF EARLY LUNG INJURY CAUSED BY EXPOSURE TO ARSENIC.

S. Han[1, 2], J. Nath[2] and V. Vallyathan[1]. *[1]Pathology and Physiology Research Branch, Health Effects Laboratory Division, NIOSH, Morgantown, WV and [2]Genetics and Developmental Biology, West Virginia University, Morgantown, WV.*

Heat shock proteins (HSPs) are a family of highly conserved proteins that are induced by stress, temperature, redox status, heavy metals, and inflammation. HSPs play a major role within a cell from folding of synthesized proteins or its degradation by proteosome. Intracellular transport and defects in folding can cause accumulation of these proteins resulting in several disease processes. HSP expression can be interpreted as an early and sensitive biomarker of cells in stress. Arsenic (As) is a naturally occurring metal that is distributed widely in the environment and is used in several industries. Exposure to As is associated with the development of pulmonary and skin cancers. The present study was undertaken to evaluate the expression levels of Hsp70 protein and mRNA induced by exposure to As which could be a sensitive and early biomarker. In addition, the cellular and molecular mechanisms of Hsp70 expression by As were investigated in the human bronchial cell line BEAS-2B. Cytotoxicity, lipid peroxidation and hydrogen peroxide generation were measured as indicators of cell injury and perturbed oxidative metabolism by As. Exposure of BEAS-2B cells to As(III) was associated with increased expression of Hsp70 protein and mRNA in a time and dose dependent manner. Hsp70 protein expression showed a significant five-fold increase by Western blot analysis and was increased 20-fold using an ELISA assay at a 50 uM As(III) concentration with a 6 hr exposure and an 8 hr recovery time. Hsp70 mRNA expression showed a 28% increase compared to controls. Cytotoxicity resulting from As(III) increased with a longer exposure time (48 hr). Lipid peroxidation increased six-fold at a concentration of 20 uM As(III) for 24 hr exposure, while H2O2 generation showed a two-fold increase. These results suggest that the induction of Hsp70 was the most sensitive indicator of cell injury by As(III), and Hsp70 may be a valuable indicator of oxygen free radical-induced lung cell injury.

1150 ROLE OF CALCIUM AND MITOGEN-ACTIVATED PROTEIN KINASES ON CADMIUM-MEDIATED GROWTH ARREST AND CASPASE-3 ACTIVATION IN MURINE MACROPHAGES.

J. Kim and R. P. Sharma. *physiology and pharmacology, University of Georgia, Athens, GA.*

Cadmium (Cd) is a well-known carcinogen and immunotoxic metal commonly found in cigarette smoke. Proliferation and apoptosis is regulated to provide appropriate cell number and to avoid tumor growth. Present study was designed to deter-

mine the possible involvement of calcium ($Ca2+$) and mitogen-activated protein kinases (MAPKs) signaling pathways on Cd-induced growth arrest and apoptosis in J774A.1 macrophage cells. Concentration-dependent apoptosis was detected; response reached a peak at 16 hr and then declined. Inhibitors of caspase-3, -8, -9 and general caspases suppressed Cd-induced caspase-3 activation and apoptosis indicating the importance of caspase-3 on Cd toxicity. The Cd-induced growth arrest, caspase-3 activation, and cytotoxicity were attenuated when intracellular $Ca2+$ chelator 1, 2-bis(2-aminophenoxy)-ethane-N, N, N', N'-tetraacetic acid tetrakis (acetoxy-methyl) ester and extracellular $Ca2+$ chelator ethylene glycol-bis(beta-aminoethyl ether)-N, N, N', N'-tetraacetic acid were used. Protein kinase C (PKC) activator phorbol myrisate acetate inhibited Cd-induced apoptosis and cytotoxicity but potentiated growth arrest, suggesting that an increased intracellular and extracellular $Ca2+$ levels and PKC activity status are important in regulation of proliferation and apoptosis. Pretreatment with cell-permeable Cd chelator N, N, N', N-terakis-(2-pyridylmethyl)ethylenediamine that has a low affinity for $Ca2+$ inhibited cytotoxicity but did not affect Cd-induced caspase-3 activation, indicating that intracellular Cd is not responsible for the induction of apoptosis. Use of selective MAPK inhibitors suggested that all three MAPKs, c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase and p38 protected the Cd-induced cytotoxicity, but only JNK was involved in Cd-induced caspase-3 activation. Our findings suggest that $Ca2+$ elevation triggers growth arrest and JNK and caspase-3 activation may be interrelated with $Ca2+$ to modulate Cd toxicity. (supported by UGA center for Academic Excellence in Toxicology and Fred Davison Endowment Fund)

1151 ARSENIC ALTERS THE SCAFFOLDING PROPERTIES OF THE MEKK4 KINASE.

University. M. Halfter, Z. E. Derbyshire and R. R. Vaillancourt. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

Toxicity of arsenite is well documented in whole organism studies where environmental exposure, i.e. mostly in drinking water and food, has caused elevated levels of cancers. Skin cancer, hyperpigmentation and hyperkeratosis or blackfoot disease are reported. We are interested in arsenite effects on cellular signal transduction mechanisms to document early effects of arsenite toxicity. We are studying the regulation of the mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 4 (MEKK4) signaling pathway. Using immortalized human keratinocytes, HaCaT cells, as a model system we are investigating the regulation of the serine/threonine protein kinase MEKK4. Our previous evidence suggests that this pathway regulates cyclooxygenase II expression levels. The inflammatory mediator, interferon gamma, has been reported to activate cyclooxygenase II expression. Indeed, we demonstrate an interferon gamma dependent tyrosine phosphorylation of MEKK4. The tyrosine kinase Pyk2 co-immunoprecipitates with MEKK4, suggesting that this tyrosine kinase may mediate the phosphorylation of MEKK4. Employing calcium chelators we demonstrate the significance of calcium for the activation of the MEKK4 kinase. Annexin II is a calcium binding protein. We have identified annexin II being co-immunoprecipitated with MEKK4 suggesting its role as a mediator of the calcium dependency. Here we present our study of the effects of arsenite on the regulation of the MEKK4 pathway. Serial dilutions of sodium arsenite applied to HaCaT cells affect MEKK4 tyrosine phosphorylation. Moreover, we found the composition of components of the MEKK4 signaling complex are altered in response to arsenite exposure of keratinocytes. To corroborate data obtained with immortalized keratinocytes we, in addition, employ normal human epidermal keratinocytes (NHEK).

1152 TUMOR PROMOTER ARSENITE STIMULATES HISTONE H3 PHOSPHOACETYLATION AT C-*fos* AND C-*jun* IN HUMAN DIPLOID FIBROBLASTS.

Y. Liu[1, 2], J. Li[2], M. Gorospe[2] and J. Barnes[2]. [1]*Children's Hospital, Children's Research Institute, Columbus, OH and* [2]*Laboratory of Cellular and Molecular Biology, NIA-IRP, NIH, Baltimore, MD.* Sponsor: C. Smith.

BACKGROUND: Although epidemiological studies have clearly established that elevated arsenic levels in drinking water are associated with increased incidences of skin, lung, bladder, kidney, and liver cancers, the carcinogenic mechanism remains elusive. Recent studies have suggested that arsenic may act as a tumor promoter by perturbing key signaling pathways. We have shown that arsenite potently induces proliferation-associated genes, including c-*jun* and c-*fos*, through a pathway regulated by EGF receptor. Recent studies have demonstrated that chromatin remodeling mediated by histone H3 phosphoacetylation plays an important role in the induction of c-*fos* and c-*jun*. OBJECTIVE: To understand the molecular mechanisms underlying the tumor-promoting properties of arsenic and test the hypothesis that histone H3 phosphoacetylation are involved in the induction of c-*fos* and c-*jun*. DESIGN/METHODS: Early passage normal human lung fibroblast WI-38 cells were stimulated with arsenite. Expression of c-*fos* and c-*jun* was examined by Northern blot analyses. Histone H3 phosphorylation and acetylation at the global levels were assessed by immunofluorescence and Western blot analyses. Histone H3 phosphorylation and acetylation at the loci of c-*fos* and c-*jun* were measured by chromatin immunoprecipitation (ChIP) and real-time PCR assays. RESULTS: Both c-*fos* and c-*jun* by arsenite can be substantially inhibited by the MEK-selective inhibitor, but not by the p38 inhibitor. Arsenite dramatically induced the phosphorylation and acetylation of histone H3 preceding the mRNA induction of c-*fos* and c-*jun*. ChIP assays revealed that arsenite markedly induced histone H3 phosphorylation/acetylation at the c-*fos* and c-*jun* loci through an ERK-dependent pathway. CONCLUSION: Our results suggest that arsenic-triggered alterations in chromatin structure perturb gene transcription and may contribute to the carcinogenic process.

1153 SODIUM ARSENITE INHIBITS FOCAL ADHESION COMPLEX FORMATION AND DYNAMICS IN LIVE CELLS.

S. L. Yancy[2], E. A. Shelden[1] and M. J. Welsh[1]. [1]*Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI and* [2]*Environmental Health Sciences (Toxicology), University of Michigan School of Public Health, Ann ARbor, MI.*

Arsenic is an environmental pollutant. It is converted in organisms to other forms, the most common of which is arsenite. Arsenite binds to protein thiols and its toxicity is thought to be non-specific. However, some thiols may be more critical for cell function than others. We previously showed that arsenite can inhibit FAK phosphorylation and cell motility. Here, we tested the hypothesis that arsenite decreases the dynamics and inhibits formation of focal adhesions (FAs) in live cells. H9C2 myoblast cells were transiently transfected with GFP-paxillin to allow observation of FAs. To ensure proper labeling of FAs by GFP-paxillin in living cells, cells were also prepared for immunofluorescence localization of the FA protein vinculin. Cells expressing GFP-paxillin were treated with 0, 1, 2.5 or 5 uM sodium arsenite for 24 hours. MTT cytotoxicity assay showed these doses did not cause reduced cell *via*bility. Cells were observed for 6 hours by time-lapse video fluorescence microscopy. Sodium arsenite-treated cells exhibited dose-dependent reductions in FA migration rates and reduced numbers of FAs per cell. FA complex formation rate was decreased, as measured by recruitment of FAK, vinculin and paxillin to FAs of cells treated with RGD-beads. Sodium arsenite-treated cells exhibited a significant reduction in ability to attach to a substrate over a 1 hour period. Sodium arsenite caused a reduction in cell contraction, indicating that arsenite might affect actomyosin-mediated contraction in H9C2 cells, a process necessary for FA formation. These data indicate that sodium arsenite alters FAs by interfering with FA formation, FA dynamics and cell contraction thereby interfering with cell behaviors dependent on FAs including cell attachment and migration and presumably other aspects of FA function and integrin signaling. Supported by NH grants P01-ES11188 to MJW and F31-HL68424 to SLY.

1154 CADMIUM-INDUCED APOPTOSIS, ACTIVATION OF MAPK SIGNALING PATHWAYS AND ACCUMULATION OF UBIQUITINATED-PROTEIN-CONJUGATES IN PRIMARY RAT NEONATAL SERTOLI-GONOCYTE CO-CULTURES.

X. Yu, J. S. Sidhu, S. Hong and E. M. Faustman. *Env. & Occ. Health Sciences., IRARC, University of Washinton, Seattle, WA.*

In this study, we employed our recently established and well characterized *in vitro* Sertoli-gonocyte co-culture system to examine whether Cadmium (Cd) affects development of the neonatal testis at low doses and to investigate the underlying mechanisms associated with such perturbations. We hypothesized that exposure to Cd during neonatal periods *in vivo* reduces the number of the Sertoli and gonocyte cell numbers by inhibiting proliferation and inducing apoptosis; and furthermore, that the activation of MAPK signaling pathways and the alterations in the ubiquitin-proteasome pathway (UPS) are involved in such perturbations. We investigated the time- and dose-dependent induction of stress signaling pathways, and the disruption of UPS function to explore the association with the corresponding induction of apoptosis. We also compared these effects to two known proteasomal inhibitors (MG132 and Lactacystin). Our results demonstrated that Cd exposure resulted in dose- and time-dependent alterations of morphology consistent with the induction of apoptosis as demonstrated by the increase of caspase-3 like activity and cleaved caspase-3 protein expression. We further observed that Cd treatment, in a dose- and time-dependent manner, resulted in activation of p38 MAPK, SAPK/JNK, c-jun and ATF-2, and accumulation of high molecular weight ubiquitin conjugated proteins (HMW-Ub). Furthermore, we identified two specific targets of UPS induced by Cd, namely p53 and Nrf 2. Nrf 2 is an important transcription factor in regulating the expression of detoxifying and anti-oxidant enzyme genes. In summary, the current study suggests that Cd-induced UPS dysfunction may be a critical mechanism underlying the toxicity associated with *in vivo* developmental testicular toxicity. Future studies will address these specific targets which render the developing neonate testis sensitive to environmental toxicants. Support: NIEHS ES09601-02, ES10613-01 and ES07033 and EPA R 826886-01-0.

1155 DEFINING P53-DEPENDENT AND INDEPENDENT MECHANISMS OF CADMIUM-INDUCED CYTOTOXICITY, STRESS SIGNALING, APOPTOSIS AND UBIQUITIN PROTEASOME PATHWAY PROCESSING.

J. S. Sidhu, S. Hong, X. Yu, E. Kim, A. Erickson, J. F. Robinson, S. Kim, M. Vredevoogd and E. M. Faustman. *Env. & Occ. Health Sciences., IRARC, University of Washington, Seattle, WA.*

The activity of the tumor suppressor oncoprotein p53, a critical cell cycle checkpoint modulating genotoxic stress, is regulated *via* the concerted function of the Ubiquitin Proteasome pathway (UPP) with stress-induced disruption leading to increased accumulation of p53 and resulting growth arrest. Recent studies have suggested that aberrant cellular responses observed with certain environmental contaminants, such as cadmium (Cd), is associated with the accumulation of high molecular weight poly-ubiquitinated protein conjugates (HMW-Ub). In the present study we investigated the role of p53 status in determining the sensitivity to Cd exposure and whether induction of stress signaling responses and perturbation of the UPP were central in mediating the metal-induced cytotoxicity and apoptosis. Utilizing synchronous cultures of p53 transgenic mouse embryonic fibroblasts we treated +/+ and -/- cells with increasing concentrations of Cd (0.5-20 μM) for 24h. We compared the metal response to two classic proteasomal inhibitors, MG 132 and lactacystin as well as a non-metal stress-inducing agent, anisomycin. Cd-induced cytotoxicity, assessed by disruption of cellular morphology and attenuated neutral red dye uptake, was partially dependent on p53 status with the wild type exhibiting a 2-fold greater sensitivity. Parallel analyses of cell extracts confirmed a Cd dose-dependent modulation of stress signaling responses (as evidenced by p38 MAPK and SAPK/JNK phosphorylation) and these responses corresponded to an accumulation of HMW-Ub as well as induction of apoptosis (as evidenced by western and activity determination of cleaved caspase-3 expression). We conclude that although Cd-mediated cytotoxicity is partially p53 dependent, the effects of this metal on induction of stress signaling responses, accumulation of HMW-Ub and resulting apoptosis appear to be independent of p53 status. Support: EPA and NIEHS R 826886-01, ES09601-02, ES07033 and ES10613-01.

1156 THE MECHANISM OF NF-κB SUPPRESSION IN CADMIUM-INDUCED APOPTOSIS IN RAT KIDNEY EPITHELIAL CELLS.

J. Xie and Z. A. Shaikh. *Department of Biomedical Sciences, University of Rhode Island, Kingston, RI.*

Cadmium (Cd) exposure induces apoptosis in different cell types including kidney cells. The oxidative stress elicited by Cd appears to exert a central role in promoting apoptosis. Redox sensitive transcription factors, such as NF-κB, are reported to participate in this process. However, the mechanism of NF-κB involvement in Cd-induced apoptosis has not yet been elucidated. In the present study, semiconfluent culture of a rat kidney epithelial cell line, NRK-52E, were exposed to 10-20 μM $CdCl_2$ for 5 hr and maintained for up to an additional 12 hr period. While apoptotic DNA fragmentation was detected 11 hr after starting the Cd exposure, both NF-κB activity and protein level were decreased at 5 hr of Cd exposure. The effect of Cd on NF-κB, assessed by EMSA and Western blotting, was dependent upon the dose and duration of Cd treatment. In comparison with Cd, other metals such as copper, zinc, lead and mercury increased rather than decrease NF-κB DNA binding activity. Preincubation of nuclear extract with 1-100 μM $CdCl_2$ showed that Cd had no direct effect on NF-kB DNA binding activity. Pretreatment of cells with antioxidants, U8386E and BHT, prevented loss of NF-κB DNA binding activity. Furthermore, Cd exposure inhibited TNF-α-induced IκB kinase α, the enzyme that phosphorylates IκB and releases NF-κB from NF-κB-IκB complex. In addition, RT-PCR analysis showed that Cd caused a decline in NF-κB mRNA level. Suppression of NF-κB by Cd also down regulated NF-κB target genes, cIAP-1 and cIAP-2, and activated caspase -3, 7 and 9. These results suggest that in kidney cells suppression of oxidative stress-sensitive transcription factor, NF-κB, is an early event in Cd-induced apoptosis and that the mechanism appears to involve both inhibition of NF-κB release and its synthesis. (Supported by RI-BRIN grant 5P20RR016457 from NCRR)

1157 OXIDATIVE STRESS AND CYTOKINES CO-STIMULATES METALLOTHIONEIN EXPRESSION IN CADMIUM TREATED HEPG2 CELLS.

C. Escobar, V. Souza, L. Bucio, E. Hernandez, L. Gomez-Quiroz and C. Gutierrez-Ruiz. *Ciencias de la Salud, Universidad Autonoma Metropolitana, Mexico, DF, Mexico.*

The liver is the target organ of acute cadmium poisoning and the principal site of synthesis of metallothionein (MT), a metal-binding protein that is purposed to cause resistance to Cd-induced hepatotoxicity. MT works as a defense mechanism against oxidative stress and some reports indicate that reactive oxygen species and some cytokines could induce it. The aim of this study is to determine the effect of IL-1β, IL-6 and TNF-α and oxidative stress on the expression of MT-II mRNA in HepG2 cells. Cell *via*bility was determined by MTT assay in a wide range of cadmium concentrations. HepG2 cells were exposed to 5 μM CdCl2 for 6 h, because cell *via*bility is higher than 90% in presence of this concentration. MT-II induction was determined by Northern blot. IL-1β, IL-6 and TNF-α (2.5ng/ml in each case) were added to HepG2 cells and MT-II induction was determined. A one hour pretreatment with anti-IL-1β (5 ng/ml) and anti-TNF-α (10 ng/ml) in Cd-treated HepG2 was also evaluated. In order to explore the oxidative stress in MT-II induction, HepG2 cells were treated with 0.25 mM H2O2 or with 2mM BSO that depletes cell glutathione content. Antioxidants pretreatment effect (10 mM NAC, 10 mM TMTU, and 2U/ml catalase) was also determined in Cd exposed cells. Cd increased 5-fold MT-II mRNA control values. All cytokines tested, increased also MT-II expression comparing with control cells values (266% TNF-α, 228% IL-6 and 239% IL-1β). Pretreatment with anti-IL-1β and anti-TNF-α of HepG2 Cd-treated cells diminished 23% MT-II expression. H2O2 and BSO induces two times MT-II mRNA. NAC and TMTU decreased 40% Cd-induced mRNA-MT-II, while a 44% decrease was obtained with catalase treatment. Oxidative stress and cytokines participate in Cd induced MT-II in HepG2 cells. The mechanism of MT-II induction as response of Cd damage must be a multi-factorial process in which cytokines and oxidative stress participates in order to protect cells of being damaged as consequence of metal exposure. CONACYT No. 400200-5-30671-M.

1158 OXIDATIVE STRESS INDUCED BY LEAD, CADMIUM AND ARSENIC MIXTURES: 30-, 90-, AND 180-DAY DRINKING WATER STUDIES IN RATS.

M. H. Whittaker, M. Lipsky, G. Wang, X. Chen and B. A. Fowler. *Toxicology Program, The University of Maryland, Baltimore, Baltimore, MD.*

Lead, cadmium, and arsenic warrant continued scrutiny because all three are found at 95% of Superfund sites. Moreover, they are associated with adverse health effects such as cancer and nephrotoxicity. Oxidative stress is thought to play a major role in the development of adverse health effects from exposure to these trace elements, and may occur through the generation of free radicals due to the accumulation of aminolevulinic acid (ALA) and free iron. In order to investigate mechanisms of trace element-induced oxidative stress, 8 groups of male Sprague-Dawley rats were exposed to deionized water, single trace elements (Pb (25 ppm as lead acetate), Cd (10 ppm as cadmium chloride), As (5 ppm as sodium arsenite)), or element mixtures for 30-, 90-, or 180-days. These doses were selected from a series of dose-response studies at LOEL dose levels. After 30 days, urinary ALA (mg/day) was increased in all groups compared to control (+18%-+287%). After 90 days, urinary ALA was increased in 6 groups (+15%-+52%). After 180 days, urinary ALA was increased in 4 groups (+25%-+77%). Urinary iron (μg/day) was decreased in all groups after 30 days (-8%—50%); after 90 days urinary iron was increased only in the Pb and As groups (+12%-+17%). After 180 days, urinary iron was decreased in all test groups (-3%—30%). When expressed in units of μg Fe/ml urine, urinary iron was increased in 4 of the 7 groups after 30 days (Pb, PbxCd, CdxAs, PbxCdxAs)(+14%-+77%); after 90 days, iron was increased in 6 of the 7 groups (all except PbxAs)(+10%-+30%); and after 180 days, iron was increased in 3 of the 7 groups (PbxAs, CdxAs, PbxCdxAs)(+8%-+30%). These data indicate that ALA and iron accumulate after acute exposure to trace elements or their mixtures. The accumulation of these two oxidative stress precursors appears to level off over 180 days, suggesting that some type of adaptation to oxidative stress occurs following repeat exposures to trace elements or their mixtures [Supported by USEPA Star Grant R827161-01-0].

1159 INTERACTIONS OF LEAD, CADMIUM AND ARSENIC IN RAT KIDNEYS.

G. Wang, X. Chen, M. M. Lipsky, W. H. Margaret and B. A. Fowler. *Toxicology Program, University of Maryland, Baltimore, MD.*

Lead, Cadmium and Arsenite are renal toxicants on an individual basis. This study explored the interactive effects of Pb, Cd, and As at empirically determined LOEL dose levels (Pb, 25 ppm, Cd, 10 ppm, As (As3+), 5 ppm) in drinking water for 30, 90 or 180 days using a factorial design. Histopathological evaluation of Sprague-Dawley rat kidneys showed no specific pathological changes for most treatments but increased kidney histopathology was observed in the combination groups at 180 days. Erythrocyte zinc protoporphyrin (ZPP) concentration increased in all exposure groups except the As group at 30 days and increased in the As and Pb*Cd*As groups at 180 days. No change in ZPP was seen in any treatment group at 90 days. Blood delta-aminolevulinic acid (ALAD) activity was inhibited in all treatment groups at 30 and 90 days. At 180 days, blood ALAD activity was inhibited only in the Pb group and was increased in all combination treatment groups. Kidney ALAD activity was inhibited in all groups at all time points except the

Pb*As group at 30 days and the As and Cd*As groups at 90 days. Kidney 8-hydroxy-2'-deoxyguanosine (8-OHdG), an oxidative DNA adduct, increased in only the Cd*As treatment group at 180 days. This adduct was decreased in all combined treatment groups at 90 days. Heme oxygenase (Hsp 32) expression displayed an increase in only the Pb*Cd*As group at 180 days. Metallothionein (MT) was induced in P1 and P2 segments of the proximal tubules in the Cd and Pb*As treatment groups at all three time points. MT staining intensity from high to low was: Pb*Cd*As=Cd*As=Pb*Cd=Cd>Pb*As>As=Pb=control. These data suggest that at 90 days there was a compensatory response to the toxic effects of these elements. Concomitant Cd*As exposure at 180 days produced significant oxidative DNA damage, not observed with Cd or As alone. The general compensatory response observed at 90 days and increased oxidative damage in combination treatment groups at 180 days cannot be explained solely by MT expression levels but must involve other regulators of oxidative damage. (Supported in part by EPA STAR grant # 827161).

1160 ACUTE RENAL FAILURE INDUCED BY CHROMATE TREATMENT AND CLAUDIN-2 EXPRESSION IN MURINE KIDNEY.

L. Arreola-Mendoza[1], J. L. Reyes[2], D. Martin[2], M. C. Namorado[2], J. C. Luna[2] and L. M. Del Razo[1]. [1]*Toxicology Section, Cinvestav-IPN, Mexico D.F, Mexico and* [2]*Physiology, Biophysics & Neurosciences, Cinvestav-IPN, Mexico D.F., Mexico.*

Renal epithelia and endothelia have tight junctions (TJs) that regulate the passage of ions, water and molecules through the paracellular pathway. Integrity of TJs is responsible for the passive permeability of polarized epithelia. Chromate (Cr6+) administration induces nephrotoxicity in humans and experimental models. It also increases formation of reactive oxygen species and oxidative damage. Oxidative stress compromises TJs, but the mechanisms are unknown. Our objective was to study the oxidative damage and effects on renal function and structure of TJs induced by chromate administration. Wistar female rats received potassium dichromate (15 mg/kg, SC, single dose). Renal function, lipid peroxidation, and claudin-2 (a protein of TJs) immunohistochemical pattern were monitored on days 0, 1, 2, 3, 7, 10 y 14. Confocal microscopy immunofluorescence of claudin-2 showed a clear presence at the cell borders of proximal tubules. This pattern was severely disorganized after chromate administration with spontaneous recovery after day 7. In agreement with morphological alterations, renal function was maximally altered on day 3 and thereafter recovery ensued. Glomerular filtration decreased and glucose and sodium fractional excretions increased. Oxidative damage showed a similar time-course. Our results suggest that chromate administration induces acute renal failure, oxidative damage and disorganization of the claudin-2 renal pattern. The excelent technical support of Gerardo Sierra is acknowledgement.

1161 SELECTIVE SIGNALING PATHWAYS FOR CHROMIUM(VI) INDUCED PATTERNS OF TRANSCRIPTION FACTOR BINDING IN EXPOSED AIRWAY EPITHELIAL CELLS.

K. A. O'Hara[1, 2], L. R. Klei[1, 2], R. J. Vaghjiani[2] and A. Barchowsky[1, 2]. [1]*Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA and* [2]*Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH.*

Inhaled hexavalent chromium (Cr(VI)) promotes pulmonary disease and lung cancer through poorly defined mechanisms. To explore these potential mechanisms, Beas-2B airway epithelial cells were used to investigate the hypothesis that non-toxic Cr(VI) exposures selectively activate cell signaling pathways which lead to changes in the profile of transcription factor binding to specific *cis*-elements in DNA. These studies demonstrated that non-toxic doses (1-5 μM) of Cr(VI) increased the abundance of tyrosine kinases. Two of these kinases, bone marrow X kinase (Bmx) and focal adhesion kinase (Fak), were detected by western blot analysis, and protein tyrosine kinase 2 (Pyk2) was detected by a Kinetworks™ protein kinase screen. As the levels of Pyk2 increased, so did its association with Fyn, a Src family kinase member. Futher, the kinase activities of both Fyn and Lck increased in response to Cr(VI). The DNA binding of various transcription factors (TF) are responsive to upstream signaling cascades that are altered in response to Cr(VI). Two protein/DNA array systems were used to demonstrate that protein binding to 9 out of a total of 150 targeted *cis*-elements changed over a 24 h exposure period. Significant increases were seen in both Stat 5 and AP-1 binding, which may be related to activation of tyrosine kinase activity by Cr(VI). The most significant decrease was seen in the level of nuclear protein that binds to the antioxidant/electrophile response element (ARE). These findings suggest that airway cell phenotype may be changed as low concentrations of Cr(VI) selectively activate signaling pathways that lead to prolonged changes in transcription factor binding. *Supported by NIEHS grant ES10638.*

1162 MODULATORY EFFECT OF THE GREEN TEA FLAVONOID EPIGALLOCATECHIN 3-GALLATE ON ARSENITE- AND HYDROGEN PEROXIDE-STIMULATED MITOGEN-ACTIVATED PROTEIN KINASE PHOSPHORYLATION AND HEME OXYGENASE-1 EXPRESSION IN HUMAN DERMAL FIBROBLASTS.

K. J. Trouba, J. D. Britton, A. C. Smith, Y. D. Rebolloso and D. R. Germolec. *NIEHS, Research Triangle Park, NC.*

Reactive oxygen species (ROS) are proposed to contribute to arsenic carcinogenesis and in skin ROS activate mitogen-activated protein kinases (MAPKs) that regulate cell growth. Green tea polyphenols are effective cancer chemopreventive agents and exert their scavenging effects against ROS and also modulate MAPK activity. Because carcinogenesis, ROS, and MAPKs can be modulated by polyphenols, we examined the activation (phosphorylation) of p42/44 and p38 MAPKs in human dermal fibroblasts (HDF) by sodium arsenite (AsIII) alone and in the presence of the small molecular weight green tea polyphenol epigallocatechin 3-gallate (EGCG). Induction of the cellular antioxidant enzyme and stress sensor heme oxygenase-1 (HO-1) also was examined under these conditions. Western blot analyses indicate that noncytotoxic AsIII (< 5 μM) and hydrogen peroxide ([H202] positive ROS control) stimulate p42/44 and p38 phosphorylation and elevate HO-1 protein expression in HDF. Short-term (3 hr) EGCG treatment (< 40 μM) inhibited p42/44 phosphorylation independent of AsIII or H202 exposure. In addition, it reduced p42/44 and p38 phosphorylation at 15 and 30 minutes post-H202 treatment. No effect on HO-1 induction stimulated by AsIII or H202 was observed after short-term EGCG treatment. Long-term (24 hr) EGCG treatment (< 40 μM) had no inhibitory effect on p42/44 or p38 phosphorylation, or the induction of HO-1 stimulated by either AsIII or H202. These data indicate that EGCG has little to no effect on AsIII-stimulated MAPK activation and HO-1 induction in our experimental system. It is anticipated that data from this study will contribute to a better understanding of arsenic skin carcinogenesis and the mechanisms of polyphenols as cancer chemotherapeutics.

1163 DIFFERENTIAL REGULATION OF AKT SIGNALING PATHWAY IN P53 WILD AND MUTATED CELLS AND RESISTANCE TO COPPER TOXICITY.

E. Ostrakhovitch and G. Cherian. *Pathology, UWO, London, ON, Canada.*

Previous studies have suggested that cells may differ in their response to metal stress depending on their p53 status. Human breast cancer epithelial cells, MDA-MB-231 cells (ER-/p53-) with mutated p53 are resistant to copper and cadmium, while, MCF-7 cells (ER+/p53+) with wild type p53 are sensitive with high degree of apoptosis. This study was undertaken to investigate the role of PI3K/Akt signaling pathway in metal resistance in these cells with different p53 status. Exposure to copper (25uM) and zinc (25uM) increased Akt phosphorylation with its nuclear localization only in MDA-MB-231 cells. The effect of zinc was transient and copper effect was persistent. Cyclin D1 expression and cell cycle progression followed the metal-induced Akt phosphorylation. Treatment with LY294002, an inhibitor of PI3K, abrogated these effects, suggesting the requirement of PI3-kinase for up-regulation of Akt and cyclin D1. In contrast in MCF-7 cells, metals increased the phosphorylation of p53 at serine 15 with transactivation of p53. In addition, metals up-regulated p21 expression and resulted in cell cycle arrest at G1 phase with apoptosis in MCF-7 cells. Addition of LY294002 had little effect on metal-induced p53 and p21 protein expression. These results demonstrate that copper induced apoptosis in MCF-7 cells is p53 dependent while the metal resistance in MDA-MB-231 cells may be due to activation of Akt phosphorylation and cell cycle progression in the absence of a functional p53 in these cells. (Supported by grants from CIHR, Canada).

1164 EFFECTS OF TRIBUTYLTIN ON APOPTOSIS IN LEYDIG CELLS: CYTOCHROME C RELEASING AND CASPASE-3 ACTIVATION BY DISTURBANCE OF CA2+.

J. Choi[1, 2], K. Lee[1, 2] and H. Jeong[1, 2]. [1]*Pharmacy, Chosun University, Kwangju, Kwangju, South Korea and* [2]*Research Center for Proteineous Materials, Chosun University, Kwangju, Kwangju, South Korea.*

Tributyltin (TBT) used world-wide in antifouling paints for ships is a widespread environmental pollutant. In this study, we investigated that the mechanisms underlying apoptosis induced by TBT in rat leydig cell line, R2C. Effects of TBT on intracellular Ca2+ level and reactive oxygen species were investigated in R2C cells by fluorescence detector. TBT significantly induced intracellular Ca2+ level in a time-dependent manner. The rise in intracellular Ca2+ level was followed by a time-dependent generation of reactive oxygen species at the cytosol level. Simultaneously, TBT induced the release of cytochrome c from the mitochondrial membrane into

the cytosol. Furthermore, ROS production and the release of cytochrome c were reduced by BAPTA, an intracellular Ca2+ chelator, indicating the important role of Ca2+ in R2C during these early intracellular events. In addition, Z-DEVD FMK, a caspase-3 inhibitor, decreased apoptosis by TBT. Taken together, the present results indicated that the apoptotic pathway by TBT might start with an increase in intracellular Ca2+ level, continues with release of reactive oxygen species and cytochrome c from mitochondria, activation of caspases, and finally results in DNA fragmentation.

1165 AMPLIFICATION OF THE CELLULAR RESPONSE TO PARTICULATES THROUGH TUMOR NECROSIS FACTOR AUTOCRINE SIGNALING.

B. chin, G. Holtom, C. chen and B. D. thrall. *Molecular Biosciences Division, Pacific Northwest National Laboraoty/Battelle, Richland, WA.*

The synthesis and secretion of tumor necrosis factor α (TNF) by macrophages plays an important role in mediating protective responses to a known environmental hazard, particulate matter (PM). However, when exacerbated, release of TNF from macrophages may also contribute to tissue damage. We investigated the relationship between particle uptake and TNF secretion in the cellular response to PM. Using model fluorescent particles of a defined diameter (500nm), we found a dose dependent relationship between total intracellular particle ingestion and extracellular TNF release in RAW 264.7 cells. Analysis of intracellular TNF protein levels by quantitative fluorescent immunocytochemistry revealed newly synthesized TNF intensely localized in the Golgi compartment. Interestingly, despite the fact that PM ingestion was complete within 24 hours, TNF synthesis and shedding remained elevated for at least 48 hours. However, co-treatment with either a neutralizing antibody that sequesters shed TNF, or with a metalloprotease inhibitor that blocks shedding of TNF, attenuated intracellular TNF protein levels. RT-PCR and western blot analysis showed that TNF stimulates its own synthesis through an ERK-dependent mechanism. Thus, the results demonstrate an intracellular amplification mechanism in response to PM insult, mediated through autoregulation of TNF synthesis and autocrine signaling. We hypothesize that this mechanism of signal amplification plays an important role in mediating toxicity at the tissue level in response to localized PM insult

1166 SILENCING OF THROMBOSPONDIN-1 EXPRESSION BY ESTROGEN IS REQUIRED FOR ESTROGEN-INDUCED ENDOTHELIAL CELL PROLIFERATION AND MIGRATION AND IS MEDIATED THROUGH NONGENOMIC ER-MAPK-JNK SIGNALING PATHWAY.

K. Sengupta, B. Snigdha, N. Saxena and S. K. Banerjee. *Hematology and Oncology, University of Kansas Medical Center & VA Medical Center, Kansas City, KS.*

The natural hormone 17β-estradiol (17β-E2) induces tumor angiogenesis in various target organs by activating positive regulators of angiogenesis. In this study, first time we show that in human umbilical vein endothelial cells (HUVECs) 17β-E2 transiently down-regulates the expression of a potent negative regulator of angiogenesis, thrombospondin-1 (TSP-1). Furthermore, this inhibitory effect of estrogen is mediated through an estrogen receptor (ER) and can be blocked by U0126, a mitogen activated protein kinase kinase (MAPKK) inhibitor as well as by an inhibitor of JNK/SAPK, a down stream signaling protein of MAPK in these cells. These findings together suggest that the down-regulation of TSP-1 expression by 17β-E2 is mediated through non-genomic ER-MAPK-JNK/SAPK signaling pathways. However the effect is lost with prolonged exposure to 17β-E2, as the level of TSP-1 protein becomes stabilized. We also show that, addition of recombinant TSP-1 protein significantly reduced the migration and proliferation of HUVECs stimulated by 17β-E2, and indicated a negative regulatory role of TSP-1 in estrogen-induced endothelial cell proliferation and migration and provides a framework for understanding the role of TSP-1 in estrogen-induced angiogenesis.

1167 SP600125, AN ANTHRAPYRALOZOLONE INHIBITOR OF JNK, INHIBITS B LYMPHOMA GROWTH.

M. Gururajan[1, 3], R. Chui[3], A. K. Karuppannan[2, 3] and S. Bondada[1, 2, 3]. *[1]Graduate Center for Toxicology, University of Kentucky, lexington, KY, [2]Microbiology & Immunology, University of Kentucky, Lexington, KY and [3]Center on Aging, University of Kentucky, Lexington, KY.* Sponsor: M. Vore.

The c-Jun NH2-terminal kinase (JNK), one of the three major groups of MAP Kinases, can cause cell death of many cell types by activating the mitochondrial apoptotic pathway. JNK is a stress-activated protein kinase that can be induced by inflammatory cytokines, bacterial endotoxin, osmotic shock and UV radiation. To understand the role of JNK in B lymphoma cells, we used a panel of B cell lines viz., BKS-2, CH31 and CH12-LX of murine; Ramos and BJAB of human origin. Treatment with the recently identified pharmacological JNK inhibitor SP600125 with concentrations ranging from 0 to 20 μM induced a dose dependent reduction in the proliferation and growth arrest at G1 phase of cell cycle in B lymphoma cells as opposed to vehicle treated cells. Moreover, the CH31 B lymphoma cells underwent apoptosis as determined by Annexin V binding. Inhibition of ERK MAP Kinase using PD98059 or UO126 had no effect on cell proliferation in BKS-2 and BJAB cells. In addition, proliferation of normal murine splenic B cells induced by a variety of mitogens was also inhibited by SP600125. We have reported earlier that inhibition of Egr-1, a pro-survival transcription factor, leads to growth arrest in BKS-2 B lymphoma. Hence we hypothesized that JNK mediated survival signals may be Egr-1 dependent. Egr-1 protein levels were reduced in BKS-2 cells treated with SP600125. Additionally, ICAM-1, a target gene of Egr-1 is downregulated in SP600125 treated BKS-2 cells suggesting that JNK mediated signals involve Egr-1. These findings reveal a pro-survival role for JNK in both murine and human models of B lymphoma. Interestingly, SP600125 had no effect on a hybridoma cell line AK11, suggesting its therapeutic potential in treating B cell lymphomas. Current studies are focused on identifying pro-apoptotic and pro-survival targets of JNK in SP600125 treated B lymphoma cells (supported by NIH grants to SB).

1168 STRUCTURAL STABILIZATION OF CELLS DETECTED BY FTIR IS POSSIBLY DUE TO TYROSINE PHOSPHORYLATION BY RHO KINASE IN SPLENOCYTES: EFFECT OF WATER EXTRACT OF THUNBERGIA LAURIFOLIA LINN.

P. Sinhaseni[1], V. Taechakitiroj[1], T. Suramana[2], T. Posayanonda[3], N. Nuntharatanapong[1], R. Sindhuphak[1], S. Chivapat[4], P. Chavalittumrong[4] and N. Dusitsin[1]. *[1]Institute of Health Research, Chulalongkorn University, Bangkok, Thailand, [2]Pharmacology and Toxicology Unit, Faculty of Sciences, Rangsit University, Pathumthani, Thailand, [3]Food Control Division, Food and Drug Administration, Nonthaburi, Thailand, [4]Department of Medical Science, Medicinal Plant Research Institute, Nonthaburi, Thailand and [5]Department of Pharmacology, Faculty of Pharmaceutical, Chulalongkorn University, Bangkok, Thailand.*

Rho is a small molecular weight GTP-binding protein shuttling between the GDP-bound inactive form and GTP-bound active form. Rho-kinase is required for sustained ERK signaling and the consequent mid G1 phase induction of cyclin D1 in fibroblasts, as well as for stress fiber formation. We earlier reported that Balb/c3T3 cocultured with 25 μM arsenite and 25-100 μM apigenin (a potent flavone from Thunbergia laurifolia Linn.) could block the disruption of vinculin and actin expression induced by arsenite. Water extract of leaves of T. laurifolia was orally given to rats at 20, 200, 1000 and 2000 mg/kg/day for 180 days. Rat spleens showed FTIR shifts at amide I and phosphodiester bond when exposed to the extract at 1000 mg/kg/day. In addition, the water extract at 2000 mg/kg/day could also lead to the increase in spleen weight ($p<0.05$). Similar FTIR shifts were observed in the nerve dysfunction by tyrosine specific phosphorylation and tighter packing of phosphorylated proteins induced by botulinum neurotoxins. Since apigenin could inhibit *in vitro* effects of arsenite - which acts through EGFR signaling, Rho-kinase, and non-receptor tyrosine kinase - on actin disruption of fibroblast stress fiber observed on confocal microscope, effects of apigenin on spleens detected by FTIR could possibly be explained by the downstream effect of serum response factor (SRF)-dependent Rho A and Rho kinase leading to cellular activation and regulation of smooth muscle gene-expression. This study suggests that FTIR could be proposed as a tool used to screen/study the Rho GTPase regulation of transcription factor SRF and toxicant activated stress-signaling pathways.

1169 DOES THALIDOMIDE ALTER HAVE THE ABLITIY TO ALTER PROTEIN KINASE C SIGNALING TRANSDUCTION PATHWAY.

T. N. Ezell. *Biology, Morgan State University, Baltimore, MD.*

In immune cells, calcium, Protein Kinase A, and Protein Kinase C (PKC) pathways are highly implicated in the expression of cytokine genes. Previous studies have shown that the over production of TNF-alpha, produced by immune cells is involved in inflammatory conditions as such septic shock, leprosy, tuberculosis, and rheumatoid arthritis. Studies have shown that thalidomide, an anti TNF- alpha agent, is know to decrease cell proliferation and decrease cell-cell adhesion potential treatment for inflammatory disorders. Yet little is known about its cellular and molecular mechanism of action. The present study explores whether thalidomide alters the PKC pathway in human monocytic cells, U937 cells and adenocarcinoma cells, HeLa cells . U937 cells at a concentration of 2 x 106 cells/ml were supplemented with RPMI 1640 medium without serum and harvested in 6 well culture plates. Next, the cells were transfected with EGFP-PKC vector using the transfection reagent DMIR-C. HeLa cells at a concentration of 1 x 105 cells/ml were supple-

mented with meduim without serum and harvested in 12-well culture plates. The HeLa cells were transfected with EGFP-PKC vector using the transfection reagent Lipofectin and Plus Reagent. After 72 hours, the transfected cells were isolated by adding 50 ng/ml of neomycin to each culture well and a stable transfected cell line was established. The stable cell lines were then treated with 0, 10, 25, and 50 ug/ml thalidomide and incubated for 24, 48, and 72 hours. The cells were then observed, photographed using fluorescence microscopy and analyzed by flow cytometry to measure alterations in PKC expression. Thalidomide treated cells exhibited a dose dependent decrease in EGFP-PKC expression.This data suggest PKC maybe involved in thalidomide-induced antinflammatory actvities. (Supported by RCMI grant # G12RR017581).

1170 PCB ACTIVATION OF EXTRACELLULAR SIGNAL-REGULATED KINASES (ERKS) IS MEDIATED THROUGH EPIDERMAL GROWTH FACTOR RECEPTOR AND INTRACELLULAR CALCIUM.

B. V. Madhukar, G. Chen and B. F. Wood. *Pediatrics/Human Development, Michigan State University, East Lansing, MI.*

Signal transduction pathways play a central role in cell proliferation, differentiation and many physiological processes. Aberrant activation of signaling cascades may lead to failure of growth control mechanisms and neoplastic growth. In ongoing studies with polychlorinated biphenyls (PCBs) we observed that orthochlorine substituted congeners activated the extracellular signal-regulated kinases (ERKs) a subgroup of mitogen activated protein kinase (MAPK) family of signal transduction kinases in liver epithelial cells. In this study, we examined the potential mechanisms of ERK activation 2, 2', 4, 4'-tetrachlorobiphenyl (TCB) a diothochlorinated PCB. Using pharmacological inhibitors and immunoblot analysis we examined the upstream targets of PCB interaction leading to activation of ERKs. TCB caused a 6- to 8-fold increase in liver cells after one-hour treatment at 20 µ?M. Pre-treatment of the liver cells with an inhibitor of ERK kinase University-0126, or a tyrosine kinase inhibitor genistein, or compound-56 an inhibitor of epidermal growth factor receptor activity, or an antagonist of calcium release from endoplasmic reticulum TMB-8 prior to TCB exposure abrogated ERK activation by more than 90%. ERK activation by the TCB was not inhibited by bisindolylmaleimide (inhibitor of protein kinase C) or by the src-kinase inhibitor PP1. These results suggest that ERK activation by TCB is mediated by activation of EGF receptor tyrosine kinase. We believe that the EGF receptor activation is achieved *via* activation of a calcium regulated tyrosine kinase (supported by NIEHS/Superfund grant no. ES-04911 and a Philip Morris Research grant).

1171 CALCIUM REGULATION OF BRAIN MITOCHONDRIAL CALCIUM / CAMP RESPONSE ELEMENT BINDING PROTEIN (CREB).

R. A. Schuh, T. Kristian, C. Chinopoulos and G. Fiskum. *Anesthesiology, University of Maryland Baltimore, Baltimore, MD.*

Calcium-mediated signaling regulates nuclear gene transcription by CREB *via* calcium-dependent kinases and phosphatases. This study tested the hypothesis that CREB is also present in mitochondria, interacts with the mitochondrial genome, and is subject to calcium-dependent modulation of its phosphorylation state. Antibodies to CREB and phosphorylated CREB (pCREB) were used to demonstrate the presence of both in highly purified mitochondrial fractions isolated from rat brain. Gel mobility shift assays using oligonucleotides containing a partial CREB response element (CRE) located in the displacement loop regulatory region of the mitochondrial DNA (mtDNA) heavy strand indicated that mitochondrial extracts contain proteins capable of binding to this site. When energized mitochondria were exposed to physiological levels of Ca2+, pCREB levels were reduced while total CREB remained constant. Additionally, the calmodulin antagonist calmidazolium decreased pCREB levels. Conversely, EGTA, okadaic acid, cyclosporin A and FK506 treatment increased pCREB levels suggesting phosphatase activity plays a role in mitochondrial pCREB/CREB levels. Exposure of mitochondria to the pore-forming molecule alamethicin resulted in osmotic swelling, disruption of the outer membrane, and a reduction in CREB but not pCREB mitochondrial immunoreactivity. These results suggest that pCREB is located exclusively in the mitochondrial matrix or inner membrane while CREB resides in the intermembrane space. Thus, a calcium-dependent phosphatase such as calcineurin may regulate the intramitochondrial distribution and possibly the transcriptional activating activity of mitochondrial pCREB/CREB. This form of regulation may affect mitochondrial gene expression following acute brain injury as early, post-ischemic pCREB dephosphorylation and delayed pCREB hyperphosphorylation were observed in brain mitochondria isolated following global cerebral ischemia and reperfusion. (Supported by NIH NS34152, DAMD 17-99-1-9483 and AHA 0256359U)

1172 COX-2 INDUCTION BY BOVINE TYPE I COLLAGEN IN MACROPHAGES VIA C/EBP AND CREB ACTIVATION.

M. Cho, Y. Cho, G. Lee and S. Kim. *College of Pharmacy, Seoul National University, Seoul, South Korea.*

Bovine type I collagen (Col-I) is utilized for medical purposes such as cosmetic surgery and wrinkle removal. Cyclooxygenase-2 (COX-2) plays roles in pathophysiological processes including inflammation and tumorigenesis. This study examines the effects of Col-I on the COX-2 expression in macrophages and the signaling pathways responsible for the enzyme induction. Col-I increased the levels of COX-2 protein and mRNA in Raw264.7 cells in a time- and concentration-dependent manner. Treatment of cells with Col-I increased protein binding to the CCAAT/enhancer binding protein (C/EBP) binding oligonucleotide. Antibody supershift experiments revealed that C/EBP DNA binding activity induced by Col-I depended largely on C/EBPβ and C/EBPδ. Col-I caused C/EBPβ translocated to the nucleus and overexpression of the dominant-negative mutant form of C/EBP abolished COX-2 induction by Col-I. Col-I increased binding of other transcription factors CREB and NF-κB to DNA. Inhibition of focal adhesion kinase (FAK) or downstream PI3-kinase and p70S6 kinase by specific chemical inhibitors prevented COX-2 induction by Col-I and also C/EBP and CREB from binding to their consensus DNA oligonucleotides. Experiments using chemical inhibitors or dominant negative mutant vectors showed that the MAP kinase pathways including p38 kinase and ERK1/2, but not JNK1, simultaneously regulated COX-2 induction by Col-I. This was in agreement with inhibition of Col-I-inducible C/EBP and CREB DNA binding in the cells concomitantly treated with SB203580 and PD98059. These results provide evidence that Col-I induces COX-2 in macrophages and that the multiple cell signaling pathways involving FAK, PI3-kinase and MAP kinases regulate COX-2 induction by Col-I *via* C/EBPβ and CREB activation.

1173 GSTA2 GENE REPRESSION BY GR-ASSOCIATED SMRT BINDING TO ACTIVATING C/EBPβ AND NRF2.

S. Ki, I. Cho, D. Choi, M. Cho and S. Kim. *College of Pharmacy, Seoul National University, Seoul, South Korea.*

The expression of glutathione S-transferase (GST) gene, whose induction accounts for cancer chemoprevention, is regulated by activation of CCAAT/enhancer binding protein-β (C/EBPβ) and NF-E2-related factor-2 (Nrf2). The present study investigated the repressing effect of ligand-bound glucocorticoid receptor (GR) on C/EBPβ- and Nrf2-mediated induction of the GSTA2 gene and explored the mechanistic basis for the gene repression. Dexamethasone (10-100 nM) that activates GR persistently inhibited constitutive GSTA2 expression in H4IIE cells. Induction of GSTA2 by oltipraz or tert-butylhydroquinone (t-BHQ) was also markedly repressed by dexamethasone. Inhibition of the constitutive and inducible GSTA2 expression by dexamethasone was abolished by treatment of cells with a GR antagonist RU486. Dexamethasone-GR activation did not suppress nuclear translocation of C/EBPβ or Nrf2 nor their DNA binding activities induced by oltipraz or t-BHQ, but suppressed inducible luciferase reporter-gene activity in the cells transfected with the -1.65 kb flanking region of the GSTA2 gene. Deletion of the distal glucocorticoid response element (GRE) abolished dexamethasone inhibition of the GSTA2 gene induction. Immunoprecipitation-immunoblot and GST pulldown assays revealed that silencing mediator for retinoid and thyroid-hormone receptors (SMRT), a corepressor recruited to steroid-GR complex, bound to activating C/EBPβ and Nrf2. Decreases in the constitutive and inducible GSTA2 promoter-luciferase activities by transfection of cells with the plasmid encoding SMRT directly evidenced the functional role of SMRT in GSTA2 repression. These results demonstrate that dexamethasone antagonizes C/EBPβ- and Nrf2-mediated GSTA2 gene induction *via* ligand-GR binding to the GRE and that steroid-mediated GSTA2 repression involves inactivation of C/EBPβ and Nrf2 by SMRT recruited to steroid-GR complex.

1174 REGULATION OF NRF-2 BY GLUTATHIONE AND THIOREDOXIN.

J. M. Hansen[1], W. H. Watson[2] and D. P. Jones[1]. *[1]Biochemistry, Emory University, Atlanta, GA and [2]Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.*

Nrf-2 is a redox-sensitive transcription factor implicated in the regulation of genes controlled by the antioxidant response element (ARE). While oxidative signals appear necessary for initial Nrf-2 activation in the cytoplasm, extensive oxidation would result in the loss of DNA binding in the nucleus. Reducing environments, which are largely maintained by glutathione (GSH) and other small biothiols, effectively block Nrf-2 activation. HeLa cells transfected with an ARE4-Luciferase reporter were treated with BSO to deplete GSH and showed a 93% increase in Nrf-2 activity, while N-acetylcysteine (NAC) and glutathione monoethylester (GSHM)

treatments decreased Nrf-2 activity significantly. The addition of tert-butyl hydroquinone (TBHQ), a Nrf-2 activator, increased Nrf-2 activity by 20%. BSO pretreatment prior to TBHQ treatment resulted in a 173% increase in Nrf-2 activity, but NAC and GSHM pretreatments were not significantly different from non-TBHQ treated cells. Immunocytochemistry verified BSO stimulation and NAC and GSHM inhibition of Nrf-2 activation and translocation to the nucleus. Thioredoxin-1 (TRX1) expression vectors, both the wild type and the active site mutant (C35S), also affected Nrf-2 activity. TRX1 increased Nrf-2 activity by 70% in unstimulated cells, while C35S TRX1 decreased activity by 44%. The addition of TBHQ to TRX1 transfected cells resulted in a 95% increase as compared to control, but there was no change in cells transfected with C35S TRX1. Because TRX1 overexpression does not inhibit Nrf-2 activity it indicates that TRX1 regulation most likely occurs at a step other than activation in the cytoplasm. Transfection with a TRX1 construct containing a nuclear localization sequence potentiated Nrf-2 activity both with and without TBHQ by 220 and 140%, respectively, implicating TRX1 to play a specific role in the nucleus. These findings suggest Nrf-2 activation in the cytoplasm is primarily regulated by GSH while Nrf-2/DNA binding is primarily regulated by TRX1.

1175 *HMGA1A* BEHAVES AS A STRESS-REGULATED TRANSCRIPTION FACTOR IN VASCULAR SMOOTH MUSCLE CELLS.

L. R. Chapman[1, 2], C. D. Johnson[1, 2], M. H. Falahatpisheh[1, 2] and K. S. Ramos[1, 2]. [1]*Biochemistry and Molecular Biology, University of Louisville, Louisville, KY and* [2]*Center for Genetics and Molecular Medicine, University of Louisville, Louisville, KY.*

High mobility group A1a (HmgA1a) is a non-histone chromosomal protein that preferentially binds in a non-sequence specific fashion to the minor groove of A-T rich DNA *via* highly conserved motifs called "A-T hooks". HmgA1a functions as an architectural transcription factor that regulates gene expression by controlling the assembly of stereo-specific multi-protein complexes in the regulatory region of target genes. The interaction of HmgA1a with DNA removes the intrinsic bend of the double helix to allow binding of transcription factors in the opposing major groove. Overexpression of *Hmg* genes correlates with neoplastic transformation and increased metastatic potential in cancer cells and chromosomal rearrangements in benign tumors of human mesenchymal cells. Here we report that oxidative injury by benzo(a)pyrene (BaP) modulates expression of the *HmgA1a* gene in vascular smooth muscle cells (vSMCs). In these experiments, murine vSMCs were established in serial culture using standard procedures and challenged with 3 μM BaP for 1, 2, 8, 16 and 24 hr. At the end of the desired treatments, cultures were processed for measurements of *HmgA1a, HmgA2, HmgB1, HmgB2, HmgB3 and HmgN1* gene expression by Real Time PCR. BaP increased *HmgA1a* expression in a time-dependent manner with a greater than seven-fold increase in mRNA levels observed by 8 hr and a return to control levels by 16 hr. These findings are consistent with recent DNA microarray experiments in the laboratory showing that the adaptive response of vSMCs to BaP injury is associated with redox-independent increases in the expression of the *HmgA1a* gene. Only transient increases in the expression of the *HmgB2* and *HmgB3* genes were observed by 2 hr in BaP-treated cells. These findings implicate HmgA1a as a stress-regulated architectural transcription factor in the response of vSMCs to atherogenic stimuli. (Supported by NIH grants ES04849, ES09106 to KSR, ES012117 to CDJ and ES012542 to MHF).

1176 GROWTH FACTOR-INDUCED REACTIVE OXYGEN SPECIES OXIDIZE GLUTATHIONE AND THIOREDOXIN-1 IN A DIFFERENTIAL MANNER.

P. J. Halvey[1, 3], W. H. Watson[2], Y. Go[1], J. M. Hansen[1] and D. P. Jones[1]. [1]*Biochemistry, Emory University, Atlanta, GA,* [2] *Environmental Health Sciences, Johns Hopkins University, Baltimore, MD and* [3]*Biochemistry, NUI Galway, Galway, Connacht, Ireland.*

During oxidative stress conditions, the redox state of specific thiol redox couples is altered in proportion to the applied stress. Glutathione (GSH) and thioredoxin-1 (TRX1) are two such couples. GSH is the most abundant non-protein thiol, while TRX1 is a 12kDa oxidoreductase involved in regulation of redox-dependent proteins. Epidermal growth factor (EGF) signaling leads to the production of reactive oxygen species (ROS). Here we examine the effects of EGF-signaling on GSH and TRX1 in human keratinocytes (HaCaT). Using a dichlorofluorescein assay, we observed a 22% increase in ROS in EGF (200 ng/ml) treated cells after five minutes. GSH and the disulfide form (GSSG) were quantified by HPLC. EGF did not cause any significant oxidation of the GSH/GSSG redox couple compared to untreated controls. A Redox Western blot methodology for quantification of TRX1 redox state revealed a more robust oxidation of cytoplasmic TRX1 compared with nuclear TRX1 after EGF treatment. The redox potential of cytoplasmic TRX1 increased by 15mV, whereas a much smaller increase was noted in the nucleus (<2mV) at two minutes EGF treatment. Nuclear TRX1 also recovers faster than cytoplasmic TRX1. Nuclear TRX1 redox state returned to control levels after 10 minutes, but cytoplasmic TRX1 redox state remained more oxidized than untreated controls even after 30 minutes. Taken together these results suggest that TRX1 is a specific target for oxidation during EGF-signaling, while the GSH pool is not. The differential response of nuclear and cytoplasmic TRX1 to EGF-induced ROS has implications for growth factor signaling. Low nuclear TRX1 oxidation may confer a protective effect on the nucleus during proliferation, thus insuring that all TRX1-dependent processes function normally.

1177 A ROLE FOR P38 MAPK IN ROS-INDUCED ONCOTIC CELL DEATH IN RENAL CELLS.

J. Dong, S. Ramachandiran, S. S. Lau and T. J. Monks. *Department of Pharmacology & Toxicology, University of Arizona Health Sciences Center, Tucson, AZ.*

Reactive oxygen species (ROS) are associated with a variety of human diseases, and toxicities mediated by redox-active chemicals and/or their metabolites. 2, 3, 5-*tris*-(glutathion-S-yl)hydroquinone (TGHQ), a reactive metabolite of the nephrotoxicant hydroquinone, induces the ROS-dependent activation of p38 mitogen activated protein kinases (MAPK) in renal proximal tubule epithelial cells (LLC-PK_1). TGHQ-induced histone H3 phosphorylation precedes oncotic cell death following MAPK activation. Cell death and histone H3 phosphorylation are both attenuated by inhibition of p38 MAPK (SB202190). To further investigate the role of p38 MAPK in TGHQ-induced ROS-dependent cell death, we transfected LLC-PK_1 cells with dominant negative p38 MAPK expressing plasmid (pcDNA3-DNp38), and functional p38 MAPK expressing plasmid (pcDNA3-p38). Although pcDNA3-DNp38 transfected cells express identical levels of constitutive p38 MAPK as pcDNA3-p38 transfected cells, TGHQ fails to induce p38 MAPK phosphorylation only in the pcDNA3-DNp38 transfected cells. TGHQ also fails to induce phosphorylation of MAPK activated protein kinase-2 (MK-2), a kinase activated by p38 MAPK, only in pcDNA3-DNp38 transfected cells, indicating loss of function of p38 MAPK. In pcDNA3 and pcDNA3-p38 transfected LLC-PK_1 cells, Hsp27, a downstream effector of MK-2, is intensively phosphorylated after TGHQ treatment, whereas in pcDNA3-DNp38 transfected cells, TGHQ fails to induce Hsp27 phosphorylation. Immunocytochemical staining of p-Hsp27 revealed association of p-Hsp27 with cell death in TGHQ-treated LLC-PK_1 cells. Thus, p38 MAPK activation by TGHQ leads to activation of two downstream signaling cascades: histone H3 and Hsp27 phosphorylation, which have in common the potential ability to remodel chromatin. Because inappropriate chromatin condensation has been linked to TGHQ-induced cell death, p38 MAPK activation and downstream signaling factors may play important roles in ROS-mediated oncotic cell death in LLC-PK_1 cells. (DK59491)

1178 EFFECTS OF BENZOQUINONE (BQ) ON CELL PROLIFERATION VIA ERK/MAPK SIGNALING PATHWAY ACTIVATION AND ROS PRODUCTION.

R. Ruiz-Ramos[1], M. E. Cebrian[1] and E. Garrido[2]. [1]*Toxicology Section, CINVESTAV IPN, Mexico City, D.F, Mexico and* [2]*Genetics and Molecular Biology, CINVESTAV-IPN, Mexico City, D.F, Mexico.*

Benzene (BZ) is a hydrocarbon present in the atmospheric air, and is a common component of gasoline, tobacco smoke and other daily used petroleum derivative products as cosmetics, medicines, etc. In the last few years, BZ has been associated to blood and bone marrow pathologies, expressed as lymphocytopenia, pancytopenia, aplastic anemia and acute myelogenous leukemia. BZ metabolism produces BQ as well as free radicals and other reactive oxygen species (ROS), which are believed to be involved in the activation of signaling pathways that regulate transcription factors and the expression of genes that regulate proliferation, differentiation and apoptotic processes. BQ acute exposure induced cell growth, as measured by BrdU incorporation, in human promyelocytic HL-60 cells. This effect was related with increases in reactive oxygen species (ROS) levels, as measured by 2, 7-dichloro fluorescein diacetate oxidation. In addition, we detected ERK activation by using specific antibodies against phosphorylated ERKs proteins, suggesting the potential involvement of this pathway in BZ/BQ-induced proliferation.

1179 INDUCTION OF ANTIOXIDANT AND DETOXIFICATION RESPONSE WITH H_2O_2 IN CARDIAC CELLS.

S. E. Purdom[1], J. A. Johnson[2] and Q. M. Chen[1, 3]. [1]*Graduate Program in Genetics, University of Arizona, Tucson, AZ,* [2]*School of Pharmacy, University of Wisconsin, Madison, WI and* [3]*Department of Pharmacology, University of Arizona, Tucson, AZ.*

Oxidants have been shown to contribute to the toxicity of various environmental toxicants and to a number of diseases including heart disease. The two major cell types in the heart are cardiomyocytes and fibroblasts. While cardiomyocytes de-

velop hypertrophy, fibroblasts contribute to fibrosis during heart injury. We tested the response of these two cell types to oxidants by analyzing their gene expression profiles and identifying the transcription factors responsible for the changes in gene expression. Microarray analyses using Affymetrix chips containing 20K genes found that a sublethal dose of H_2O_2 (100 μM) caused about 1% of genes to increase expression to 2-fold or higher in cardiomyocytes and cardiac fibroblasts. Among those induced are antioxidant and detoxification genes containing the antioxidant response element (ARE) in their promoters, such as NAD(P)H:quinone oxidoreductase-1 (NQO1) and heme oxygenase-1 in cardiomyocytes and NQO1 and glutathione *S*-transferase in fibroblasts. ARE promoter-luciferase reporter assays demonstrated that the ARE is activated in an H_2O_2 dose-dependant manner in cardiomyocytes. The peak activation of ARE, an average of about 5 fold, occurred at 4 hrs post 10-min treatment with 100 μM H_2O_2. This level of activation was comparable to the positive control *tert*-butylhydroquinone. No induction was detected with a luciferase reporter plasmid containing an empty or a mutant ARE promoter after H_2O_2 treatment. NF-E2-related factor 2 (Nrf2) is a basic leucine zipper transcription factor that can bind to the ARE. Expression of dominant-negative Nrf2 abolished ARE activation by H_2O_2. A dominant negative form of c-Jun, which is thought be a dimerization partner of Nrf2 in certain experimental systems, did not affect ARE activation by H_2O_2. These data suggest that oxidants can induce survival or adaptive responses by activating the expression of antioxidant and detoxification genes in cardiomyocytes and cardiac fibroblasts.

1180 DIFFERENTIAL REGULATION OF THE NRF2- AND AP-1-RESPONSE ELEMENTS BY 4-HYDROXY-2-NONENAL, t-BUTYLHYDROQUINONE AND PHORBOL 12-MYRISTATE 13-ACETATE IN CULTURED HEPATIC STELLATE CELLS.

J. F. Reichard and D. R. Petersen. *Toxicology, University of Colorado Health Sciences Center, Denver, CO.*

Stellate cell activation is a central event in liver fibrosis characterized by increased extracellular matrix production and cell proliferation. Oxidative stress has been implicated as an activator of the bZIP transcription factors Nrf2 and AP-1 through shared kinase signaling pathways that purportedly mediate stellate cell activation. The present study examined Nrf2- and AP-1-mediated gene expression following oxidative stress in cultured hepatic stellate cells. Reporter constructs generated by mutation of the AP-1- and Nrf2-binding elements of the human nqo1 promoter demonstrated that the Nrf2 element was fully responsible for mediating inducible gene expression in response to the prooxidants 4-hydroxy-2-nonenal and tert-butylhydroquinone. Contrastingly, the AP-1 element was incapable of mediating inducible expression in response to either oxidative stress or PMA, despite the presence of Fos- and Jun-related nuclear proteins. When present together, the Nrf2 and AP-1 elements acted synergistically to mediate high-level basal promoter activity. Identification of AP-1 family proteins revealed heterodimeric binding complexes consisting predominantly of Fra-1, Fra-2, JunB and JunD proteins, with little c-Fos, FosB or c-Jun. With exception of a 2-fold increase in nuclear c-Fos following PMA treatment, neither electrophiles nor PMA treatment appreciably altered the presence of AP-1 proteins. Taken together, these data demonstrate that, although overlapping kinase-signaling pathways activates AP-1 and Nrf2 transcription factors, oxidative stress discretely activated Nrf2, while AP-1 was insufficient to convey independent reporter induction. This observation is significant in context of the physiologic importance attributed to the AP-1 in mediating essential profibrogenic gene expression during liver fibrogenesis.

1181 INDUCTION OF CYCLOOXYGENASE-2 BY H_2O_2 AND CORTICOSTERONE IN CARDIOMYOCYTES.

H. Sun and Q. M. Chen. *Pharmacology, University of Arizona, Tucson, AZ.*

Heart disease is the number one cause of natural death worldwide. A large body of evidence has indicated a role of oxidants in myocardial infarction and other types of heart disease. Although cyclooxygenase-2 (COX-2) can contribute to an inflammatory response, induction of COX-2 has been shown to be cytoprotective in cardiomyocytes *in vitro* and *in vivo*. Oxidants and the stress hormone corticosterone are thought to be detrimental to the heart. However, our gene array work has found COX-2 and a number of cytoprotective genes induced by these two stimuli in cardiomyocytes. An increase of COX-2 mRNA and protein was detected with either oxidants or corticosterone treatment by RT-PCR and Western blot. The optimal dose for COX-2 induction was 50-100 μM for H_2O_2 and 0.25-1 μM for corticosterone. The peak increase of COX-2 protein occurred at 2-4 hour after 1 hour treatment with 100 μM H_2O_2 or at 32 hour in cells treated with 1 μM corticosterone. While the induction of COX-2 is transient and returned to basal level within 10 hours for H_2O_2, elevation of COX-2 is sustained for 3 days with corticosterone treatment. The p38 MAPK inhibitor SB202190 but not inhibitors of JNKs, ERKs or PI3K prevented H_2O_2 from inducing COX-2 elevation. In contrast, the glucocorticoid receptor antagonist mifepristone inhibited COX-2 induction by corticosterone. Using the TranSignal array technique to screen for activated transcription factors, we found that oxidants and corticosterone activated several transcription factors in common. The roles of these transcription factors in elevation of COX-2 expression are under investigation.

1182 INDUCTION OF METALLOTHIONEIN I BY PHENOLIC ANTIOXIDANTS REQUIRES METAL-ACTIVATED TRANSCRIPTION FACTOR 1 (MTF-1) AND ZINC.

Y. Bi[1], R. D. Palmiter[2], K. M. Wood[1] and Q. Ma[1]. *[1]HELD/CDC, TMBB/NIOSH, Morgantown, WV and [2]Biochemistry, Howard Hughes Medical Institute, University of Washington School of Medicine, Seattle, WA.*

Phenolic antioxidants, such as tert-butyl-hydroquinone (tBHQ), induce metallothionein (Mt1) gene expression. Induction of Mt1 mRNA correlates with oxidation-reduction functions of the antioxidants. Here we analyzed the biochemical pathway of the induction. Induction depends upon the presence of MTF-1, a transcription factor that is required for metal-induced transcription of Mt1, but does not require Nrf2, a tBHQ-activated CNC bZip protein that is responsible for regulating genes encoding phase II drug-metabolizing enzymes. Moreover, tBHQ induces the expression of MRE-bGeo, a reporter gene driven by five metal regulator elements (MREs) that constitute an optimal MTF-1 binding site. Reconstitution of Mtf1-null cells with MTF-1 restores induction by both zinc and tBHQ; however, reconstitution with only the DNA-binding domain of MTF-1 fused to VP16 activation domain allows in induction by zinc but not by tBHQ. Unlike activation of phase II genes by tBHQ, induction of Mt1 expression does not occur in the presence of EDTA, when cells are cultured in zinc-depleted medium, or in cells with reduced intracellular 'free' zinc due to overexpression of ZnT1, a zinc-efflux transporter, indicating that induction requires zinc. These findings establish that phenolic antioxidants activate Mt1 transcription by a zinc-dependent mechanism that involves MTF-1 binding to MREs.

1183 PI3-KINASE REGULATES CA^{2+}-DEPENDENT NRF2 TRANSLOCATION FROM THE CYTOPLASM TO THE PLASMA MEMBRANE PRIOR TO ITS NUCLEAR TRANSLOCATION.

K. Kang[1, 2] and S. Kim[2]. *[1]College of Pharmacy, Chosun University, Gwang-Ju, South Korea and [2]College of Pharmacy, Seoul National University, Seoul, South Korea.*

Expression of phase II detoxifying genes is regulated by NF-E2-related factor 2 (Nrf2)-mediated antioxidant response element (ARE) activation. Phosphatidylinositol 3-kinase (PI3-kinase) regulates nuclear translocation of Nrf2 through the rearrangement of actin microfilaments in response to prooxidants. We further studied the role of PI3-kinase signaling pathway in the rise of cellular Ca^{2+} and the translocation of Nrf2 to the plasma membrane. Immunocytochemistry and subcellular fractionation analyses revealed that pretreatment of cells with tert-butylhydroquinone (t-BHQ) inhibited the translocation of Nrf2 to the plasma membrane, whereas treatment of cells immediately after addition of PI3-kinase inhibitors (control experiment) caused Nrf2 to migrate to the plasma membrane before nuclear translocation. t-BHQ treatment stimulated a rise in $[Ca^{2+}]i$, which was prevented by PI3-kinase inhibition. Chelation of cellular calcium suppressed Nrf2 migration to the plasma membrane. Conversely, A23187 a calcium ionophore, allowed cytoplasmic Nrf2 to move to the plasma membrane in the cells exposed to both t-BHQ and PI3-kinase inhibitor. Immunoblot analysis confirmed that either PI3-kinase inhibition or Ca^{2+} chelation prevented GSTA2 induction by t-BHQ. These results demonstrated that the PI3-kinase pathway regulates Ca^{2+}-dependent translocation of Nrf2 from the cytoplasm to the plasma membrane prior to its nuclear translocation.

1184 NICOTINE USES T CELL ANTIGEN RECEPTOR-INDEPENDENT AND -DEPENDENT SIGNALING PATHWAYS TO AFFECT INFLAMMATORY AND ADAPTIVE IMMUNE RESPONSES.

N. C. MISHRA, S. Razani-Boroujerdi, S. Singh, R. Langley, R. Kalra, J. C. Pena-Philippides and M. L. Sopori. *Immunology Program, Lovelace Respiratory Research Institute, Albuquerque, NM.*

Development of adaptive T cell immunity requires activation of the T cell antigen receptor (TCR), leading to increased intracellular Ca2+ levels ([Ca2+]i), T cell proliferation, and differentiation, while activation (nuclear translocation) of the transcription factor NF-κB is the hallmark of an inflammatory response. Activation of TCR through ligation by anti-TCR antibodies, increases Ca2+ and nuclear localization of NF-κB. Chronic exposure to cigarette smoke/nicotine suppress both

adaptive and inflammatory responses. Immunosuppression by nicotine is causally associated with constitutive activation of protein tyrosine kinases (PTKs) and the impaired Ca2+ response. We now show that chronic NT treatment of rats *via* subcutaneously implanted Alzet pumps constitutively activates the Src-like PTKs, Fyn and Lck, and nuclear localization of the transcription factor NF-κB in T cells. Mammalian T cells have α7-nicotinic acetylcholine-like receptors, and exposure of rat T cells or the human T cell line (Jurkat cells) to nicotine or anti-TCR antibodies activates Fyn kinase activity, increases the intracellular Ca2+ concentration ([Ca2+]i), and promotes nuclear localization of NF-κB. However, neither anti-TCR antibodies nor nicotine treatment stimulate Fyn kinase or the Ca2+ response in CD3-ζ or Lck deficient Jurkat cells, indicating that effects of nicotine on the Ca2+ response in T cells requires a functionally intact TCR. Interestingly, in the mutant Jurkat cells, nicotine promoted the activation of ERK and nuclear localization of NF-κB. These results strongly suggest that while activation of TCR induces both Ca2+ and NF-κB responses, nicotine may affects the two responses through different signaling pathways. Moreover, acute effects of nicotine on T cell function may be distinct from its chronic effects.

1185 THE EFFECTS OF THALIDOMIDE ON C-MYB SIGNALING.

N. A. Thadani[1] and L. M. Winn[1, 2]. *[1]Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada and [2]School of Environmental Studies, Queen's University, Kingston, ON, Canada.*

Despite its devastating birth defects thalidomide is being used therapeutically to treat a number of diseases, including leukemia. While thalidomide's mechanism of action remains unknown, it is postulated that exposure to thalidomide leads to increased ROS, which can inhibit NF-kappa B, which upregulates the anti-apoptotic kinase Pim-1. Pim-1 signaling involves the transcription factor c-Myb, which is involved in the regulation of differentiation and apoptosis. We hypothesize that thalidomide acts by interfering with the c-Myb signaling pathway and ultimately leads to increased apoptosis. To investigate this hypothesis, human K-562 cells were transfected with a Myb-responsive luciferase reporter plasmid derived from the chicken mim-1 promoter, along with control DNA and a plasmid expressing c-Myb. Subsequently, cells were exposed to thalidomide (0-80 μg/ml) for 1 hour. Luciferase activities were then measured to evaluate c-Myb activity using a dual-luciferase protocol. Results showed that cells exposed to thalidomide (20-80 μg/ml) had significantly decreased c-Myb activity compared to cells exposed to the vehicle control. A one hour pre-incubation of these cells with either the anti-oxidative enzyme catalase (1600 units/ml), or the NF-kappa B inhibitor SN-50 (20 μM) prevented the decrease in c-Myb activity, suggesting a role for ROS and NF-kappa B in the c-Myb signaling pathway. In addition, western blot analysis on thalidomide exposed (20-80 μg/ml) untransfected cells showed a decrease in Pim-1 expression, but not in NF-kappaB expression. Futhermore, in cells transfected with a plasmid containing the c-myb gene we found that thalidomide (20-80 μg/ml) caused a decrease in phosphorylated c-Myb expression. These results suggest that the decrease in Pim-1 and phosphorylated c-Myb expression may be responsible for the observed decreases in c-Myb activity. This study supports the hypothesis that ROS, NF-kappa B and Pim-1 are factors in the thalidomide-induced changes on c-Myb, which may mediate thalidomide's toxicity and therapeutic action. Support:CIHR

1186 ESTABLISHMENT OF THE PUBLIC SAMPLE BANK TO MONITOR A LONG-TERM TREND OF HUMAN EXPOSURE TO PERSISTENT ORGANIC POLLUTANTS (POPS).

K. Inoue, A. Koizumi, T. Yoshinaga, K. Harada and N. Saito. *Health Environmental Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan.* Sponsor: R. Reitz.

The Pops is a class of chemicals that are persistent in the environments. A large majority of current Pops is banned or under tight regulations but new classes of Pops, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are emerging. To evaluate risks of these chemicals and efficacy of regulations and management, long-term trends of human exposures to Pops should be evaluated.The aim of the present study is to establish a public sample bank for monitoring human exposures. Materials and methods:Samples-The samples were composed of three groups.Group I: Samples were composed of human serums and meals. Those samples were collected twice at 55 sites in Japan in the early 1980s and middle of 1990s. Serums were collected from those who donated meals. Group II: Samples are composed of breast milk and serums. These were collected local health centers. Three centers (Sendai, Akita and Yokote) donated the samples. Samples were collected from the late 1970s to 1990s. Group III: Samples are composed of current samples, consisting of breast milk, meals, and serums. Sampling sites will cover all over Japan. Results and Discussion: Group I samples were composed of 1, 700 samples. These samples were suitable for long term biological monitoring for Pops. Group II samples were composed of 90 breast milk samples and 3, 000 serum samples. The group III is now under construction and its sampling sites will cover all over Japan. This will provide the basis of modern samples. These samples were stocked at -20C.Volumes of serum and meal samples were 1-2ml and 1kg. Therefore, we should develope analytical methods using trace amounts of samples for monitoring.(This project is supported grant-in aids by Research on Environmental Health, H15-KAGAKU-004).

1187 CHRONIC TOXICITY INCLUDING CARCINOGENICITY OF HPCDD OBEYS THE C X T = K PARADIGM.

K. K. Rozman[1, 3], M. Lebofsky[1] and D. M. Pinson[2]. *[1]Pharmacology, Kansas University Medical Center, Kansas City, KS, [2]Pathology, Kansas University Medical Center, Kansas City, KS and [3]Toxicology, GSF, Neuherberg, Germany.*

Prior to this study, the carcinogenicity of TCDD, but not of 1, 2, 3, 4, 6, 7, 8-heptachloro-dibenzo-p-dioxin (HpCDD), had been reported in great detail. It was expected that under conditions of continuous exposure the spectrum of chronic effects of HpCDD would be identical to those of TCDD even in single dose experiments because of the long half-life(314 days)of HpCDD. The hypothesis tested was that chronic toxicities of HpCDD also occur according to the c x t = k paradigm. A total of 338 female SD rats (180 days old, bw 270-310 g) were housed individually and provided feed and water ad libitum. HpCDD was administered as a single dose *via* oral gavage at 4 mL/kg in four dose rates at 12 hour intervals due to low solubility of the HpCDD in corn oil. The last dose rate was considered day 0 of each experiment. The doses and number of animals per group (in parenthesis) were: 0(36), 1.0(30), 2.5(30), 2.8(60), 3.1(30), 3.4(32), 3.8(30), 4.1(30), 5(30), and 10(30) mg/kg. Rats were allowed to live out their allotted life span. Each rat was necropsied and bone, kidneys, lungs, liver, GI-tract and heart were procured and stored in 10% neutral buffered formalin for histological analysis. Doses of 2.5 mg/kg and higher decreased life expectancy in a highly predictable fashion. Average life expectancy of control animals was 540 days (+ 180 days = day 0 of study). For survivors of the acute toxicity, the chronic c x t product was remarkably constant (1180 ± 52.6 mg/kg·day) with a mere 4.5% variability. In contrast, the lowest dose (1.0 mg/kg) prolonged the life of rats by about 2 months. Squamous cell carcinoma of the lungs was the most sensitive and most frequent (up to 73.3%) endpoint of toxicity. There was neither lung nor liver cancer at the lowest dose and overall cancer rate was reduced.

1188 COMPARISON OF OVERALL METABOLISM OF 1, 2, 3, 7, 8-PENTACHLORODIBENZO-P-DIOXIN (PECDD) IN CYP1A2 (-/-) KNOCKOUT (KO) AND C57BL/6N PARENTAL STRAINS OF MICE.

J. J. Diliberto[1] and H. Hakk[2]. *[1]PKB, ETD, NHEERL ORD, USEPA, Research Triangle Park, NC and [2]ARS, BRL, USDA, Fargo, ND.* Sponsor: L. Birnbaum.

The toxic dioxin congener, PeCDD, induces hepatic cytochrome CYP1A2 to which it subsequently binds. Metabolism of PeCDD is very limited. Whether it is a poor substrate for metabolizing enzymes or unavailable for metabolism due to its strong affinity to CYP1A2 is not known. Thus, we tested the hypothesis that sequestration of PeCDD by CYP1A2 makes PeCDD unavailable for metabolism that would readily occur in its absence. Male C57BL/6N mice which possess or lack the CYP1A2 gene were given orally 116 ug [14C]PeCDD/kg. After 4 da, mice housed in metabolism cages with separate collection of urine and feces every 24h were killed & tissues collected. Tissue deposition & overall metabolism in urine/feces were quantitated. Similar to previously reported studies, liver:fat ratios were different, 5.8 (C57BL/6N) vs 0.4 (KO). Slightly higher 14C-derived PeCDD levels were excreted in C57BL/6Ns feces at each time pt compared to KOs; similar levels were excreted in urine of both strains. Overall level of metabolism of PeCDD was determined as sum of 14C in 0-96h urine, non-extractable feces, & metabolites in extractable feces. Parental strain had greater overall metabolism than KO mice: 15.0% vs 7.8% dose, respectively. Lower overall metabolism in KO than parental strain is probably due to low hepatic retention & rapid redistribution of PeCDD into lipophilic tissues for storage, which made PeCDD unavailable to hepatic metabolizing enzymes. The present results provide a basis for understanding the pharmacokinetic behavior of toxic dioxins at low environmental exposures, levels that are insufficient to induce hepatic CYP1A2. In conclusion, the data contradicts the hypothesis but confirms that PeCDD has an inherently slow metabolism in mammals, perhaps *via* the inducible CYP1A1, 1A2, and 1B1 isozymes and/or non-P450 dependant mechanisms.(This abstract does not reflect USEPA.)

1189 DEVELOPMENT OF A PBPK MODEL FOR HEXACHLOROBENZENE IN THE CONTEXT OF ITO'S MEDIUM-TERM LIVER FOCI BIOASSAY.

Y. LU, M. Reddy, M. Lohitnavy, O. Lohitnavy, A. Ashley and R. S. Yang. *Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Colorado State University, Fort Collins, CO.*

Hexachlorobenzene (HCB) is an important environmental contaminant. Due to its high lipophilicity and persistence in the environment, human exposure to HCB has continued despite the curtailment of its usage. HCB is carcinogenic to laboratory

animals with the liver as one of the primary target organs. We have incorporated the study of the relationship between pharmacokinetics and pharmacodynamics into the Ito's medium-term liver foci bioassay. Thus, we describe here the development of a PBPK model for HCB in the context of Ito's bioassay protocol. The model was built based on two data sets: one from a single intravenous dosing study (Scheufler et al. 1984) and the other from a single oral gavage study (Yamaguchi et al. 1986). It was then modified to simulate the exposure conditions in the Ito's bioassay and verified with data from our laborotary. The PBPK model includes five compartments: blood, liver, fat and rapidly and slowly perfused tissues. As HCB can bind to red blood cells, the blood compartment was divided into red blood cells and plasma. The fat compartment was divided into two subcompartments, one with a higher perfusion rate and lower partitioning and the other with a lower perfusion rate and higher partitioning. Uptake of HCB was diffusion-limited in the fat compartments and flow-limited in the others. HCB was eliminated by metabolism in the liver and by passive transfer from plasma to the intestinal lumen. Reabsorption of HCB from the intestine was incorporated in the model. The resultant model adequately described the available data sets. This model may be linked with a clonal growth model to quantitatively describe the relationship between an external exposure to HCB and the consequent preneoplastic liver foci development. (Supported by NIOSH/CDC grant 1 RO1 OH07556 and NIEHS Training Grant 1 T32 ES 07321)

1190 TOXICITY AND RELATIVE POTENCY OF 1, 2, 3, 4, 6, 7-HEXACHLORONAPHTHALENE (PCN66) AND 1, 2, 3, 5, 6, 7-HEXACHLORONAPHTHALENE (PCN67)IN FEMALE SPRAGUE-DAWLEY RATS.

L. M. Fomby[1], M. Hejtmancik[1], D. Vasconcelos[1], A. Fuciarelli[2], M. Vallant[3], R. Chhabra[3], H. Toyoshiba[3], N. Walker[3] and M. Hooth[3]. *[1]Battelle Columbus, Columbus, OH, [2]Battelle Northwest, Richland, WA and [3]NIEHS, Research Triangle Park, NC.*

Polychlorinated naphthalenes (PCNs) have been shown to produce toxicity similar to 2, 3, 7, 8-tetrachlorodibenzo-ρ-dioxin (TCDD). PCN 66 and PCN 67 have been found in human liver, adipose, and breast milk samples. The purpose of this study was to determine the toxicity of PCN 66 and PCN 67 and to evaluate the potency relative to TCDD in female Sprague-Dawley rats. Formulations of PCN 66 or PCN 67 in corn oil:acetone (99:1) were administered by gavage at dosages of 0, 500, 1500, 5000, 50, 000, and 500, 000 ng/kg for up to 5 days a week for 12 days. TCDD in corn oil:acetone (99:1) was similarly administered by gavage at dosages of 1, 3, 10, 100, and 300 ng/kg. Rats receiving 500, 000 ng/kg of PCN 66 or PCN 67 failed to gain weight. Histopathologic changes were observed in the livers of rats receiving ≥ 100 ng/kg TCDD, 500 ng/kg PCN 66, and 50, 000 ng/kg PCN 67. Thymus atrophy was observed in rats receiving ≥ 10 ng/kg TCDD, 5000 ng/kg PNC 66 and 500, 000 ng/kg PCN 67. Significantly increased hepatic CYP1A1 activity was observed in rats receiving≥ 1 ng/kg TCDD, 500 ng/kg PCN 66, and 1500 ng/kg PCN 67. Significantly increased hepatic CYP1A2 activity was observed in rats receiving ≥ 10 ng/kg TCDD, 5000 ng/kg PCN 66, and 50, 000 ng/kg PCN 67. Likelihood ratio analysis indicated that the sigmoidal dose response curves (Hill model) for induction of CYP1A1 activity were not significantly different between each of the three compounds. Relative potency factors (RPFs) were calculated as the ratio of the ED50s of PCN to TCDD when all data were fit using common dose response parameters. Relative potency factors for PCN 66 and PCN 67 for increases in CYP1A1 activity were 0.00151 and 0.00036, respectively. These are comparable to RPFs calculated using previously published *in vitro* data. Based on these RPFs, PCN 66 and PCN 67 would not be predicted to contribute significantly to the human body burden of dioxin-like activity.

1191 ROLE OF THE AROMATIC HYDROCARBON RECEPTOR (AHR) IN CAUSING NEONATAL LETHALITY IN MICE EXPOSED TO COPLANAR HEXABROMOBIPHENYL (CHBB).

C. P. Curran, K. Miller, T. Dalton, M. Miller, H. G. Shertzer and D. W. Nebert. *Environmental Health, University of Cincinnati, Cincinnati, OH.*

Polybrominated biphenyls (PBBs) are part of a larger class of brominated fire retardants and exhibit toxic effects similar to those by polychlorinated biphenyls. PBBs were the primary component of a commercial product, Firemaster-BP6, which was accidentally mixed with cattle feed in Southern Michigan in 1973. Widespread contamination led to toxicity and many deaths among cattle, pigs and chickens, and neurological symptoms (somnolence, amnesia, confusion) among humans consuming farm products. To determine the cause of PBB neurotoxicity, we have studied two stereoisomers - coplanar 3, 3', 4, 4', 5, 5' hexabromobiphenyl (cHBB) and noncoplanar 2, 2', 4, 4', 6, 6' hexabromobiphenyl (ncHBB) - in C57BL/6J (B6, having the high-affinity AHR) and B6.D2-Ahrd (having the DBA/2J poor-affinity AHR in a B6 genetic background) mice. Pregnant dams were given either cHBB or ncHBB (100 mg/kg, i.p.) on GD5; although all pups appeared normal at birth, all cHBB-treated B6 died within 72 h. Using Hepa-1 cells containing stably transfected AHRDtk-LUC, which is activated when exposed to AHR ligands, we confirmed that cHBB and not ncHBB binds to the AHR. Further, cHBB-treated B6 fetal liver and brain at GD18 showed (*via* RT-PCR) induced CYP1A1 mRNA (cHBB-treated B6.D2-Ahrd and ncHBB-treated B6 and B6.D2-Ahrd did not), evidence that cHBB given on GD5 crosses the placenta and has a persistent effect 13 days later. Significant lethality occurred in cHBB-treated B6 newborns at doses as low as 2.5 mg/kg, whereas all cHBB-treated B6.D2-Ahrd and Ahr(-/-) and ncHBB-treated B6 and B6.D2-Ahrd survived until the end of the experiment (90 days) at the 100 mg/kg cHBB dose. No histopathology or increases in oxidative stress parameters were evident in the liver or brain of the GD18 fetuses or newborns that die 2-3 days later. We conclude that neonatal lethality of the cHBB-treated B6 mice is mediated by the AHR receptor, but the precise cause of death remains under investigation. -Supported, in part, by NIH grants P30 ES06096, R01 ES06321 and R01 ES08147.

1192 NEONATAL EXPOSURE TO PCB 180 AFFECTS THE PREPUBERTAL REPRODUCTIVE ORGAN DEVELOPMENT IN FEMALE RATS BY ANTIESTROGENIC ACIVITY.

G. Rhee[1], S. Kim[1], R. Lee[1], S. Kwack[1], K. Lim[1], H. Yhun[1], G. Lee[2], E. Jeung[2] and K. Park[1]. *[1]Specialized Toxicology, National Institute of Toxicological Research, KFDA, Seoul, South Korea and [2]Veterinary Medicine, Chungbuk National University, Chung-Ju, South Korea.* Sponsor: J. Hong.

Many environmental chemicals have hormonal activity, and thus can adversely affect the reproductive and developmental systems of wildlife and mammals including human. Polychlorinated biphenyls (PCBs) including 2, 2', 3, 4, 4', 5, 5'-Heptachlorobiphenyl (PCB 180) are environmental persistent chemicals and are now widespread in the world due to their high lipophilicity and chemical resistance, providing potential for encironmental exposure. This study investigated the developmental effect of neonatal exposure to PCB 180 in the prepubertal reproductive organ development and its action mechanism in Sprague-Dawley female rat. Neonatal exposure to PCB 180 at 1 to 5 days after birth caused reductions in uterine and ovarian wet weights at prepuberty (PND 18) and this effect was statistically significant at certain doses. In addition, meonatal treatment with PCB 180 led to decreases in serum E2 concentration and uterine and ovarian ERa mRNA expressions on PDN 18. These results suggest the antiestrogenic effect of this chemical on female reproductive system. Therefore, we examined the potential antiestrogenic activity of PCB 180 by immature rat uterotrophic and estrogen-reponsive calbindin-D9k gene expression assays. In the uterotrophic assay using 18-day old female rat, subcutaneous treatment with PCB 180 (0.1 mg/kg/day) for 3 days led to significantt decreases in absolute and relative uterine wet weights and inhibited the E2-induced weight increases. Northern blot analysis showed the reduction of calbindin-D9k mRNA expression in response to PCB 180 as well as E2. Our results indicated that PCB 180 affects the prepubertal development of reproductive system in female rat, and this effect may be due to its antiestrogenic activity.

1193 AROCLOR 1254 CHEMICAL MODEL FOR REYE'S SYNDROME.

K. Ebner[1, 2, 3]. *[1]09ZC, Abbott Labs, Abbott Park, IL, [2]Department of Pharmacol, Ohio St University, Columbus, OH and [3]Department Pharmacol/Toxicol, Mich St. University, East Lansing, MI.*

PCBs were tested in male Wistar/Sprague-Dawley rats for changes in hepatic mitochondria (Hm) NH_4^+ metabolism associated with RS. PCB (PCB vs C, $p<0.05$, 30-300 mg/kg-d in corn oil & 1-4 d) elicited fatty-enlarged liver, hypoglycemia with suppressed gluconeogenesis and high blood urea with increased ureagenesis after ip NH_4Acetate, urea excretion, and negative nitrogen balance with wasting. HM after PCBs *in vivo* exhibited 1) general ion leakiness to K^+, H^+, Cl^- & Acetate$^-$ in energy-dependent swelling or passive contraction, respiratory control & ATP synthesis with younger rats being more susceptible than older, larger rats; 2) HM (PCB vs fasting) carbamoylphosphate synthesis was stimulated with extra-M ATP but not ADP; 3) HCO_3^- (>5mM) inhibited sonicated HM (cp) synthesis; 4) sensitized octanoate (9 uM) inhibition (doses:0.03-300 mg/kg-d, 4d)of HM CP synthesis; and 5) altered HM NH_4^+-α-KG dismutase regulated CP synthesis after dietary nitrogen (8, 24 and 70%) adaptations. HM uncoupler SARs for hydroxylated PCBs (i.e., inner membrane leakiness) increased directly with chlorination, diazomethane treated PCBOHs were ineffective, and uncoupling was not dependent on planar configuration and pKa. Non-lytic ARO concentrations facilitated HM energy-dependent swelling, RC and transmembrane potential but potentiated PCBOH HM uncoupling. These finds support a pharmacodynamic interaction of environmental PCBs with their obligatory PCBOH metabolites to uncouple HM NH_4^+ regulation and gluconeogenesis leading to endocrine disruption and wasting

toxicity. The energy-linked transhydrogenase and reverse electron transport are the most likely uncoupling sites since they are more sensitive to uncoupling than F_1-ATPase and are physiologically poised to drive reductive amination through GDH and nitrogen assimilation and de novo amino acid, protein, and pyrimidines syntheses. Hyperammonemia is characteristic and a prognostic indicator of RS. The NH_4Acetate probe with biomarker responses could benefit as a RS diagnostic test to determine HM uncoupling of ammonia metabolism.

1194 EFFECTS OF PCB 126 ON LIVER ENZYMES AND THE THYROID AXIS.

T. L. Almekinder[2], J. L. Campbell[2], S. Muralidhara[2], J. V. Bruckner[2], D. C. Ferguson[3], M. Mumtaz[4], H. El-Masri[4] and J. Fisher[2]. *[1]Environmental Health Science, University of Georgia, Athens, GA, [2]Interdisciplinary Toxicology Program, University of Georgia, Athens, GA, [3]Physiology and Pharmacology, University of Georgia, Athens, GA and [4]Division of Toxicology, ATSDR, Atlanta, GA.*

PCB 126 is known to interfere with thyroid hormone (TH) homeostasis by inducing microsomal enzymes and increasing TH excretion. The aim of the present study is to evaluate the temporal effects of PCB 126 (3, 3', 4, 4', 5-pentachlorobiphenyl) on the thyroid system, as mediated by induction of liver cytochrome P450 (total P450 and CYP1A1) and UDP-glucuronosyltransferase. A pharmacodynamic model for PCB 126-induced effects on the thyroid system is also under development. Adult male Sprague-Dawley rats were administered a single oral bolus dose of 0. 7.5, 75, or 275 μg PCB 126/kg bw dissolved in corn oil. The rats were killed periodically over 59 days for evaluation. PCB 126 caused a dose-related decrease in body weight gain. Thyroid weights, normalized to body weight, increased in a dose- and time-related fashion compared to controls. Total P450 levels were elevated for 59, 22, and 5 days for the 275, 75, and 7.5 μg/kg dosage groups, respectively. Ethoxyresorufin-O-deethylase activity, a marker for CYP1A1, was increased for 35, 5, and 1 days in the 275, 75, and 7.5 μg/kg groups, respectively. Analysis of serum thyroid hormones is ongoing. These data demonstrate that PCB 126 interferes with thyroid homeostasis. Funded by ATSDR (Grant No: U61/ATU472105-01).

1195 BIOMEDICAL APPLICATION OF ACCELERATOR MASS SPECTROMETRY (AMS): PLACENTAL AND LACTATIONAL TRANSFER OF PCB 126 IN SPRAGUE-DAWLEY RATS.

B. Buchholz[2], S. Lee[1], M. B. Reddy[1], K. H. Liao[1], M. Lohitnavy[1], J. Vogel[2] and R. Yang[1]. *[1]Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO and [2]Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore, CA.*

In earlier pharmacokinetic studies in our laboratory, we had great difficulty quantifying PCB 126 by gas chromatography in both adult rats and fetuses/pups because: (1) PCB 126 uniquely accumulated in the adult liver; (2) The dose was limited because of high toxicity; and (3) Tissue samples available from fetuses/pups were too small. To overcome analytical limitations on perinatal pharmacokinetics of environmental contaminants with trace amounts in minute fetus or pup tissue samples, accelerator mass spectrometry (AMS) was used to measure 14C-PCB 126 in the tissues of pregnant/lactating rats and their fetuses/pups during perinatal developmental stages. AMS is a highly sensitive method which can quantify attomole levels of isotopically labeled compounds in milligram-sized tissue samples to a precision of <5%. In this study, 14C-PCB 126 (20 nCi, 10 ug/kg) was given to pregnant rats on gestational day (GD) 9 *via* oral gavage and the maternal and fetal/pup's tissues were collected on GD 14 and postnatal day (PND)2. Preliminary results showed that PCB 126 was successfully quantified in all tissues we collected and the data will be highly useful in building PBPK models for perinatal pharmacokinetics. We believe that this biomedical application of AMS will greatly aid our understanding of perinatal transfer of PCB 126 and other persistent environmental pollutants. (Supported by NIEHS R03 ES 10116, NIH RR 13461 and ATSDR Cooperative Agreement U61/ATU 881475. This work was performed in part under the auspices of the US Department of Energy, National Nuclear Security Administration by the University of California, Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.)

1196 DIETARY AROCLOR 1254-INDUCED CHANGES IN GLOBAL GENE EXPRESSION IN FISHER RATS.

T. M. Basford and J. C. Means. *Environmental Institute & Department of Chemistry, Western Michigan University, Kalamazoo, MI.*

Dietary intake is a primary route for biomagnification of environmentally persistent polyclorinated biphenyls (PCBs) within humans and animals, as these hydrophobic compounds partition to and accumulate in lipid tissues. Exposure to PCBs *in vivo*is associated with varied toxic responses such as alterations in blood and lipid biochemistry, immune suppression, neoplastic lesions, and impaired nerological function (Safe, 1993; Waring, et.al., 2001). To date, no microarray studies of the impact of PCB ingestion on global gene expression, at either acute or chronic levels, have been reported. Changes in expression of 8800 hepatic genes in Fisher 344 male rats were assessed by Affymetrix microarray analysis to identify important physiological pathways affected by dietary PCBs. Six week old animals were fed control food or food containing 10 ppm or 50 ppm Aroclor 1254 for 7 days (acute) and 84 days (chronic). Two arrays were prepared from liver mRNA for each dose and timepoint; a threefold or greater change in signal between control and treated genes was considered significant. Blood serum levels of lipids (cholesterol, triglycerides), bilirubin, and enzyme markers of liver health (ALT, AST, ALP, GGT) were also measured and correlated to changes in gene expression. Safe S., Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. Environ Health Perspect. 1993 Apr;100:259-68. Waring JF, et.al.Clustering of hepatotoxins based on mechanism of toxicity using gene expression profiles. Toxicol Appl Pharmacology 2001 Aug 15;175(1):28-42.

1197 MULTI-FACTOR ANALYSIS OF TEQ-EQUIVALENT AROCLOR AND TCDD TREATED RATS REVEALS DIFFERENTIAL GENE EXPRESSION PROFILES.

K. Illouz[1], A. Possolo[1], M. Zhao[1], S. B. Hamilton[2], G. M. Hoffman[3], Y. Huang[4], S. Goodwin[4], T. R. Sutter[4] and J. B. Silkworth[1]. *[1]Global Research Center, GE, Schenectady, NY, [2]Corporate Environmental Programs, GE, Fairfield, CT, [3]Huntingdon Life Sciences, East Millstone, NJ and [4]Feinstone Center for Genomic Research, University Memphis, Memphis, TN.*

Toxicogenomics has the great potential to enhance our understanding of PCB and TCDD tumorigenesis in rats. However, current methods of data analysis and interpretation present a challenge and require validation. This study was designed to compare current findings with previously conducted chronic studies that showed that female SD rats are more susceptible than males to Aroclor 1254 induced hepatotumorigenesis. TCDD and Aroclor doses were TEQ-matched to each other and to the previous study. Rats (n=4) were administered corn oil or 0.3 or 3.0 μg/kg TCDD or 0.6, 6.0, or 60 mg/kg Aroclor 1254 mg/kg po for 3 consecutive days. Day 4 hepatic cDNA was hybridized to Affymetrix RG U34 arrays. A novel multifactor, additive model was fitted to each gene probe set, with background adjusted, normalized, and log transformed perfect match intensities as the response variable. The model included treatment effects (corn oil, TCDD, Aroclor), gender effects, gender-treatment interactions, and probe affinity effects. Preliminary results for the control and highest doses indicated that, using cut-off criteria of ≥ 2-fold induction and p≤ 0.001, TEQ equivalent doses of TCDD or the Aroclor 1254 mixture induced 53 and 92 early response genes, respectively, with similar expression of most P450 1A genes between treatments. Eight genes were unique to TCDD, while 48 genes, including many Phenobarbital-related genes, were unique to the Aroclor mixture. At ≥1.5-fold and p≤ 0.001, CYP1B1 and CYP3A genes were induced only in females. There were 59 male specific genes responsive to Aroclor 1254 and 8 to TCDD. These preliminary results suggest that equivalent TEQ doses induce combinations of genes that are both strongly similar and strongly dissimilar between both genders and treatments.

1198 DIFFERENTIAL ACTIVATION OF GENES BY TCDD AND RELATED COMPOUNDS: ANALYSIS BY MICROARRAYS.

S. Khan[1], P. Beremand[2], T. Thomas[2] and S. Safe[1]. *[1]Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX and [2]Biology, Texas A&M University, College Station, TX.*

The toxic equivalency factor (TEF) approach has been used for risk assessment of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and related polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs). The toxic or dioxin equivalents mixture is the sum of the concentrations of the individual components times the functional potency (TEF) relative to TCDD. This methodology is based on the common aryl hydrocarbon receptor (AhR) mediated mechanism of action for the major toxic PCDDs, PCDFs and PCBs. The validity of this approach has been tested in Ah-responsive MCF-7 and other cancer lines treated with equipotent (for CYP1A1 induction) doses of 2, 3, 7, 8-TCDD, 1, 2, 3, 7, 8-pentaCDD, 2, 3, 4, 7, 8-pentaCDF, 3, 3, '4, 4', 5-pentachlorobiphenyl (PCB126) and the AhR active flavonoid, chrysin. All five compounds induced CYP1A1 and CYP1B1 gene expression and four other genes; however chrysin induced or decreased 800 and 826 genes, respectively, and the pattern significantly differed from the halogenated aromatics. 2, 3, 7, 8-TCDD, 1, 2, 3, 7, 8-pentaCDD, 2, 3, 4, 7, 8-pentaCDF and PCB126 increased/decreased 82/11, 114/10, 105/32 and 100/29 genes, respectively (> 2-fold increase or 0.5-fold decrease). Compared to TCDD as a reference compound the percentage of common genes induced by 1, 2, 3, 7, 8-

pentaCDD, 2, 3, 4, 7, 8-pentaCDF and PCB126 were 32, 28 and 27%, respectively, and the percentage of common genes decreased were 60, 11 and 10%, respectively. Moreover although all four congeners induced 22 common genes, approximately 40-60% of the genes induced by each compound were not induced by any of the other congeners. These results suggest that even for structurally-related AhR-active halogenated aromatics, their pattern of induced/decreased gene expression is highly compound specific. (Supported by NIEHS ES04917 and the American Chemistry Council)

1199 SEX-DEPENDENT LIVER GENE EXPRESSION PROFILES OF TCDD-TREATED SPRAGUE-DAWLEY RATS.

Y. Huang[1], S. Goodwin[1], K. Illouz[2], J. B. Silkworth[2], G. M. Hoffman[3] and T. R. Sutter[1]. *[1]Feinstone Center for Genomic Research, University of Memphis, Memphis, TN, [2]Global Research Center, General Electric, Schenectady, NY and [3]Huntingdon Life Sciences, East Millstone, NJ.*

In rats, the sensitivity to the hepatotumorigenic effect of TCDD (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin) is sex-dependent. Female rats are more sensitive than male rats. In order to determine whether sex-specific gene expression profiles are present in rats treated with TCDD, we used oligonucleotide micoarray analysis (Affymetrix RG U34A arrays) to examine global gene expression in rodent liver tissue samples. Four groups of rats (n=4 per group) were studied. Males and females were orally administered either corn oil or 3ug/kg/day TCDD for 3 days. We used the Affy and Multtest packages of Bioconductor (www.bioconductor.org) to compare RMA (robust multiple average), dChip and MAS5 procedures for calculating expression values. When using the Westfall-Young procedure for controlling Family-wise error rate for the multiple hypothesis tests (F-test), 67.7 percent genes in the gene list of RMA were also present in that of dChip. However, only 46.6 percent genes in the gene list of RMA were present in that of MAS5. When using RMA with Westfall-Young correction, we generated a gene list of 282 genes having an adjusted p-value smaller than 0.05. The gene expression levels across the 16 samples were used to generate a 282x4 data matrix based on the 4 groups for SOM (self organizing map) analysis (www.genome.wi.mit.edu). The 282 genes segregated into 12 partitions. The resulting SOM automatically revealed genes that displayed either and both sex- and treatment-dependent expression levels. The identification of these specific gene clusters supports the hypothesis that sex-dependent gene expression may, in part, account for the reported sex differences in hepatic sensitivity to TCDD in rats.

1200 TCDD-INDUCED OXIDATIVE STRESS IN DIFFERENT BRAIN REGIONS OF RATS : MODULATION BY VITAMIN E SUCCINATE AND ELLAGIC ACID.

E. A. Hassoun, J. Vodhanel, A. Abushaban and M. Al-Ghafri. *Pharmacology, University of Toledo, Toledo, OH.*

The effects of vitamin E succinate and ellagic acid on TCDD-induced oxidative stress in different brain regions of rats, have been studied. Groups of female Sprague-Dawley rats were administered either vitamin E succinate (80 mg/kg) or ellagic acid (1 mg/kg), p.o, every other day during a treatment period of 90 day. Half of the animals in each group were also treated p.o, with daily doses of 46 ng TCDD/kg. Control groups received the vehicles used to dissolve TCDD, ellagic acid and vitamin E succinate. At the end of the treatment period, the rats were sacrificed and their brains were removed and dissected into various brain regions, including, hippocampus (H), cerebral cortex (Cc), brain stem (BS) and cerebellum (C). Production of superoxide anion (SA) and lipid peroxidation (LP), as well as the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were determined in those regions. Administration of vitamin E succinate and ellagic acid resulted in significant decreases in TCDD-induced LP and SA production in Cc and H, significant increases in SOD and CAT activities in those two regions and significant increases in GSH-Px activity in H, Cc, Bs and C. Statistical analyses of the effects of vitamin E succinate and ellagic acid on TCDD-induced oxidative stress in brain regions of rats have revealed better protective effects provided by vitamin E succinate than ellagic acid. The results suggest that vitamin E succinate and ellagic acid can protect against TCDD-induced oxidative stress in different brain regions of rats by increasing the activities of different antioxidant enzymes in those regions. (Supported by NIH/NIEHS grant ES11048).

1201 TCDD ALTERS THE p53 RESPONSE TO DNA DAMAGE.

M. Viluksela[1], University. Stenius[2], R. Pohjanvirta[3, 4, 1] and J. Hogberg[2]. *[1]Department of Environmental Health, National Public Health Institute, Kuopio, Finland, [2]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [3]National Food and Veterinary Research Institute, Kuopio, Finland and [4]Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland.*

Tumor suppressor p53 plays a key role in the prevention of cancer and developmental defects. Carcinogenicity and developmental defects are characteristic effects of dioxins, but the role of p53 in dioxin-induced toxicity has not been clarified. We studied the effects of TCDD pretreatment on the expression of p53 in response to diethylnitrosamine (DEN) induced DNA damage. We used TCDD sensitive Long-Evans (*Turku/AB*; L-E) rats and TCDD resistant Han/Wistar (*Kuopio*; H/W) rats as well as cultured human HepG2 hepatocytes. TCDD attenuated the DEN-induced increase in p53 protein levels in both rat strains. In addition, TCDD treatment increased the amount of phosphorylated MDM2 protein that is involved in the degradation of p53. Similar results regarding p53 and MDM2 were also obtained in TCDD-treated HepG2 cells. In addition, a deceased p21 response after TCDD treatment was seen. It seems likely that alterations in MDM2 levels and phosphorylation patterns in this protein resulted in reduced p53 response. The data indicate that TCDD affects p53/MDM2 regulation and suggest that interactions between TCDD and genotoxic carcinogens may result in a deteriorated repair or control of DNA damage.

1202 MITOCHONDRIAL PROTEIN EXPRESSION IN MOUSE LIVER FOLLOWING TCDD EXPOSURE.

X. Shi, D. Shen, S. N. Schneider, M. Genter, D. W. Nebert, T. P. Dalton and H. G. Shertzer. *Department of Environmental Health and Center for Environmental Genetics, University of Cincinnati Medical Center, Cincinnati, OH.*

2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD; dioxin) is a persistent environmental contaminant that can cause diverse pathological outcomes. The mechanism of TCDD toxicity results, in part, from its stimulation of a mitochondrial respiration-dependent oxidative stress response. In order to examine the mechanism of TCDD-stimulated mitochondrial reactive oxygen production, we compared changes in protein composition in liver mitochondria from C57BL/6J mice treated with TCDD (15 μg/kg body weight) with vehicle-treated controls. After one week, liver mitochondria were isolated; proteins were separated by pH-molecular weight (MW) 2-dimensional electrophoresis. Gels were stained with CYPRO Ruby, scanned, and analyzed with INVESTIGATE HT Analyzer software (Genomic Solutions). More than 550 individual spots were identified in mitochondria from control or TCDD-treated mice. Of these, 160 appeared only in control mitochondria, while 116 appeared only in TCDD-treated mitochondria. While most proteins distributed in the pH=4-6 range, TCDD treatment shifted the distribution toward pI values greater than 7. Among the 287 common spots, the 2-fold, 3-fold, 6-fold and 10-fold up- or down-regulated expression following TCDD treatment included 20 proteins up-regulated/27 proteins down-regulated, 11/35, 2/7, and 4/4, respectively. Spots matched for pI and MW were examined using the ExPASy Molecular Biology Proteomics Server. Candidate proteins associated with large increases in protein expression levels were associated with complex 3 electron transport, associated with the urea cycle, and associated with high rates of protein turnover. Thus, TCDD produces major changes in the protein composition of mouse liver mitochondria, which may be related to TCDD-mediated physiological and biochemical alterations in mitochondria. (Supported by grants R01 ES10133, R01 ES06321, R01 ES08147 and P30 ES06096)

1203 HYDRONEPHROSIS AND RENAL CYP1A1 INDUCTION IN THE RAT KIDNEY BY LACTATIONAL EXPOSURE TO DIOXIN.

N. Nishimura[1, 2], J. Yonemoto[1, 2], Y. Takeuchi[1], C. Yokoi[1, 2], H. Nishimura[3] and C. Tohyama[1, 2]. *[1]NIES, Tsukuba, Japan, [2]CREST, Kawaguchi, Japan and [3]Aichi Mizuho University, Toyota, Japan.*

Cleft palate and hydronephrosis are the major phenotype of teratogenicity in mice induced by a relatively high dose of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (40 μg/kg TCDD) during pregnancy. We investigated the critical period of TCDD exposure for the occurrence of hydronephrosis in TCDD-exposed rat pups by cross-fostering studies, and also a possible involvement of the arylhydrocarbon receptor (AhR) in the developmental of hydronephrosis by using AhR-null mice. In the cross-fostered pups at the weaning (PND21), hydronephrosis was detected in the C/T (lactational exposure only), and T/T (both prenatal and lactational exposure) groups with a high incidence, while no incidence of hydronephrosis was observed in pups from the C/C (control) and T/C (prenatal exposure only) groups. Serum and liver in the C/T and T/T groups showed higher concentrations of TCDD, as compared to the T/C group. No differences in TCDD concentrations were observed among the cortex, outer medulla and inner medulla. Milk in the stomach from the T/T and C/T groups on PND1 contained a large amount of TCDD compared to the C/C and T/C groups. The RT-PCR analysis of the renal cytochrome P450 (CYP)1A1 mRNA showed that it was greatly induced in the C/T and T/T groups in contrast to nearly no expression in the C/C and T/C groups. Immunohistochemical staining for CYP1A1 was found in a very limited area of the kidney, the ascending Henle's loop, from the C/T and T/T groups. Pups from TCDD-exposed AhR-null mice did not manifest hydronephrosis but those from AhR-heterozygotes (AhR+/-) mice showed an incidence of 37.5 %. Taken together, we conclude that lactational exposure to TCDD is primarily responsible for the renal developmental malformation in neonates and that this teratogenicity is mediated by the AhR. Despite the even distribution of TCDD in kidney, region-specific

expression of AhR and CYP1A1 in the developing kidney may suggest that AhR-dependent genes or CYP1A1-related cellular events that might lead to the occurrence of hydronephrosis.

1204 EFFECTS OF *IN UTERO* AND LACTATIONAL 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) EXPOSURE ON THE PROSTATE AND ITS RESPONSE TO CASTRATION IN SENESCENT C57BL/6 MICE.

W. A. Fritz, T. Lin and R. E. Peterson. *School of Pharmacy, University of Wisconsin, Madison, WI.*

In utero and lactational (IUL) TCDD exposure inhibits ventral, dorsolateral, and anterior prostate development in C57BL/6 mice. To determine if these effects persist into senescence, mice born to dams given TCDD (5 μg/kg, po) or vehicle on gestation day 13 were examined at 100 and 510 days of age. Androgen responsiveness was also assessed by castrating half the mice ten days prior to necropsy and sham operating on the other half. In TCDD-exposed mice the ventral prostate was absent at 100 days of age, but a few individuals had rudimentary ducts in senescence. Castration of vehicle-exposed mice caused far greater reductions in organ weight, epithelial cell height, and androgen-dependent gene expression (MP25 and probasin) in young mice than in senescent ones. Responses to castration were similar in vehicle- and TCDD-exposed mice at 100 days of age. At 510 days, however, TCDD-exposed mice were substantially more responsive to castration than vehicle-exposed mice. These results suggest that IUL TCDD exposure protects mice against the decline in androgen responsiveness that normally occurs with aging. The only result that did not follow this pattern was proliferating cell nuclear antigen (PCNA) labeling, where the age-dependent change in the response to castration was not affected by TCDD. The percentage of dorsolateral prostate ducts with cribriform structure was 2-3% in vehicle-exposed senescent mice regardless of castration, but was 16% in sham-operated TCDD-exposed senescent mice and 7% following castration. Collectively, these results demonstrate that effects of IUL TCDD exposure on the prostate persist through senescence, and suggest that IUL TCDD exposure retards the aging process in the prostate. However, because cribriform structures are often considered to be a pre-cancerous lesion, these results also suggest that TCDD exposure early in development may increase susceptibility to prostate cancer. (Supported by NIH Grant ES 01332.)

1205 DECREASED CARDIOMYOCYTE PROLIFERATION AND DOWN-REGULATION OF CELL-CYCLE-SPECIFIC GENES FOLLOWING *IN UTERO* 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN EXPOSURE.

E. A. Thackaberry and M. K. Walker. *College of Pharmacy, University of New Mexico, Albuquerque, NM.*

2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) has been demonstrated to alter proliferation in a number of cell lines. While some cell types, such as keratinocytes, respond with increased proliferation, other cell types, such as thymocytes and cardiomyocytes, have been shown to experience a reduction in proliferation. Previous work in our laboratory has demonstrated that fetal mice treated *in utero* (day 14.5) with 6 ug/kg TCDD have reduced heart weights by d17.5 of gestation. Furthermore, cardiac proliferation is reduced, as measured by proliferating cell nuclear antigen (PCNA) staining. These results suggest that TCDD may alter cell cycle control in the developing heart. While the majority of the cardiac volume is made up by cardiomyocytes, cardiac fibroblasts can account for up to 50% of the total cell number in the heart, and represent a second possible target of TCDD-induced growth arrest. In the current studies, we have sought to investigate this effect, and determine the cell types and mechanisms involved. Real-time PCR quantification analysis using whole heart samples revealed that the G1/S cyclins A2 and E2 were reduced, suggesting a delay in this cell cycle checkpoint. In order to determine if the cardiomyocytes are the primary cell-type with reduced proliferation following TCDD treatment, two cardiomyocyte cell lines, the murine HL-1 and the rat H9C2 are being used in an attempt to reproduce this effect. Growth curves for both cell lines have been conducted, and preliminary dose-response studies suggest a decrease in cardiomyocyte proliferation. These *in vitro* models will be used to dissect the mechanism by which TCDD causes cardiomyocyte growth arrest. (Supported by ES09804 to MKW, ES012855-01 to EAT, and NMNIEHS Center ES12072)

1206 LOSS OF ARNT2 IS INSUFFICIENT TO PROTECT AGAINST TCDD DEVELOPMENTAL TOXICITY IN ZEBRAFISH.

A. L. Prasch, W. Heideman and R. E. Peterson. *Molecular and Environmental Toxicology Center and School of Pharmacy, University of Wisconsin, Madison, WI.*

ZfAHR2 has been identified as the receptor that is essential for mediating the developmental toxicity caused by 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the zebrafish embryo. *In vitro* molecular evidence demonstrates that one form of zfARNT2, zfARNT2b, forms a functional heterodimer with zfAHR2 that specifically recognizes DREs in gel shift experiments and induces DRE-driven transcription in COS-7 cells treated with TCDD. However, whether zfARNT2b is the physiological dimerization partner for zfAHR2 in developing zebrafish has yet to be determined. An insertional mutagensis screen performed in the lab of Nancy Hopkins from the Massachusetts Institute of Technology has generated a line of mutant zebrafish which lack expression of the *zfarnt2* gene. We have used the *zfarnt2*$^{-/-}$ mutant zebrafish to test the hypothesis that zfARNT2 is required for mediating the developmental toxicity of TCDD. Adult fish heterozygous for the insertion were pair mated and the eggs treated with TCDD within 4 hpf. Surprisingly, immunostaining results performed at 96 hpf demonstrate that the *zfarnt2*$^{-/-}$ embryos show a similar pattern of TCDD-induced zfCYP1A expression as WT embryos. TCDD dose response studies were also performed and the embryos assessed at 96 hpf for three endpoints of TCDD toxicity: pericardial edema, reduced trunk blood flow, and shortened lower jaw. For all three endpoints *zfarnt2*$^{-/-}$ mutant embryos showed a similar response to TCDD as WT embryos. RT-PCR results confirm that the *zfarnt2*$^{-/-}$ mutants do, in fact, lack expression of all splice forms of zfARNT2 mRNA. These results demonstrate that zfARNT2 is not essential for mediating several responses to TCDD in developing zebrafish and suggest that alternate dimerization partner(s) exist for zfAHR2 *in vivo*. (Supported by UW Sea Grant).

1207 TCDD INHIBITS REGRESSION OF THE COMMON CARDINAL VEIN IN DEVELOPING ZEBRAFISH.

S. M. Bello, W. Heideman and R. E. Peterson. *Pharmacy, University of Wisconsin - Madison, Madison, WI.*

A role for the aryl hydrocarbon receptor (AHR) in vascular development has been implicated by studies in *Ahr* null mice. Select fetal vascular structures that normally regress during development fail to regress in *Ahr* null mice. We hypothesized that 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) would affect regression of vessels programmed to regress in developing zebrafish. Several vessels have been identified in zebrafish that undergo regression during normal development including the common cardinal vein (CCV) and subintestinal vein / hepatic portal vein (SIV/HPV). Fli1-eGFP transgenic zebrafish[1] embryos were exposed to TCDD for 1h between 1 and 6 hpf and the effects on programmed regression monitored. The CCV, the inflow tract of the zebrafish heart, begins to regress around 74 hpf as the position of the heart within the pericardium migrates dorsally. This regression is inhibited in larvae exposed to 1 or 10 ng TCDD/ml. Knocking down expression of AHR2 with a zebrafish *ahr2* specific morpholino (*zfahr2*-MO) protected against the TCDD induced inhibition of regression. However, use of the *zfahr2*-MO alone did not significantly affect regression of the CCV. The SIV develops over the yolk with connections to the posterior cardinal vein and the CCV beginning around 60 hpf. Between 84 and 96 hpf the anterior portion of the SIV becomes the HPV as the hepatic sinusoid develops. During the development of the SIV/HPV, sprouts grow both dorsally and ventrally off the horizontal portion of the SIV/HPV. These ventral sprouts normally regress during development disappearing by 96 hpf. TCDD exposure (1 ng/ml) results in retention of these ventral sprouts past 96 hpf. Use of *zfahr2*-MO protects against the TCDD induced retention of these ventral sprouts. However as with inhibition of regression of the CCV, the *zfahr2*-MO alone does not inhibit regression of the ventral sprouts. These results demonstrate that TCDD can inhibit regression of vessels programmed to regress in an AHR2 dependent manner. (UW SeaGrant, NRSA) [1]Provided by B. M. Weinstein and N. D. Lawson, NIH.

1208 WATER PERMEABILITY AND TCDD-INDUCED EDEMA IN EARLY LIFE STAGES OF ZEBRAFISH.

A. J. HILL, S. M. BELLO, A. L. PRASCH, R. E. PETERSON and W. HEIDEMAN. *School of Pharmacy, University of Wisconsin, Madison, WI.*

A common response to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) exposure in teleost embryos is blue sac disease, characterized by pericardial and yolk sac edema. The cellular and extracellular fluids of freshwater fish are hyperosmotic compared to the surrounding water. In order to maintain osmotic balance, freshwater fish must have a barrier to minimize water entry and be able to excrete excess water. It was hypothesized that edema observed between 72 and 168 hours postfertilization (hpf) in zebrafish exposed for 1 h to TCDD (0.1, 0.5, 1, 5 or 10ng/ml) was due to degradation of water barrier function. To test this hypothesis, the osmotic gradient was decreased by increasing the osmolarity of the surrounding water with 250 mM mannitol. This significantly reduced pericardial and yolk sac edema. Late addition of mannitol only partially reversed edema already formed. An alternate hypothesis is that TCDD impairs water excretion. *In situ* hybridization studies showed that expression of the key pronephric developmental genes *sim 1*, *pax 2a*, and *wt 1* were unaffected by TCDD exposure. Histological analysis revealed a significant alteration in glomerular shape (flattened) from 52 hpf. However, in larvae treated with 175 mM mannitol, this change in TCDD-exposed larvae was not evi-

dent and hence was thought to be dependent on edema. Likewise, blocking edema formation using *zfahr2*-morpholino also prevented flattening of the glomerulus. At 56 hpf, glomerular filtration of injected fluorescently labelled dextran from the bloodstream was similar in both control and TCDD-exposed larvae. Expression of Na^+/K^+ ATPase in the pronephric ducts and membrane polarity of the ductal epithelium, indicated through alkaline phosphatase expression, was unaffected in TCDD-exposed larvae. These results tend to rule out a role for dioxin-induced malfunction of the pronephros in causing edema. In conclusion, TCDD exposure may inhibit the function of a permeability barrier in the skin to water that is critical for maintaining osmotic balance in early development. (UW SeaGrant, NRSA).

1209 MORPHOLINO KNOCKDOWN OF CYP1A DOES NOT PROTECT AGAINST 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN INDUCED EMBRYOTOXICITY IN ZEBRAFISH.

S. Carney, W. Heideman and R. E. Peterson. *University of Wisconsin, Madison, WI.*

In zebrafish embryos exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) during a developmental window before 96 hpf reduces blood flow throughout the embryo and causes pericardial edema. These endpoints of toxicity occur upon activation of the AHR2 signaling pathway and can be blocked in embryos injected with an AHR2 morpholino prior to treatment with TCDD. The TCDD-induced upregulation of cytochrome P4501A (CYP1A) is also blocked in AHR2 morphants. Therefore it is possible the toxicity that results from TCDD activation of AHR2 is mediated by CYP1A. To test this hypothesis, a morpholino that blocks translation of CYP1A was injected into one-cell stage embryos and compared to embryos injected with the AHR2 morpholino or a control morpholino. Embryos from each group were exposed to TCDD or vehicle within 4 hpf, and CYP1A protein levels were measured in each group from 48 to 96 hpf. Blood flow in the trunk segmental vessels and development of pericardial edema were then measured at 72 and 96 hpf to assess effectiveness of the two morpholinos in blocking TCDD toxicity. From 48 to 96 hpf the CYP1A morpholino blocked the TCDD-dependent induction of CYP1A even more effectively than the AHR2 morpholino. Blood flow in AHR2 morphants treated with TCDD was no different from vehicle controls at 72 and 96 hpf. Despite the lower levels of CYP1A protein in embryos injected with the CYP1A morpholino these morphants had decreased blood flow in the trunk segmental vessels at 72 and 96 hpf when treated with TCDD. Likewise CYP1A morphants treated with TCDD developed pericardial edema starting at 72 hpf while AHR2 morphants treated with TCDD did not develop pericardial edema even at 96 hpf. Both morpholinos blocked TCDD induction of CYP1A through 96 hpf. However, the AHR2 morpholino blocked TCDD-induced decreases in blood flow and development of pericardial edema while the CYP1A morpholino did not. Therefore CYP1A is not a mediator of these endpoints of TCDD developmental toxicity in zebrafish. (UW Sea Grant)

1210 CHRONIC EXPOSURE TO LOW DOSES OF TCDD ALTERS REPRODUCTIVE SUCCESS IN ZEBRAFISH.

T. King Heiden, B. Wimpee, R. Hutz and M. J. Carvan. *UW-Milwaukee Great Lakes WATER Inst and NIEHS Marine and Freshwater Biomedical Sciences Center, Milwaukee, WI.*

Dioxins are prevalent within the environment and are formed as by-products from several anthropogenic processes. 2, 3, 7, 8-TCDD (TCDD) in particular is especially toxic, and has been shown to act as a teratogen, embryotoxin, carcinogen, immune system suppressor, and endocrine disruptor. Fish embryos are among the most sensitive vertebrates to the toxic effects of TCDD. Wild fish populations exposed to high environmental levels of TCDD have reduced reproductive success. The long-range goal of this project is to facilitate a better understanding of the mechanisms by which TCDD modulates reproductive function in fishes, using zebrafish (Danio rerio) as a model organism. The first objective was to determine the effects of chronic exposure to low concentrations of TCDD on the reproductive success of zebrafish. Adult female zebrafish were fed different concentrations of TCDD for a period of three weeks, and subsequently spawned with untreated males to determine the effects of TCDD exposure on egg production and offspring survival. While females did not exhibit an overt toxic response, there was a significant decrease in the ovosomatic index and a slight decrease in overall egg production. Additionally, there was a dose-dependent increase in early life-stage toxic response, including yolk sac edema, cranial edema, morphological anomalies, and mortality. Ongoing investigations into the molecular mechanisms by which TCDD alters reproductive success of zebrafish will contribute to our understanding of the dangers that endocrine disrupting chemicals such as TCDD have on vertebrates systems, and provide insight into ways that we can protect wildlife and human populations from such toxicants.

1211 EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON MATRIX METALLOPROTEINASE (MMP) EXPRESSION IN A FISH MODEL OF WOUND REPAIR.

K. Hogan[1], B. Wall[2], K. Cooper[1, 2] and L. White[1, 2]. [1]*Joint Graduate Program in Toxicology, Rutgers/UMDNJ, Piscataway, NJ and* [2]*Department of Biochemistry, Rutgers, New Brunswick, NJ.*

MMPs, a family of zinc-dependent enzymes, degrade the extracellular matrix (ECM) at critical stages of wound repair. The present studies investigate the effect of the ubiquitous environmental toxicant TCDD on expression of MMP-2 and -9 in the wounded caudal fin of the Japanese medaka fish. Exposure to TCDD has been shown to: 1) increase expression of MMPs in cultured human keratinocytes; and 2) inhibit angiogenesis, an MMP-dependent process, during fin regeneration in zebrafish. Because medaka are both TCDD-responsive and capable of caudal fin regeneration, we believe these fish are an ideal model to test the hypothesis that exposure to TCDD alters expression of MMPs during wound repair. In the present studies fish were administered two doses of TCDD (25 or 50 pg/g food x 4 days) and recovered (~2d) prior to surgical ablation of a portion of the caudal fin (~2mm). Total RNA was isolated from tissue collected from the margins of wounded tail fins 3, 6, 12, and 24h post-wounding. Quantitative real time RT-PCR revealed temporal and dose-dependent changes in expression of MMPs in wound margins of TCDD and vehicle-treated fish. At 3h post-wounding, dose-dependent expression of MMP-2 (~7-12 fold) was observed in fish pretreated with TCDD. No changes were observed at 6h post-wounding. At 12h post-wounding, increased expression of MMP-2 (~6 fold) and MMP-9 (~3 fold) were observed in the wound margins of fish treated only with vehicle control. Fish exposed to the lower dose of TCDD (25 pg/g) showed expression of MMP-2 at 12h and MMP-9 at 3 and 24h post-wounding. In these studies, real time RT-PCR reactions were performed in triplicate on RNA extracted from ~15 caudal fin tails/treatment group/time point. Data were normalized to actin and are represented as fold change over vehicle control. These studies are the first to show an effect of TCDD on MMP expression following wounding in an alternative animal model.

1212 TCDD DECREASES RESPONSIVENESS OF THE CHICK EMBRYO HEART TO ISOPROTERENOL BUT NOT TO AGENTS EFFECTING DOWNSTREAM EVENTS OF THE BETA-ADRENERGIC RECEPTOR.

R. J. Sommer[1] and M. K. Walker[2]. [1]*Biology Department, Bates College, Lewiston, ME and* [2]*College of Pharmacy, University of New Mexico, Albuquerque, NM.*

2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) causes cardiovascular toxicity in laboratory animals, including decreased contractility and heart rate, decreased myocyte proliferation, increased cardiac apoptosis and inhibition of coronary vasculogenesis. Beta-adrenergic receptor (β-AR) signaling plays an important role in each of these processes, thus, our laboratory investigated the effects of TCDD on β-AR signaling. Fertile chicken eggs were injected with vehicle (corn oil) or 0.3 pmol TCDD/g egg prior to incubation (D0). On D10 embryos were anesthetized and silver wire electrodes, connected to a bioamplifier, were inserted through the shell. Embryos were placed in a 37°C incubator and ECGs were recorded digitally for 10 min followed immediately by injection of vehicle or isoproterenol (β-AR agonist), forskolin (activates adenylate cyclase), theophylline (inhibits cAMP degradation), or BayK8644 (opens L-type calcium channels). Baseline heart rates (HR) of control (219.9 ± 4.0 bpm, n=39) and TCDD-exposed (221.8 ± 4.4 bpm, n=36) embryos were similar. Isoproterenol (30-120 μg/egg) significantly increased HR of controls (16.1 ± 6.8-29.5 ± 11.2 bpm above baseline, n=5-6 per dose) compared to saline controls (-0.5 ± 2.3 bpm change from baseline, n=5). TCDD-exposed embryos did not increase HR in response to isoproterenol. However, TCDD-exposed embryos increased HR similar to control values following pharmacological agents that stimulate signaling downstream of β-AR. Theophylline (2.5 mg/egg) and BayK8644 (10 μg/egg) increased HR of control and TCDD-exposed embryos 17.2 ± 6.3 and 14.0 ± 2.0, and 34.8 ± 5.6 and 30.0 ± 7.4 bpm above baseline, respectively. TCDD-exposed embryos were more sensitive than controls to forskolin (25 μg/egg), increasing HR 21.8 ± 3.5 vs control at 6.3 ± 2.7 bpm above baseline. These data suggest that TCDD decreases the response of the chick embryo heart to β-AR agonists upstream of adenylate cyclase. Supported by NIH R15 ES011806.

1213 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) EXPOSURE AFTER ANGIOBLAST DIFFERENTIATION INHIBITS CORONARY VASCULOGENESIS.

I. D. Ivnitski-Steele, M. M. Friggens, M. K. Chavez and M. K. Walker. *College of Pharmacy, University of New Mexico, Albuquerque, NM.*

TCDD exposure on day 0 (d0) of embryo development reduces coronary vascular outgrowth in chick embryos *in vivo* and in heart explants *in vitro*. In this study, we investigated whether TCDD reduces coronary vasculogenesis by affecting (a) the

proepicardial organ, which is the source of angioblast precursors, and/or (b) VEGF-A-dependent angioblast assembly into coronary endothelial tubes. Fertile chicken eggs were treated with control (corn oil) or TCDD (0.3 pmol TCDD/g) on d5 after coronary angioblasts have differentiated. On d6, cardiac ventricle explants were cultured on a collagen gel and coronary endothelial tube outgrowth assessed. On culture day 4, recombinant VEGF-A (rcVEGF-A) or soluble flt-1 receptor plus rcVEGF-A were added to explant media. In a second experiment, condition media from control explants was added to TCDD explants, while conditioned media from TCDD explants was added to control cultures. Lastly, VEGF-A protein in condition media was measured by ELISA. TCDD exposure on d5 reduced coronary artery number *in vivo* by 53±8% and reduced the length and number of outgrowing coronary endothelial tubes from explant cultures by 30±3% and 58±2%, respectively, compared to controls. The soluble flt-1 receptor blocked outgrowth in control cultures and blocked rcVEGF-A-mediated rescue of outgrowth from TCDD explants. Additionally, media from control explants rescued coronary outgrowth from TCDD explants, while media from TCDD explants had no effect on controls. Finally, VEGF-A protein secreted into media from TCDD explants was 43±3% lower than from control explants. These data suggest that 24 hr TCDD exposure is sufficient to inhibit coronary vasculogenesis and that the proepicardial organ is not involved in this inhibition. Further, TCDD does not stimulate the secretion of an inhibitory factor, but significantly reduces cardiac VEGF-A secretion. Finally, media from control cultures containing physiological levels of VEGF-A can rescue TCDDs inhibitory effect. Supported by ES09804 and NM Center ES12072.

1214 ARSENIC DISRUPTION OF CELL CYCLE: MECHANISMS AND EFFECTS ON APOPTOSIS, DIFFERENTIATION AND CARCINOGENESIS.

J. States[1] and M. J. McCabe[2]. *[1]Pharmacology & Toxicology, University of Louisville, Louisville, KY and [2]Environmental Medicine, University of Rochester, Rochester, NY.*

Epidemiological studies indicate that arsenic is a human carcinogen, but the mechanism of arsenic carcinogenesis is unknown and a subject of great debate. Paradoxically, arsenic is an effective anti-leukemia agent. There is great interest in understanding and exploiting the potential chemotherapeutic effects of arsenic compounds to treat other malignancies. Recent discoveries detailing arsenic's effects on cell cycle regulation may provide insight into the mechanisms underlying its carcinogenic and chemotherapeutic activities. Collectively, recent studies suggest that arsenic may influence cell cycle regulators at either the transcriptional or post-translational levels, and effects on specific protein-protein interactions also have been postulated. Arsenic recently has been shown to interfere with protein ubiquitination — an important finding given the role of ubiquitination of cell cycle regulatory proteins (e.g., cyclins) in normal cell cycle control. Disruption of normal cell cycle checkpoints is thought to be a key feature of the carcinogenic process. Similarly, disrupting cell cycle control is a contemporary strategy for chemotherapy. Determining which cell cycle regulators are targeted by arsenic and how such targeting is linked to cellular processes (i.e., differentiation, apoptosis) will provide an understanding of the carcinogenic mechanism and molecular targets that may be useful for chemotherapeutic benefit. This symposium will present an overview of the role of protein ubiquitination in cell cycle regulation, as well as the latest research investigating arsenic effects on protein ubiquitination and on regulation of genes and signaling pathways controlling cell cycle, differentiation and apoptosis. The presentations will discuss mechanisms that may explain the paradoxical effects of arsenic as both a cancer causing and a chemotherapeutic agent.

1215 UBIQUITINATION IN THE CONTROL OF CELL CYCLE, GROWTH AND ONCOGENESIS.

A. Banerjee. *Inst. of Env. Health Sciences, Wayne State University, Detroit, MI.*
Sponsor: J. States.

Regulatory signals as well as environmental pollutants/carcinogens trigger cells to enter a new cell division cycle. Knowledge of mechanistic controls of cell cycle is critical for understanding the underlying defects in developmental abnormalities as well as in diseases such as cancer. Cell cycle regulatory mechanisms involving proteins include synthesis, degradation, phosphorylation, binding of inhibitors, and checkpoints. As a regulatory mechanism, ubiquitin-dependent proteolysis is required for cell division, as well as for differentiation, oncogenesis, DNA repair, transcription, and removal of abnormal proteins. Ubiquitination targets proteins for degradation by the 26S proteasome. Our efforts are directed to address the following questions: i) is there a particular kind of domain for recognition by each ubiquitination sub-pathway catalyzed by a specific ubiquitin conjugating enzyme (E2s); ii) whether proteins that have undergone conformational changes in the presence of substrates or ligand/protein partner binding can be recognized by the same ubiquitination sub-pathway as in the absence of substrates or ligand/protein partner binding. Efforts are directed to unravel the mechanism of action of E2s and the kinetics of association and activation of E2 associated multi-protein ubiquitin ligation complex (E3s). For the former, protein structures of about 200 E2s (homology models and crystal structures) were aligned and centered on a 96Å^3 virtual grid. The hydrophobic potentials of the E2s were computed and analyzed. Investigation is underway to analyze the electrostatic potential similarity between the specific amino acid surface patches that have been identified and their functional significance is being examined. These are also being exploited for their potential to bind small molecule inhibitors and thereby for the development of specific E2 enzyme inhibitors, by *in silico* small molecule screening. Leads from the development of *in vitro* inhibitors would provide a method for the development of *in vivo* inhibitors as experimental tools, and potentially as therapeutic agents. (Supported by NIH grant GM59467.)

1216 EFFECTS OF *IN VITRO* EXPOSURE TO ARSENIC ON THE UBIQUITIN PATHWAY IN HUMAN RENAL AND BLADDER CELLS.

A. J. Gandolfi, D. S. Kirkpatrick, T. G. Bredfeldt and X. H. Zheng.
Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

The ubiquitination of proteins within a cell plays a key role in maintaining the appropriate regulatory balance of processes such as cell cycle, apoptosis, and stress response. During cellular stress, such as following arsenic exposure, the ubiquitin pathway rapidly degrades damaged and misfolded proteins to maintain the fidelity of the cellular machinery. In studies performed in rabbit renal cortical slices, HEK293 cells, and UROtsa cells, low-level arsenic (0.5 uM - 10 uM) causes an accumulation of ubiquitin modified proteins within cells. Microarray analysis of arsenic exposed cells has shown a number of alterations in ubiquitin family genes that help to explain the changes seen. Studies from the rabbit slices and HEK293 cells show that 20S proteasome activity, but not ubiquitin-conjugating activity, was affected by arsenic. Depletion of glutathione with buthionine-L-sulfoximine during coexposure with As (III) greatly increases the levels of ubiquitin-conjugated proteins compared to arsenic treatment alone. This suggests overlapping possibilities that glutathione is directly preventing arsenic mediated protein damage, or that oxidants are involved in the damage of proteins by arsenic. Understanding the effects of low-level arsenic on protein homeostasis will be instrumental to further characterization of the mechanisms behind arsenic carcinogenicity. Because the kidney and bladder are exposed to significant levels of arsenic during urine processing, and because exposed human populations are at increased risk of developing bladder cancer, these organ systems appear to be well suited for the study of the proteotoxic effects of arsenic.

1217 ARSENIC-INDUCED DISRUPTION OF MITOTIC PROGRESSION: IMPLICATIONS FOR CARCINOGENESIS AND POTENTIAL FOR CHEMOTHERAPY.

J. States[1], S. C. McNeely[1] and M. J. McCabe[2]. *[1]Pharmacology & Toxicology, University of Louisville, Louisville, KY and [2]Environmental Medicine, University of Rochester, Rochester, NY.*

SV40 transformed human cells and p53 deficient tumor cells are sensitive to arsenite induced mitotic arrest followed by apoptosis. In contrast, arsenite induces only a mitotic delay in diploid human cells. Abrogated p53 function is a common feature of arsenite sensitive cells. Therefore, we investigated the potential for p53 to modulate the response to arsenite using TR9-7 human fibroblasts in which p53 is under exogenous control (tet-off). Effects of high (p53hi), low (p53lo) and no (p53-) p53 expression on arsenite induced cytotoxicity and mitotic arrest were examined. TR9-7 p53hi cells grew very little and were relatively resistant to arsenite induced toxicity. In asynchronous TR9-7 p53- cells, 5 µM arsenite caused a 3-fold increase in mitotic index and induced apoptosis as indicated by morphological criteria. TR9-7 p53lo cells were resistant to arsenite induced increase in mitotic index. Rather, arsenite treated p53lo cells passed through mitosis and accumulated in G1 as indicated by bromodeoxyuridine incorporation and DNA content analyses by flow cytometry. In TR9-7 cells released from G2 synchronization, arsenite treatment caused a p53 independent delay of entry into mitosis. However, exit from mitosis was p53 dependent in arsenite treated cells. Microarray analyses of gene expression in synchronized cells exiting G2 and entering mitosis indicate that arsenite treatment alters the expression of 11-fold more genes in the presence than in the absence of p53. Genes with altered expression include stress responsive genes as well as genes involved in ubiquitin pathways. These results suggest that p53 expression prevents arsenite induced mitotic arrest but does not affect delayed exit from G2 and entry into M phase. Thus, arsenic induced carcinogenesis is likely independent of p53 inactivation. Conversely, arsenic chemotherapeutic effectiveness may be dependent on loss of p53 mediated cell cycle regulation. (Supported by ES06460, ES011314 and Kentucky Research Challenge Trust Fund).

1218 CELL CYCLE DYSREGULATION BY ARSENITE: IMPLICATIONS FOR ITS CHEMOTHERAPEUTIC ACTIONS.

M. J. McCabe and G. McCollum. *Environmental Medicine, University of Rochester, Rochester, NY.*

Arsenic has been implicated in certain human cancers. It also is being used currently as an anti-leukemia agent. Arsenite is effective in treating acute promyelocytic leukemias expressing the PML-RARα oncoprotein. However, arsenite induces apoptosis in a variety of cancer cells regardless of PML-RARα expression. We have been studying arsenite effects on U937 cells, a human promonocytic leukemia that lacks PML-RARα. Low micromolar levels of arsenite induce U937 cells to differentiate as assessed by increased CD11b expression and increased directional motility in a chemotaxis assay. Thus, the anti-leukemic effects of clinically achievable levels of arsenite may be due to the induction of cell differentiation rather than cell death. Both arsenite-induced apoptosis and differentiation are preceded by cell cycle changes. Using bromodeoxyuridine (BrdU) and flow cytometry, we have found that arsenite impairs cell cycle transit. Cell synchronization and BrdU pulse-chase experiments have established that arsenite delays progression through all cell cycle phases with the most pronounced effect being delayed transit through G2/M followed by mitotic arrest-associated, caspase-3 dependent apoptosis in a fraction of the cells. Over the long term (i.e., ~96), arsenite induces the accumulation of cells in G1. Given the importance of the cyclin-dependent kinase inhibitor p21 in regulating G1 to S phase transition and U937 cell differentiation, we have hypothesized that arsenite-induced differentiation is preceded by increased p21 activity and arrest in G1. As regulation of p21 is largely transcriptional, the effects of arsenite on p21 mRNA levels as well as other cell cycle regulators is being measured by ribonuclease protection assay. Arsenite-induced changes in p21 mRNA and protein abundance are being correlated with the onset of cell cycle arrest and differentiation, and a causal role for p21 in these processes also is being examined. These studies are providing an explanation of how disruption of distinct cell cycle stages is linked to the anti-leukemogenic effects of arsenite. (Supported by ES01247).

1219 MOLECULAR EVENTS DURING TRANSPLACENTAL INORGANIC ARSENIC CARCINOGENESIS IN MICE: ABERRANT ACTIVATION OF GENES LINKED TO CELL CYCLE DYSREGULATION.

M. P. Waalkes[1], J. Liu[1], H. Chen[1], W. E. Achanzar[1] and B. A. Diwan[2]. *[1]LCC, NCI at NIEHS, Research Triangle Park, NC and [2]SAIC, NCI-Frederick, Frederick, MD.*

Defining carcinogenic mechanisms is critical to assessing the human health hazard of arsenic exposure. As gestation is a time of high sensitivity to chemical carcinogenesis, we have performed several *in utero* exposure studies with inorganic arsenic. Pregnant mice received drinking water containing sodium arsenite at various levels, and the offspring were observed for up to 2 years. As adults, male offspring consistently developed hepatocellular carcinoma (HCC) and adrenal tumors, while female offspring showed ovarian and lung tumors, as well as uterine and oviduct preneoplasia. The livers of adult male mice bearing arsenic-induced HCC after *in utero* exposure showed marked over-expression of estrogen receptor-alpha (ER) at both the protein and RNA levels. ER is an estrogen activated transcription factor mediating cell proliferation. ER was intensely localized within the liver cell nucleus, indicating the active form. Activation of hepatic cyclin D1, which can be turned on by ER, was also seen in arsenic exposed mice. Cyclin D1 is critical in cell cycle regulation and, if over-expressed, is considered a hepatic oncogene. *In utero* arsenite induced hypomethylation of the ER promoter region DNA, probably accounting for its over-expression. The cyclin D1 promoter was constitutively unmethylated, and unaltered by arsenic exposure, indicating over-expression was a secondary event. As further evidence that ER over-expression had functional impact, a female pattern of metabolic enzymes occurred in adult male liver after *in utero* arsenic exposure, including over-expression of female-dominant CYP2A4 and CYP2B9, and reduced expression of male-dominant CYP7B1. In summary, altered gene expression occurred in the livers of adult mice bearing HCC induced by *in utero* arsenite exposure that likely resulted in cell cycle dysregulation and enhanced proliferation. Thus, aberrant estrogen signaling, possibly from methylation errors, may play a role in liver cancers induced by *in utero* arsenic exposure.

1220 OCCUPATIONAL SKIN EXPOSURE: CURRENT TRENDS AND FUTURE DIRECTIONS FROM THE FIELD TO GENOMICS.

A. A. Shvedova[1] and M. Luster[2]. *[1]HELD/PPRB, NIOSH, Morgantown, WV and [2]HELD/TMBB, NIOSH, Morgantown, WV.*

The purpose of this symposium is to address important and emerging areas of occupational skin toxicology with respect to current trends and future directions from the field to genomics. For many years, skin has been considered primarily as a route of exposures for toxic chemicals and not as a target organ. As a result, research in skin toxicology per se is extremely underemphasized and under-represented in the discipline of toxicology. Advances in cellular and molecular skin biology have provided insightful opportunities to explore dermal toxicology at different levels. There is no doubt occupational and environmental exposures play a substantial role in skin maladies. A comprehensive discussion of recent developments and trends in occupational skin toxicology will provide novel approaches to elucidate exposure outcomes. This group of selected topics will bring together leading experts representing diverse perspectives in this important field. Ample time will be allotted for full discussion of major skin programs and current findings in Europe and the US. This symposium will address innovative issues in the area of skin toxicology ranging from disease incidences and causes to dermal toxicogenomics.

1221 A RASH OVERVIEW OF OCCUPATIONAL SKIN DISEASES.

B. D. Lushniak. *DSHEFS, NIOSH, Cincinnati, OH.* Sponsor: A. Shvedova.

The importance of skin exposures in occupational settings has become more apparent. Skin can serve as both a target organ of occupational exposures as well as a route of absorption. This presentation will review the field activities of the National Institute for Occupational Safety and Health (NIOSH) involving direct effects on the skin and dermal exposure responses. It will review the wide spectrum of occupational skin diseases (OSDs), describe the epidemiology and public health importance of OSDs, emphasize the causes of contact dermatitis, and review results from NIOSH surveillance and health hazard evaluation investigations.

1222 MOLECULAR MECHANISMS OF ANTIOXIDANT DEFENSE IN THE SKIN.

A. A. Shvedova. *HELD/PPRB, NIOSH, Morgantown, WV.*

Antioxidant deficiency is a component of nutrition shortage. A growing body of evidence suggests that exposure to a number of occupational and environmental toxicants caused oxidative stress leading to short or long-term antioxidant deficiency and cellular dysfunction. Antioxidants, including vitamin E, vitamin C, glutathione and beta-carotene are among the body's natural defense mechanisms against oxidative stress. The skin is recognized as a barrier to absorption, a primary target and route of entry to the systemic circulation. The skin cells may create an oxidative or reductive environment, depending on predominance of redox processes catalyzed by both enzymic and non-enzymic systems. The talk will focus on recent advances in genetic and molecular mechanisms of chemical induced oxidative skin injury, oxidation of skin cellular constituents by reactive oxygen species leading to necrotic/apoptotic death pathways, phospholipid oxidation and signaling, bio-markers of oxidative stress determined in human cells and skin tissues obtained from alimentary vitamin E deficient and knockout tocopherol (alpha) transporter protein Ttpa mice. Value of antioxidants in occupational exposures causing skin damage will be addressed.

1223 BASIC AND CLINICAL ASPECTS OF SKIN DISEASES.

K. D. Cooper. *Case Western University, Cleveland, OH.* Sponsor: A. Shvedova.

Skin toxicants share an ability to cause activation of cell signaling, often resulting in inflammatory and immunologic mechanisms. Skin has a limited number of reaction patterns, usually represented by various specific diseases. Allergic, irritant and atopic contact dermatitis, psoriasiform dermatitis/psoriasis, sunburn/photoxicity, and physical trauma to the epidermis represent some of the reactions that can occur in response to occupational toxicant exposures. Discussed here will be the pathogenesis of allergic contact dermatitis, atopic dermatitis, psoriasis and photoinjury. Keratinocyte inflammatory mediators, cytokines and chemokines can often initiate or propagate cellular immunologic mechanisms. These include Langerhans cell migration, T cell recruitment and activation, macrophage activation, and associated epidermal changes, ranging from spongiosus to hyperplasia or dermal-epidermal junction targeted toxicity to basal keratinocytes. Psoriasis as a model of Type I T cell mediated immune reactions (IFNg, IL-12, TNF) will be compared to the Type II immune responses (IgE, IL-4, 5, 13) associated with Atopic Dermatitis. The immunomodulatory effects of skin perturbation will be exemplified by the intersecting innate and adaptive immune response mechanisms initiated by solar radiation photons in the UVA and UVB region.

1224 DISSECTING MECHANISMS OF SKIN TOXICITY IN THE AGE OF PROTEOMICS AND GENOMICS.

J. D. Laskin. *UMDNJ, Piscataway, NJ.*

This talk will describe the results of using newer techniques coupling multidimensional protein separation and liquid chromatorgraphy/mass spectrometery to characterize the proteosome, and microarray analysis to describe the transcriptosome, in defining mechanisms of skin toxicity. The ultimate goal of these studies will be to identify genes that regulate the changes that occur in tissues during skin toxicity that are likely to offer novel targets for risk assessment and therapeutic intervention. Newer techniques coupling two dimensional gel electrophoresis, liquid chromatography/mass spectrometry and micro-array analysis have provided many important new leads to understand mechanisms of skin toxicity. Characterizing genes that regulate the changes that occur in tissues during skin toxicity are likely to offer novel targets for occupational risk assessment and therapeutic intervention.

1225 EXTRA CUTANEOUS ORGAN ENDPOINTS AND DEFINING KNOWLEDGE HIATUS (CLINICAL, CONCEPTUAL AND LABORATORY) WHOSE SOLUTIONS SHOULD LEAD TO INCREASED WORKER SAFETY.

H. I. Maibach. *Department of Dermatology, UCSF, San Francisco, CA.*

Occupational dermal toxicology surged forward in the half century since publication of the Schwartz Tullipan text. With increasing clinical experience and advancing bioanalytic and other laboratory technology in Europe, Asia, and the United States, we have become increasingly aware of the strength and weakness of current workplace health knowledge. This presentation will focus on current knowledge in the field of skin diseases, e.g., irritant dermatitis syndrome, photoirritation, allergic contact dermatitis, and contact urticaria syndrome. The talk will address the ability of the skin to inhibit percutaneous penetration, and the converse, failure to protect strength and weakness of the models systems for quantifying flux as related to the workplace (vitro vs. vivo, Q S A R, and recent shortcuts, Rougier System); models systems for quantifying decontamination in theory, practice, and need; extracutaneous end points (human organ effects) from clinical medicine and how they could be avoided; and an abridged bibliographic system permitting toxicologists to efficiently follow developments in this rapidly evolving science.

1226 XENOBIOTIC-ACTIVATED RECEPTORS: BIOLOGICAL FUNCTIONS AND DISEASE PREVENTION.

R. D. Palmiter. *University of Washington, Seattle, WA.*

Xenobiotic-activated receptors comprise several classes of structurally distinct receptor/transcription factors, which sense changes in the chemical environment of cells, mediate transcriptional responses to the chemical stimuli, and thereby control the homeostasis of cells. Overactivation or dysfunction of the receptors is often associated with altered responses to chemicals, including toxicity and disease states. The rapid advances in understanding of signal transduction and molecular mechanism of action of these receptors provide new insights into the biological functions of the receptors and their relation to disease pathogenesis. Moreover, increasing evidence reveals that many xenobiotic-activated receptors represent important targets for developing effective therapeutic and preventive strategies in disease control and prevention. The objective of this symposium is to bring together leading experts to present new advances in the concept and understanding of the biological functions of a number of receptors in relation to disease development and prevention. Topics include ligand-receptor interactions and implications for therapy/chemoprotection; receptor-mediated antioxidant/oxidative responses and relation to autoimmune regulation/embryonic development; and control of metal homeostasis.

1227 A NEW CLASS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ AGONISTS: 1, 1-BIS(3'-INDOLYL)-1-(P-SUBSTITUTEDPHENYL)METHANES.

S. Safe. *Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.*

Diindolylmethane (DIM) and several ring-substituted analogs exhibit aryl hydrocarbon receptor (AhR) agonist activities and inhibit growth of breast and other cancer cell lines. Substitution of the methylene bridging carbon with various substituted phenyl groups resulted in loss of AhR-dependent activity. However, several of the resulting C-substituted DIMs containing p-substituted phenyl groups (pPhX) inhibited growth of cancer cell lines *in vitro* and carcinogen-induced rat mammary tumor growth *in vivo*. A series of 1, 1-bis(3'-indolyl)-1-(p-substitutedphenyl) methanes (DIM-pPhX) were synthesized and screened as potential ligands for receptors that bind lipophilic compounds. DIM-pPhX compounds induced a structure-dependent activation of PPARγ in MCF-7 breast and several other cancer cell lines. Analogs with p-trifluoromethy, p-cyano, p-phenyl, p-t-butyl and p-dimethylamino substituents were active (5 - 20 μM), whereas p-methyl, p-methoxyl, p-hydroxy or p-halo-substituted compounds were inactive at similar concentrations. DIM-C-pPhCF$_3$ and other active analogs and 15-deoxy-Δ12, 14-prostaglandin J2 (PGJ2) exhibited similar activities; however, in mammalian two-hybrid assay using PPARγ and coactivator constructs, DIM-C-pPhCF$_3$ but not PGJ2 induced interactions with PPARγ coactivator-1 (PGC-1). Like PGJ2, the PPARγ active compounds inhibited MCF-7 cell growth and G_0/G_1 to S phase progression, downregulated cyclin D1 and estrogen receptor α proteins, and induced apoptosis. However, the comparative mechanisms of cancer cell growth inhibition and induction of apoptosis by C-substituted DIMs, PGJ2 and other synthetic PPARγ agonists were variable and dependent on cell context.

1228 THE AH RECEPTOR:DIVERSITY IN LIGAND BINDING AND BIOLOGICAL/TOXICOLOGICAL RESPONSES.

M. Denison[1], A. Padini[3], L. Bonati[3] and S. Safe[2]. *[1]Environmental Toxicology, University of California, Davis, CA, [2]Vet. Physiol. & Pharmacology, Texas A&M University, College Station, TX and [3]di Scienze dell, University of Milano-Bicocca, Milano, Italy.*

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that mediates many of the biological and toxic effects of chemicals, including that of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). The highest affinity AhR ligands include a variety of hydrophobic toxic and carcinogenic halogenated and polycyclic aromatic hydrocarbons, however, recent evidence demonstrates that the AhR can be bound and activated by a structurally diverse range of synthetic and naturally-occurring chemicals. While activation of the AhR and AhR-dependent gene expression by TCDD and other AhR ligands are known to produce a variety of toxic effects, anticarcinogenic/antiproliferative effects of AhR ligands have also been observed in rodent uterine and mammary tumors and in breast, endometrial, prostate and pancreatic cancer cell lines. Given the potential of AhR ligands as chemotherapeutic agents, we have carried out chemical screening in order to identify novel nontoxic selective AhR modulators (SAhRMs). High throughput screening assays using cell lines containing stably transfected AhR-responsive reporter genes has identified a variety of relatively potent new AhR agonists including a variety of flavones, benzoflavones, indirubins, tryptanthrins and related chemicals. The structural diversity of AhR ligands also suggests that the AhR contains a promiscuous ligand binding domain (LBD). Using a 3D homology model of the AhR LBD based on the crystal structures of homologous PAS family members that we developed we are attempting to identify and characterize the residues involved in AhR ligand binding. Structure-activity relationships of these toxic and nontoxic SAhRMs for AhR ligand binding and analysis of the binding of these diverse ligands within the modeled AhR LBD will provide insights into the mechanism of ligand-dependent activation of the AhR by toxic and nontoxic SAhRMs and can lead to the identification of new chemotherapeutic agents.

1229 NRF2, AN ANTIOXIDANT ACTIVATED CNC BZIP TRANSCRIPTION FACTOR: MECHANISM OF ACTION AND ROLE IN AUTOIMMUNE FUNCTION.

Q. Ma. *Toxicology and Molecular Biology Branch, CDC/NIOSH, Morgantown, WV.*

NF-E2 related factor 2 (Nrf2) is a member of the cap n collar, basic leucine zipper family of transcription factors. Nrf2 mediates gene regulation by a range of chemicals with diverse structures. Activation of Nrf2 by phenolic and other antioxidants involves redox signaling. Induction of phase 2 drug-metabolizing enzyme NQO1, which catalyzes two electron reductions of quinone and quinoid chemicals, is used as a model for analyzing mechanism of gene transcription by Nrf2. Biochemical and genetic evidence demonstrate Nrf2 is required for three types of transcription of the gene: the basal expression, induction by antioxidants, and induction by AhR ligands, suggesting it serves as a master regulator of multiple signal transduction pathways in the transcription of target genes. Loss of Nrf2 function by targeted gene knock out increases the sensitivity of mice and cells to toxicity of oxidative chemicals. Moreover, Nrf2 null mice develop an early-onset, Lupus-like, autoimmune syndrome, characterized by appearance of anti-double strand DNA antibodies in young adulthood (as early as 2 month of age), multi-organ inflammatory lesions, enhanced proliferation of lymphoid cells, deposition of immunoglobulin complexes in glomerular membranes, and death due to rapid progressing, diffuse membranoglomerular nephritis. Taken together, these findings suggest Nrf2 plays critical roles in maintaining cellular homeostasis to oxidative toxicants and in physiological surveillance of autoimmune functions.

1230 THE NRF1 TRANSCRIPTION FACTOR IN OXIDATIVE STRESS RESPONSE AND DEVELOPMENT.

J. Chan. *Pathology, University of California at Irvine, Irvine, CA.* Sponsor: Q. Ma.

Nrf1 is a transcription factor of the CNC bZIP family, which includes Nrf1, 2, 3, p45NFE2, Bach1 and Bach2. Knockout studies show that Nrf1 mutants die at late-gestation secondary to anemia and presumed fetal liver abnormality. The importance of Nrf1 in antioxidant gene expression was examined. Nrf1 mutant fibroblasts exhibited a low level of glutathione content and enhanced sensitivity to the toxic effects of oxidants. Moreover, these analyses revealed a direct role of Nrf1 in the regulation of genes important in glutathione synthesis. Nrf1 is broadly expressed during development, but defects in tissues other than liver were not apparent in mutants. To gain further insights into the function of Nrf1, we generated chimeric mice using ES cells deficient in Nrf1 to determine the cell type(s) in which Nrf1 is important during development. Characterization of the chimeric mice indicated that Nrf1 mutant ES cells contribute to most tissues in adult animals including those in which Nrf1 is normally expressed at high levels. Deficiency in Nrf1 is associated with impaired contribution of ES cells to adult but not fetal hepatocytes. While Nrf1 function is not required for normal fetal liver ontogeny, liver cells in chimeric embryos undergo apoptosis late in gestation, suggesting a cell type and developmental stage- specific requirement for Nrf1 in protecting hepatocytes from apoptosis during development. Our findings suggest that Nrf1 plays an important role in maintaining redox balance in the fetal liver cells through regulating antioxidant gene expression. As oxidative stress is thought to play an important role in promoting cell death in response to various stimuli, these findings draw attention to the potential importance of Nrf1-antioxidant pathway in various pathological conditions.

1231 DEFENSE AGAINST ZINC AND CADMIUM TOXICITY.

R. D. Palmiter[1, 2]. [1]*Biochemistry, University Washington, Seattle, WA and* [2]*Howard Hughes Medical Institute, University of Washington, Seattle, WA.* Sponsor: Q. Ma.

Zinc is an essential cofactor of some metalloenzymes and a structural component of thousands of zinc-finger proteins. Zinc enters cells *via* zinc-influx transporters and calcium channels but at high concentrations it becomes toxic to cells. Cells can cope with excess zinc by transporting it out of the cell *via* zinc-efflux transporters, sequestering it in intracellular compartments or by inducing proteins such as metallothioneins that bind it tightly. Exposure of cultured cells or mice to high levels of zinc results in the induction of metallothioneins and the zinc efflux transporter, ZnT1. Induction of these proteins depends on the metal-responsive transcription factor, MTF-1. Loss of either Znt1 or Mt gene function in cultured cells results in sensitivity to zinc toxicity, whereas resistance is conferred by amplification of either of these genes. Mice lacking either Mtf1 or Znt1 gene function die as embryos, but mice lacking Mt 1 and Mt2 genes are *via*ble. A large family of membrane proteins related to ZnT1 facilitates the sequestration of zinc in various cellular compartments, including Golgi complex, secretory granules, endosomes and synaptic vesicles. Zinc in these compartments probably serves specific biological functions but it may also protect against zinc toxicity. Cadmium probably enters cells *via* the same routes as zinc but no transporters are known that transport cadmium out of the cytoplasm. Cadmium is toxic because it displaces zinc in critical metalloproteins. The major mechanism of defense against cadmium toxicity is induction of metallothioneins.

1232 OVERVIEW OF THE ILSI "AGRICULTURAL CHEMICAL SAFETY ASSESSMENT" PROJECT.

N. G. Carmichael. *Toxicology, Bayer CropScience, Sophia-Antipolis, France.*

This workshop will present and debate a series of proposals which result from a fundamental re-examination of the process of evaluating the safety of agricultural chemicals. This project has had the support and active participation of internationally recognised expert scientists from industry, international government agencies and academia. The intention was to propose a scheme which would provide a robust evaluation of these chemicals while reducing wasteful use of animals and other resources. Since October 2002 three multisector task forces have been working on proposals for a tiered approach to the evaluation of toxicology dossiers, guided by existing precedents for the evaluation of other types of chemicals and taking into accounts the specificity of agricultural chemicals. Thus, the approach is oriented towards the risk assessments which are dictated by the use of these products and the emphasis on hazard is decreased. The three groups were charged to look at potentially vulnerable life stages, the design of the systemic toxicity evaluation package and the role of ADME in study design and choice. The integration of ADME into the toxicology package is seen as key to a more modern and appropriate approach to study design and interpretation. Availability of good data from such studies is intended to permit a well informed choice of studies and a justification for avoiding unnecessary use of animals and other resources. As for any tiered evaluation, the triggers for higher tiers are critical and require thought and debate. The scheme allows the efforts to be concentrated on the studies which will be needed to perform critical risk assessments and suggests study designs that are most appropriate.

1233 DEVELOPMENT OF ADME DATA IN AGRICULTURAL CHEMICAL SAFETY ASSESSMENTS.

T. Pastoor[1] and H. Barton[2]. [1]*Syngenta Crop Protection, Greensboro, NC and* [2]*EPA Office of Research and Development-NHEERL, Research Triangle Park, NC.*

A multi-stakeholder series of discussions developed a set of general considerations, recommendations, and specific strategies to help guide generation of useful pharmacokinetic (PK) data in agricultural chemical testing. The value of this information is predicated on determining the relationship between the concentration of free compound in plasma and the biological/toxicological response. Careful, tier-wise collection of PK data would better define dose across route, frequency, and duration of exposure as well as across species and life stages. The recommendations include: Develop basic kinetic data early, before or in conjunction with initial toxicity testing. Generate more focused kinetic data based on results of endpoint-specific toxicity studies. Once toxicity data become available, then the kinetics data should be revisited to aid in interpretation and development of an understanding of mode of action (MOA) and dose-response relationships. More focused kinetic data will define a systemic dose (versus administered dose) and provide a logical means to establish route-specific reference doses. Collect appropriate kinetics data from repeated toxicity studies. The purpose of such studies is to clearly understand the disposition of a compound in the body, improve understanding of its disposition after multiple dosing and, ultimately, to be able to define systemic dose for a variety of dosing scenarios. Obtain human kinetics data to validate and compare with animal data. Kinetic studies, coupled with PK modeling, converts administered dose into an "internal" dose that can be compared from animals to humans. Integrate Dose and Response. Tying tissue dose metrics and mode of action (MOA) together has the potential to lead to more rational and accurate health risk assessments.

1234 INCORPORATING LIFE STAGE TESTING INTO AGRICULTURAL CHEMICAL SAFETY ASSESSMENT.

J. C. Lamb. *BBL Sciences, Reston, VA.*

The ILSI HESI committee has undertaken a complete re-examination of how to test for special sensitivity of life stages, while reducing the number of studies and test animals required. Current testing strategies result in thousands of laboratory animals including mice, rats, rabbits and dogs for the approval by regulatory agencies of a crop protection chemical that will be used on food crops. The committee has developed a strategy that will substantially decrease animal use, without compromising confidence that sufficient testing has been performed. The committee has developed a hierarchy of study types, endpoints, and triggers for a tiered approach that would ensure coverage of life stages. While low exposure and low toxicity would necessitate only a basic testing package, either higher exposures or evidence of higher toxicity would lead to additional testing. This is similar to the strategy already being applied by the US Food and Drug Administration. Through assessment of available study design alternatives, recommendations were made concerning the form and scope of successive tiers, including the use of exposure estimates at certain decision points in safety assessment. Options for incorporating pharmacokinetic and metabolism data are considered throughout the tiered approach. The proposed strategy will save animals, resources, while providing adequate information on life stage susceptibility.

1235 INCORPORATING SYSTEMIC TOXICITY TESTING IN AGROCHEMICAL SAFETY ASSESSMENT.

A. Moretto. *Environmental Medicine and Public Health, Universita di Padova, Padova, Italy.*

A decision tree for systemic toxicity endpoints to be used for the safety assessment of agrochemicals has been developed by the ILSI HESI Committee. The proposal was based upon examination of existing toxicological databases, and criteria and end-points used to define NOAELs and consequently to establish the reference values. The proposal takes into account recent advances in biological and toxicological sciences. The latter include the increased specificity and sensitivity of analytical methods, a better understanding of the mechanisms/mode of toxic action of chemicals, the characterisation of the time-courses and/or steps during the development of toxic lesions. In addition, consideration of the use patterns of the agrochemical was also taken into account. On this basis a tiered approach has been proposed. The

first tier comprises a set of core studies based on use patterns (i.e.: foreseeable duration and characteristics of human exposure). All basic study designs should include full clinical and pathological evaluation of animals, as well as a recovery group. The approach will be that of identifying key markers for specific effects and triggers for next tiers. In fact, other additional studies would be triggered based on evidence of toxicological concern from the shorter-term studies, and from data of the absorption, distribution, metabolism and excretion studies (ADME). More detailed ADME data than those currently requested would help in designing repeated dose studies (especially in dose selection, duration of exposure, route of exposure, life-stage, and species) and may be used to justify the waiving of some other studies. The proposed approach will lead to generation of data more suited to risk assessment, better identification of key effects, less use and improved welfare of animals and more efficient use of resources by regulatory authorities and industry.

1236 AN EPA PERSPECTIVE: A NEW TESTING PARADIGM FOR PESTICIDES.

V. Dellarco. *Office of Pesticide Programs, USEPA, Washington DC, DC.*

The current testing requirements for pesticides were put in place over 20 years ago. Since the promulgation of FQPA, the data needs for pesticides are changing with increased emphasis on, for example, life stage sensitivities, mechanisms of toxicity, and the effects of chemical mixtures. Thus, there is a need to improve and revise the toxicity testing of pesticides to ensure that data supporting pesticide registrations will represent the best science. Through the efforts of the ILSI HESI Technical Committee on Agricultural Chemical Safety Assessment, a tiered testing approach is proposed that provides an efficient and thorough assessments of potential human health risks. The ILSI HESI project represents an international perspective, which is important to EPA, given the Agency's commitment to international harmonization of testing guidelines. The proposed testing approach utilizes available information about each chemical and its toxicity to design a rational, science-based assessment strategy. The program design includes the need to reduce animal usage, the need to include new methods, the need to modify existing studies to provide coverage of life stages, a more complete and systematic evaluation of toxicokinetics, and a more focused evaluation of the potential toxicities of pesticides. Although there will be future advancements in toxicology testing (e.g., genomics and proteomics), the ILSI HESI project provides important near-term recommendations for improving the testing of pesticides.

1237 HISTOMORPHOLOGY AND BEYOND: CORRELATING NON-CLINICAL IMMUNE MODULATION WITH CLINICAL DATA.

J. Schuh[1] and L. Reid[2]. *[1]Applied Veterinary Pathobiology, PLLC, Bainbridge Island, WA and [2]Division of Reproductive and Urologic Drug Products, USFDA, Rockville, MD.*

Histopathology is an important component for assessing immunomodulation as part of immunotoxicology profiling in non-clinical studies. Histomorphological evaluation of lymphoid organs and tissues in animals captures the accumulation of both background and treatment-specific immunomodulation and careful evaluation can provide evidence of altered immune function. However, immunomodulatory changes in tissues need to be interpreted in the context of variation due to genetic modifiers, stress and degree of environmental antigenic exposure. In the last few years, experiences with expanded immunotoxicology testing have resulted in availability of data sets that allow correlation of histomorphology to functional data and clinical studies. Additionally, compartments of the mucosal immune system are also beginning to be more thoroughly analyzed. Many challenges remain in our efforts to fully understand non-clinical immunohistology relative to functional data. The increasing need for immunotoxicology assessment as part of regulatory submissions reinforces our need to continually improve our abilities to correctly interpret and apply immunotoxicology information. Periodic reassessment of completed and ongoing immunohistology and immune function studies is important to monitoring the robustness of testing guidelines, directing testing modifications and to establish predictive value of animal studies for clinical studies. The purpose of this workshop is to present and examine several available data sets that have evaluated histomorphology and immunomodulation in animals and resulting correlations to clinical data. Representative data sets from immunomodulation subsequent to chemical and pharmaceutical exposure conducted by laboratories in The Netherlands and by the NTP, USFDA and ILSI will be highlighted. Discussions on these current and ongoing experiences with correlative data sets will be relevant to individuals involved in gathering and evaluating data in all aspects of the rapidly progressing field of immunotoxicology.

1238 IMMUNOTOXICOLOGY AND THE MUCOSAL IMMUNE SYSTEM.

C. F. Kuper. *TNO Nutrition and Food Research, Zeist, Netherlands.* Sponsor: J. Schuh.

Aside from histomorphological examination of Peyer's patches (PP), mesenteric lymph nodes (MLN) and secretion of immunoglobulins, immunotoxicology studies pay little attention to the mucosal immune system. On the one hand this is hard to understand because a). mucosae are most often the first site of contact for xenobiotics, b). mucosa-associated lymphoid structures (MALT) such as bronchial (BALT) and nasal (NALT) structures and intestinal cryptopatches are strategically situated to have extensive contact with inhaled or ingested substances, and c). T-lymphocytes disseminated diffusely in the epithelium (IEL) and lamina propria (LPL) of the mucosae form the body's largest T-lymphocyte pool. It is questionable whether PP and MLN can be used as sole representatives of the mucosal immune system because the various organized mucosa-associated lymphoid structures differ from each other and the single T-lymphocyte pool is quite unique in phenotype and function. Moreover, the lymphocyte maturation process in the intestinal epithelium may be particularly sensitive to immunotoxins, as it is in the thymus. On the other hand, the mucosal lymphoid structures and the IEL and LPL are difficult to sample and examine, which may have attributed to the scarcity of reported toxic effects on the mucosal immune system. Nevertheless the rise in incidence of allergic reactions, especially those expressed in the respiratory tract, the increasing use of nasally applied medicines and enhanced studies with the respiratory allergens trimelllitic anhydride (TMA) and toluene diisocyanate (TDI), the immunosuppressive drug azathioprine, the industrial substance hexachlorobenzene (HCB) and the food contaminant vomitoxin (deoxynivalenon or DON) demonstrate the need to address the mucosal immune system in immunotoxicology studies more extensively than is currently done.

1239 PROTOCOLS AND VALIDATION STUDIES OF HISTOPATHOLOGY AND IMMUNE FUNCTION IN NON-CLINICAL STUDIES.

J. G. Vos. *Laboratory of Pathology and Immunology, National Institute for Public Health and the Environment, Bilthoven, Netherlands.*

The toxicologic pathology discipline plays an important role in the process of identifying and defining the health effects following exposure to xenobiotics. This is also true for the identification of immunomodulatory agents as part of the toxicologic profiling. In assessing immunomodulation, a two-tier testing system is usually employed in which the first tier is a general screen for (immuno)toxicity including enhanced histopathology of lymphoid organs and the second tier consists of specific immune function studies including host resistance tests or mechanistic studies. Studies with TCDD, TBTO, HCB, azathioprine and cyclosorin A will be discussed that provided data correlating histopathology with induced immune function changes. This will be followed by a discussion of i) the outcomes of enhanced histomorphology in the interlaboratory validation studies of azathioprine, cyclosporin A and HCB in the rat; and ii) the recent study of a working group of veterinary/toxicologic pathologists evaluating the sensitivity and predictability of extended histopathology in the mouse as an indicator of immunotoxicity, as compared with the NTP's functional testing battery. In the latter study, standardized slide sets from thymus, spleen and mesenteric lymph nodes were generated for 11 chemicals that had previously been evaluated for their immunotoxicity using functional tests. While overall there was good agreement between histopathology and functional tests, the antibody forming cell (AFC) assay detected immune suppression in two instances where no changes in pathology were indicated. In contrast, the AFC assay failed to detect oxymetholone as immunotoxicant, although extended histopathology indicated immunologic changes. Data collected over the past two decades on these studies in rodents have been the basis for a number of regulatory activities. These will be discussed and suggestions will be given to further improve the identification and (semi)quantitation of histopathologic changes in lymphoid organs and tissues.

1240 REGULATORY IMPLICATIONS OF NON-CLINICAL IMMUNOTOXICOLOGICAL FINDINGS DURING THE DRUG DEVELOPMENT PROCESS IN THE UNITED STATES.

D. Mellon. *Division of Anesthetics, Critical Care and Addiction Drug Products, Center for Drug Evaluation and Research, US Food and Drug Administration, Rockville, MD.* Sponsor: L. Reid.

Assessment of the potential for compounds to alter the immune system is critical to the logical and cost-effective development of new therapeutics. Although, extensive research in the field of immunotoxicology has provided essential clues to the mech-

anisms of adverse drug reactions, a unified consensus on an appropriate manner to assess changes in the immune system as part of drug development process is just beginning to be developed. To date, global harmonization has not yet been attained. The US Food and Drug Administration has published a Guidance for Industry titled "Immunotoxicology Evaluation of Investigational New Drugs" which can be found on the FDA website (http://www.fda.gov/cder/guidance/4945fnl.PDF). This presentation will discuss the rationale behind the FDA's approach toward immunotoxicology assessment, as described in the guidance. FDA ascertains that evidence of immunotoxicity can usually be observed in standard non-clinical toxicology studies. However, in some cases, additional studies are recommended. To illustrate this approach, several examples of how detailed immunohistological assessment of lymphoid tissues can be used to assess immunotoxicity will be presented. The circumstances when additional non-clinical studies should be conducted will be described.

1241 CONCORDANCE OF ANIMAL TOXICITY AND SAFETY PHARMACOLOGY DATA WITH HUMAN TOXICITIES FOR THERAPEUTIC AGENTS: FOCUS ON THE IMMUNE SYSTEM.

M. P. Holsapple[1], K. Thomas[1], J. Sanders[2] and E. Kadyszewski[3]. *[1]Health and Environmental Sciences Institute, International Life Sciences Institute, Washington, DC, [2]Aventis Pharmaceuticals, Bridgewater, NJ and [3]Pfizer Central Research, Groton, CT.*

There is a widespread perception that laboratory animals are appropriate models for predicting the adverse effects of chemicals in humans. For many classes of chemicals, testing this assumption is not practical. By contrast, for pharmaceuticals, an extensive body of relevant data exists to explore the validity of this assumption. Consequently, the ILSI HESI Non-Clinical / Clinical Safety Correlations Technical Committee is building a database to develop an improved understanding of the extent to which various types of human toxicities (HTs) could be predicted from standard toxicology studies. The database is being developed to evaluate the concordance of animal data with HTs exhibited during clinical trials for a number of pharmaceuticals. The database will also help to determine whether the range of preclinical tests that are currently performed produce data that are relevant in the design and/or interpretation of human studies. To establish a comprehensive measure of the utility of non-clinical animal studies for human risk assessment, the project features a prospective collection of compounds entering the *in vivo* toxicology stage of drug development, which ensures studies have been conducted according to modern guidelines. In addition, the details of those toxicities manifested in animals that are considered to be counterparts of the human clinical toxicity profile will be fully documented. The database also captures toxicokinetic and metabolic interspecies comparison data. Ultimately, the database provides a unique forum for examining the value of animal studies as surrogates for predicting the toxicity of drugs in humans and addressing how non-clinical tests can be optimized to improve drug safety. The presentation will focus on the existing dataset for immune system toxicity, and will provide a comparison of the concordance of immune system toxicity with toxicities in other organ systems.

1242 ZEBRAFISH-A MODEL ORGANISM FOR ASSESSING DEVELOPMENTAL TOXICITY IN DRUG DISCOVERY/ENVIRONMENTAL RISK ASSESSMENT.

R. R. Dugyala[1] and J. M. Rogers[2]. *[1]Reproductive Toxicology, Schering-Plough Research Institute, Lafayette, NJ and [2]Reproductive Toxicology Division, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC.*

Several organisms such as the worm (C. elegans), fly (D. melanogaster), frog (X. laevis) and zebrafish (D. rerio) have been discussed as alternative models for assessing developmental toxicity of environmental agents in higher mammals. Zebra fish seems to be an appropriate model for several reasons. It is a vertebrate, its genetics and development is well understood and transgenics/knockdowns can be easily made. The zebrafish life cycle is short therefore; its specific developmental phenotypes can be screened effectively. Zebrafish are easily bred throughout the year in the laboratory and individual females can give rise to hundreds of progeny on a year-round basis. Several zebrafish mutants are representative of known forms of human genetic disorders. In addition, its genomic sequence will be available from the Sanger Sequencing Center and this has already allowed easy isolation and identification of gene regions. Commercial sources of DNA libraries for microarray analysis are now available. Moreover, they can be easily exposed to environmental agents; can be used to screen environmental agents that have developmental risk in a semi or high-throughput manner. Mechanistic data involving specific genes/pathways can also be effectively generated to help better estimate the human developmental risk. Therefore, zebrafish can be an effective alternative model in the drug discovery process or environmental risk assessment.

1243 TRANSGENESIS, MICROARRAY ANALYSIS AND ANTI-SENSE KNOCKDOWNS OF ZEBRAFISH GENES-TOOLS FOR USING ZEBRAFISH AS A TOXICOLOGICAL MODEL.

E. Linney[1], L. Upchurch[1], S. Donerly[1], Q. Xhao[1], C. Lassiter[1] and E. Levin[2]. *[1]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC and [2]Department of Psychiatry, Duke University Medical Center, Durham, NC.*

There are distinct advantages to using zebrafish as an experimental, embryonic, vertebrate model: 1)it develops outside the mother as a microscopically viewable organism; 2)the DNA sequencing of the genome by the Sanger Centre is providing sequence and gene information which allows the generation of large microarrays for the analysis of gene expression during normal development and after toxicant challenge; 3)a growing number of transgenic lines of fish which express fluorescent marker proteins in response to small molecules or in specific structures(such as the developing nervous system) allows the continuous visual monitoring of changes produced by toxicant challenge; and 4)the availability of anti-sense morpholino technology allows one to combine the above advantages with selective down-regulation of specific genes. These advantages plus the sharing or regulatory pathways observed in mammalian models allows one to scan the breadth of development at various levels under normal conditions and after exposure to environmental toxicants. Transgenic models will be presented to illustrate their potential use in toxicant studies. Specifically, an example of the combined use of these technologies in exploring the effects of chlorpyrifos exposure, the effects of exposure upon acetylcholine esterase, and resulting behavioral changes in the treated, adult fish will be presented. (Supported by NIEHS Superfund and Toxicogenomics Consortium Programs).

1244 ZEBRAFISH AND DIOXIN DEVELOPMENTAL TOXICITY: POISED TO IDENTIFY CRITICAL GENES FOR SPECIFIC ENDPOINTS.

R. E. Peterson[1], A. L. Prasch[1], E. A. Andreasen[2], R. L. Tanguay[2] and W. Heideman[1]. *[1]School of Pharmacy, Molecular and Environmental Toxicology, University of Wisconsin, Madison, WI and [2]Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Zebrafish have emerged as the premier teleost species for use in molecular, genetic, and developmental studies and may reveal novel mechanisms of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (dioxin) developmental toxicity applicable to human health risk assessment. Endpoints of dioxin toxicity in the zebrafish embryo include impaired cardiac development, reduced peripheral blood flow, anemia, edema, and impaired jaw development. Some of these endpoints have also been observed in a*via*n and mammalian embryos. Since most, if not all, endpoints of dioxin toxicity are generally considered to be mediated by the aryl hydrocarbon receptor (AHR), components of the AHR signal transduction pathway have been identified and characterized in zebrafish. In contrast to mammals, zebrafish have two AHRs (AHR1 and AHR2) that are products of two distinct genes. Transcripts of both AHRs are expressed in the zebrafish embryo and larva but AHR2 mRNA is far more abundant. AHR nuclear translocator (ARNT), and the AHR interacting protein (AIP), AHR repressor (AHRR), and CYP1A have also been cloned in zebrafish. To determine the role that each zebrafish AHR may play in dioxin toxicity, their functional properties were determined revealing that AHR2 supports high affinity binding of dioxin whereas AHR1 does not, AHR2 supports sequence-specific binding to mammalian AHRE sequences but AHR1 binding is weak, and AHR2 supports dioxin induction of reporter gene expression while AHR1 does not. These findings suggest that AHR2, not AHR1, is involved in dioxin developmental toxicity. This interpretation is supported by morpholino experiments where AHR2 repression protects dioxin-exposed zebrafish embryos from toxicity. (Supported by UW Sea Grant and NIEHS)

1245 NICOTINE-INDUCED DEVELOPMENTAL TOXICITY IN ZEBRAFISH.

R. L. Tanguay[1], K. R. Svoboda[2] and S. Vijayaraghavan[3]. *[1]Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, [2]Department of Biological Sciences, Louisiana State University, Baton Rouge, LA and [3]Department of Physiology and Biophysics, University of Colorado Health Sciences Center, Denver, CO.*

Exposure of the developing fetus to nicotine from the maternal serum has been linked to a number of physical abnormalities including; an increase in induced abortions, low infant birth weights, increased need for neonatal intensive care units, perinatal disorders and deaths. Furthermore, there is increased concern that fetal exposure to nicotine is also associated with significant cognitive, intellectual and

behavioral impairments. At this time there is a gap in our understanding of precisely how nicotine leads to these serious consequences. It is accepted that the actions of nicotine are mediated *via* the activation of neuronal nicotinic acetylcholine receptors (nAChRs). The presence of both ACh and nAChRs during embryogenesis also suggest that nAChRs play a significant role during development. We are using zebrafish as a model system to gain insight into how nicotine exposure alters neuronal development and behavior. Transient exposure of embryonic zebrafish to nicotine delays development of secondary spinal motoneurons. Furthermore, there is a long-lasting alteration in axonal pathfinding in secondary motoneurons that is not ameliorated by drug withdrawal. Mammalian nicotinic receptor antagonists reversed these effects of nicotine. At the behavioral level, continuous exposure of embryos to nicotine beginning at 12hpf abolished spontaneous bending of the axial musculature, which occurs between 17hpf and 24hpf. On the other hand, transient exposure of embryos to nicotine between 22hpf and 30hpf induces a swimming-like behavior. Overall, our results show that transient embryonic exposure to nicotine leads to long lasting effects on the developing vertebrate nervous system. These results also demonstrate that the zebrafish is a useful model system to examine the effects of nicotine specifically, and drugs of abuse in general, on the development of the central nervous system in vertebrates.

1246 ZEBRAFISH AS A MODEL FOR SMALL MOLECULE SCREENING/DISCOVERY.

R. T. Peterson, D. Milan, C. MacRae, T. Peterson and M. Fishman. *Cardiovascular Research Center, Massachusetts General Hospital, Charlestown, MA.* Sponsor: R. Dugyala.

The transparency of the zebrafish embryo makes it possible to rapidly identify chemical perturbations of organ structure and function. As a result, high-throughput chemical screens can be performed to characterize systematically the biological effects of thousands of small molecules. We have used this chemical screening approach in several ways. First, we have identified small molecules that cause cardiovascular malformations. After screening through more than 10, 000 small molecules, we have identified dozens of compounds that affect cardiac size, shape, or function. By database comparisons and structure activity relationship studies, we have identified relationships between chemical substructures and the malformations they cause. We are identifying the mechanisms of action of several of these compounds. Second, we have developed automated assays for identifying small molecules that affect cardiac physiology. These assays can be used to predict the cardiotoxic effects, including QT prolongation, of hundreds of drugs and drug combinations. Finally, we have used high-throughput chemical screening to identify small molecules that reverse the effects of a genetic mutation. Compounds identified in this screen suppress a genetic vascular defect by upregulating expression of vascular endothelial growth factor. Therefore, zebrafish small molecule screens provide a systematic means of linking chemical structures with their biological activities, with applications ranging from developmental toxicology to drug discovery.

1247 EVALUATING THE TOXICITY OF ENVIRONMENTAL SAMPLES USING ZEBRAFISH DEVELOPMENTAL ENDPOINTS AND ALTERATION OF GENE EXPRESSION.

M. J. Carvan, B. A. Wimpee, T. King-Heiden, E. J. Loucks and K. A. VanDerel. *Great Lakes WATER Institute and Marine & Freshwater Biomedical Sciences Center, University of Wisconsin-Milwaukee, Milwaukee, WI.*

The dynamic progression of development often makes the embryonic and larval stages of vertebrates exquisitely sensitive to environmental stressors, including toxic chemicals. Toxic environmental chemicals often perturb interdependent pathways that regulate developmental processes in a manner that is both characteristic and predictable. Many of the most common developmental abnormalities in zebrafish are easily identified by careful observation of live embryo/larvae. These abnormalities include: delayed development, edema, pooling of blood, and a number of gross abnormalities generally of skeletal origin that involve the craniofacial and neurocranial skeleton, spine, and fins. Morphometric analysis of stained cartilaginous structures in the larval skeleton reveals very subtle teratogenic effects resulting from exposure to very low concentrations of toxic chemicals. Staining of apoptotic cells in live embryos localizes tissue-specific toxic affects that otherwise don't become apparent until much later in life. Utilization of transgenic fish expressing tissue-specific green fluorescent protein (GFP) greatly simplifies analysis of cardiovascular deficits in living larvae. We are also developing transgenic lines of zebrafish in which DNA motifs that respond to selected classes of environmental pollutants induce a GFP reporter gene. A battery of fish with different pollution-inducible enhancers will provide a very sensitive bioassay for the identification of specific chemical classes in a complex environmental mixture. Subsequent gene expression analysis using microarrays or real-time RT-PCR provides sufficient data for the identification of specific chemical(s) to which the GFP-expressing embryo/larvae have been exposed. In summary, integration of all these endpoints will identify bioavailable toxic chemical species within a complex environmental mixture, and provide strong biologically-based support for environmental risk assessment.

1248 INDIVIDUAL VARIATIONS IN CIGARETTE MAINSTREAM SMOKE BIOMARKERS OF EXPOSURE.

C. J. Smith, M. J. Morton, B. G. Brown, D. W. Bombick, D. L. Heavner, M. W. Ogden, J. H. Robinson and D. J. Doolittle. *Research and Development, RJ Reynolds, Winston-Salem, NC.*

Biomarkers of tobacco smoke exposure were measured in urine, buccal cells and buccal cell mouthwash supernatants from 56 smokers of full-flavor low "tar" (FFLT) cigarettes to evaluate the degree of individual variation. Biomarkers were measured while each individual smoked their usual brand of FFLT cigarette (UB) and after all smoked the same leading commercial FFLT cigarette brand (LC). Twenty-four hour urine collections and spot buccal mouthwash samples were taken while smoking each cigarette. Biomarkers were determined for nicotine; benzene; acrolein; the tobacco-specific nitrosamine 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); the PAH surrogate marker 1-hydroxypyrene (a metabolite of pyrene); oxidative stress (8-hydroxydeoxyguanosine [8-OHdG] and 15-F2t -isoprostane-M); carbonyl protein adducts; and the 1, 3-butadiene metabolites monohydroxybutenyl mercapturic acid (MHBMA) and dihydroxybutenyl mercapturic acid (DHBMA). The standard de*via*tions of the logarithms of the biomarker measurements did not differ while smoking the UB or LC cigarettes, indicating there was not a statistically significant difference in biomarker variability when smokers used their usual brands of FFLT or all smoked the same FFLT cigarette. Intra-subject variability in biomarker concentrations was minimal between the two cigarettes while inter-subject variability was quite large, typically spanning one or more orders of magnitude. These data demonstrate the wide range of individual exposures to cigarette mainstream smoke components experienced when different individuals smoke the same cigarette.

1249 LARGE WITHIN CHILD VARIABILITY FOR OP PESTICIDE URINARY BIOMARKERS LIMITS OUR ABILITY TO IDENTIFY HIGH EXPOSURE FARM WORKER CHILDREN.

W. C. Griffith[2, 1], C. L. Curl[1], E. M. Faustman[2, 1], C. A. Li[1] and R. A. Fenske[1]. *[1]DEOHS, University of Washington, Seattle, WA and [2]Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, WA.*

The diakyl metabolites of organophosphate (OP) pesticides have been measured in the urine of children by a number of studies. Interpretation of measurements of these urinary metabolites about population and individual exposures is complicated since a number of OP pesticides produce the same metabolites. Estimates of seasonal variability and intra and inter individual variability have been made from a longitudinal study (Koch, et al, Environmental Health Perspectives, 2002). Children, 2 to 5 years of age, of agricultural orchard workers were recruited in the Yakima Valley through a Women, Infants, and Children clinic, and 44 were followed over a 21month period with twice monthly urine measurements. Statistical analysis of levels of the diakyl OP metabolites in urine is complicated because a large fraction of the urinary metabolite levels were below the limit of detection. Statistical methods for censored data were developed for a maximum likelihood mixed effects model. The seasonal trends in the data showed a decrease in the second year in OP metabolite levels because of decreased pesticide use due to unusually cold weather, with higher dimethyl metabolites during the spray seasons. The variance in urine concentrations within children is over an order of magnitude larger than the variation between children. The large within child variance has implications for using diakyl metabolites to distinguish which children in a population may be more highly exposed. When metabolite concentrations are measured on only one or a few days the large within child variability makes it difficult to judge which children are more highly exposed. Conclusions can be drawn about the distribution of exposures for a population but not about whether the exposure of a particular child is high or low in relation to the rest of the population. Support for this work was from the Center for Child Health Risk Research NIEHS (PO1 ES09601) / EPA (R826886).

1250 A REVERSED PHASE HPLC METHOD FOR THE DETERMINATION OF EPOXIDES OF 1, 3-BUTADIENE AND OTHER PETROCHEMICAL ALKENES.

B. Kandlakunta, J. L. Allison and R. M. Uppu. *Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA.* Sponsor: J. Ward.

1, 3-Butadiene (BD) is an environmental pollutant widely distributed in petrochemical industrial areas in the US. A recent evaluation by IARC indicates that BD is a probable human carcinogen falling under group 2A. 1, 3-Butadiene is metabo-

lized by enzymes of the cytochrome P450 system, forming electrophilic metabolites, 1, 2-epoxy-3-butene (EB) and 1, 2;3, 4-diepoxybutane (DEB), which, in turn, mediate the toxicity of BD. During the course of a study undertaken to examine the role of oxidative stress and cell signaling in the establishment of cellular injury by BD, a need arose for the development of a rapid and convenient method for the estimation of EB and other epoxides. Here we describe a new reversed phase HPLC method to quantify low levels of epoxides of BD and other alkenes in cell culture. The method involves derivatization of epoxides using a 100-1, 000-fold molar excess of N, N-diethyldithiocarbamate (DTC) at 70 °C for a period of 10 min as a first step. The excess, unreacted DTC is then decomposed (to carbon disulfide and diethyl amine) by acidification with phosphoric acid (pH_{final} *ca.* 1-2). Finally, a suitable aliquot of the derivatized sample is analyzed by HPLC using a ODS column (4 mm x 150 mm; Supelco) with 40% acetonitrile in water as mobile phase and the system is programmed for a flow of 1 mL/min. The HPLC analysis is performed using a Waters 660 liquid chromatograph equipped with a Waters 996 diode array detector. Upon monitoring at 278 nm, we find linearity in the concentration range of 0.1 to 100 μM. The present method is highly reproducible with recoveries ≥96% of spiked samples, and as low as 5 pmol of the analyte could be successfully detected. (Funding support from NIEHS grant ES10018 is acknowledged. Corresponding author: Rao M. Uppu. E-mail: rao_uppu@cxs.subr.edu. Fax: 225/771-5350.)

1251 DEVELOPMENT OF AN LCMS METHOD FOR THE QUANTITATIVE MEASUREMENT OF AFLATOXIN B_1 SERUM ALBUMIN ADDUCTS.

P. F. Scholl[1], L. McCoy[2], R. Schleicher[2] and J. D. Groopman[1]. *[1]Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD and [2]National Center for Environmental Health, Inorganic Toxicology and Nutrition Branch, Centers for Disease Control and Prevention, Atlanta, GA.*

The lysine adduct formed by the reaction of aflatoxin B_1 (AFB_1) epoxide or dialdehyde with serum albumin is an established biomarker of AFB_1 exposure. HPLC with fluorescent detection, RIA and ELISA methods were previously used to detect this adduct in rats and human molecular epidemiologic studies. Here we report a new synthetic technique for preparing lysine-AFB_1 standards and an LCMS method for the quantitative measurement of lysine-$AFB_{1|}$ in serum samples. Nα-t-Boc-L-lysine-AFB_1 was synthesized by the reaction of AFB_1 dialdehyde with Nα-t-Boc-L-lysine. Deprotection was performed using 40% TFA. D4, 4, 5, 5-L-lysine-AFB_1 was prepared *via* the reaction of AFB_1 dialdehyde with Cu^{2+}(L-lysine)$_2$. Blood samples (100 μL) were drawn from rats dosed with AFB_1. Serum samples were spiked with deuterated lysine-AFB_1 as an internal standard and enzymatically digested. Digests were desalted using C18 solid phase extraction cartridges. Samples were eluted with MeOH and directly injected onto a cyano HPLC column (2 x 150 mm) for positive ESI-MS and fluorescent detection. Complete deprotection of Nα-t-Boc-L-lysine-AFB_1 was obtained in 2 hours without loss of the product lysine-AFB_1. Solid phase extraction recovery of lysine-AFB_1 was 85%. LCMS analysis was performed in 15 min and enabled the detection of lysine-AFB_1 in the serum of rats dosed with AFB_1. Optimization of LCMS assay conditions is expected to enable the high throughput and quantitative measurement of approximately 1 pg lysine-AFB_1 in 100 μL blood samples.

1252 CYCLIN AND HOX GENE EXPRESSION ASSOCIATED WITH DRUG-INDUCED BILIARY HYPERPLASIA AND CELL PROLIFERATION IN CYNOMOLGUS MONKEYS.

C. M. Bral, F. M. Goodsaid, R. J. Smith, F. Poulet and I. Y. Rosenblum. *Genetic and Molecular Toxicology, Schering-Plough Research Institute, Lafayette, NJ.*

There are no predictive biomarkers for bile duct hyperplasia (BDH) for pre-clinical or clinical applications. BDH is a consequence of cell proliferation within the hepatobiliary tree. The expression of cyclin genes can be used as a marker for cell proliferation. Expression of Hox genes is associated with tissue differentiation and with the activation of growth-regulating cyclins. We have measured by quantitative RT-PCR (qRT-PCR) cyclin and hox gene expression changes associated with BDH and cell proliferation in Cynomolgus monkeys treated with an experimental S-P compound. BrdU incorporation at days 7, 14 and 28 was measured for monkeys receiving a daily dose of this compound and for days 21 and 42 for monkeys in recovery groups. Peak BrdU incorporation and BDH concurrent with BrdU incorporation were detectable in the day 14 group. qRT-PCR measurements of these samples were made for 21 cyclin and 41 hox genes. All of the 21 cyclin amplicons and 24 of the hox gene amplicons were induced in a preliminary screen against a pool of samples from active lesions (concurrent BrdU incorporation and BDH). Gene expression measurements for samples from all the monkeys in this study were made for genes shown to be induced in this screen. Cyclins E2 and E2v2 genes showed dose-response but no correlation with BrdU incorporation. Cyclins A, B and D1 genes showed both dose-response as well as a peak signal preceding that for BrdU incorporation. Cyclins A and D1 showed peak induction on day 7, and cyclin B showed induction for samples from both days 7 and 14. Hox gene induction was detected for genes in the Hox A and B series, as well as for Pitx 2. An induction profile similar to that for cyclins A and D1 was measured for Hox A7 and Pitx 2, with induction for these genes becoming undetectable in samples from tissues with undetectable BrdU incorporation. These results suggest that it may be possible to identify genomic markers not directly associated with cell division that are indicators of BDH.

1253 DMBT1 IS A BIOMARKER OF BILE DUCT HYPERPLASIA IN F344 RATS.

D. E. Watson[1], I. Kadura[1], B. Li[1], G. Searfoss[1], K. Rodocker[2], J. Sullivan[2] and B. Berridge[2]. *[1]Global Exploratory Toxicology Team, Eli Lilly and Company, Greenfield, IN and [2]Experimental Pathology, Eli Lilly and Company, Greenfield, IN.*
Sponsor: C. Thomas.

Bile duct hyperplasia (BDH) is a hepatotoxicity caused by many compounds. The molecular mechanisms by which this toxicity arises are not well understood. Moreover, there is a lack of *in vivo* biomarkers for this condition in non-clinical and clinical settings. In an attempt to identify biomarkers of BDH, we investigated gene expression changes in rats exposed to methapyrilene, a compound that causes BDH. From the outset we were challenged by the fact that biliary epithelial cells are few in number relative to the total cell population of the liver. We therefore attempted to enrich the liver samples for bile ducts by conducting a collagenase perfusion of the liver prior to harvest. Portal vein perfusion with collagenase reduced by ~80% the wet weight of the liver through elimination of hepatocytes. Portal trees isolated by this process were examined by light microscopy and processed for RNA and protein analysis. Affymetrix® analysis led to identification of a number of genes that had promise as *in vivo* biomarkers of BDH, including Ebnerin, a secreted protein originally identified in the epithelial cells of the von Ebner gland in the tongue of the rat. Literature review and TaqMan® analyses led to the identification that the gene detected by the Ebnerin probe sets on the Affymetrix® RGU34A array was actually a gene called Deleted in Malignant Brain Tumors 1 (DMBT1). TaqMan® and western blot analyses confirmed the induction of DMBT1 mRNA and protein, respectively, in the liver of rats exposed to methapyrilene. Additional experiments with more than 20 compounds indicate that DMBT1 may be both a sensitive and specific biomarker for BDH in the rat. The known function of DMBT1 includes differentiation and proliferation of proto-epithelial cells *in vitro* and in a variety of tissues (liver, intestinal tract, kidney) and species (rat, rabbit, human) *in vivo*, lending further support to the use of DMBT1 as a biomarker of BDH.

1254 BIOMARKERS OF HEPATOTOXINS IDENTIFIED USING MURINE EMBRYONIC STEM CELL DIFFERENTIATION SYSTEMS.

Y. S. Kim[1], Y. Luo[1], O. A. Callan[1], A. Vickers[2] and H. R. Snodgrass[1]. *[1]VistaGen Therapeutics, Burlingame, CA and [2]Novartis Pharmaceuticals, East Hanover, NJ.*

The *in vitro* differentiation of embryonic stem (ES) cells represents a complex biological system which may be useful for studying the effects of compounds on a wide range of tissues and biological processes. Our goal in these studies is to identify biomarker profiles that are characteristic of heptatoxins using an early stage of differentiation of ES cells as the biological system. Training sets were selected from 20 compounds that are known to induce varying levels of liver injury (troglitazone, rosiglitazone, pioglitazone, Wy-14643, bezafibrate, fenofibrate, KRP297, AZ242, dimethylformamide, methotrexate, carbon tetrachloride, ibupofen, naproxen, etoposide, indomethacin, methapyrilene hydrochloride, arsenic, allyl alcohol, aroclor 1254, and 3-methylchloranthrene), 3 renal toxins (tobramycin, kanamycin, gentamycin), 4 neural toxins (quinolic acid, glutamic acid, kainic acid, domoic acid), and 8 mixed toxins (diquat, amiodarone hydrochloride, dimethylnitrosamine, crotaline, carbamazepine, cyclosporine, cisplatin, and dimethyl sulfoxide) and evaluated to identify predictive gene-expression biomarker profiles characteristic of tissue-specific toxicity. Initially compound doses were selected to yield a 30-70% decrease in cell number at the end of a 6-day murine embryoid body (EB) assay. The effect of each compound was evaluated by monitoring gene expression microarrays. The data were analyzed by a variety of statistical tools including analysis of variance, hierarchical clustering, principal component analysis, and class prediction algorithms. Preliminary analyses indicate that biomarkers could be identified which are 80% predictive for the three classes of tissue toxins. Furthermore, the hepatotoxic compounds form distinct clusters, many of which are related to mechanism and/or relevant biochemical pathways. These data suggest that the ES cell differentiation system may be a valuable early predictive screen for toxins, and for early information about effects of compounds on known biological and/or biochemical pathways.

1255 DIFFERENTIATION BETWEEN RENAL INJURY AND COMPENSATORY RESPONSES BY THE USE OF SPECIFIC BIOMARKERS.

A. Coyle[1], P. R. Maxwell[2] and D. Gordon[3]. *[1]Biotrin, Dublin, Ireland, [2]Biochemistry, Stobhill Hospital, Glasgow, United Kingdom and [3]Medicine, Stobhill Hospital, Glasgow, United Kingdom.* Sponsor: R. Chandra Gupta.

Based on the early release of the enzyme N-Acetyl-β-D-glucosaminidase (NAG), it was previously believed that proximal tubular injury, was an early event in diabetic renal injury. However, NAG can be up-regulated in response to proteinuria and is a marker of increased lysosomal turnover, as well as overt injury. In this study, NAG was compared with the cytosolic injury biomarkers alpha glutathione S-transferase (αGST,) and pi glutathione S-transferase (πGST). αGST is a cytosolic protein specific for the proximal convoluted tubule and πGST is found in the distal tubules. Their presence in urine indicates that cytolysis is occurring to cells in distinct parts of the nephron. 128 diabetic subjects were studied, 57 without albuminuria (group 1), 50 with microalbuminuria (group 2) and 21 with proteinuria (group 3) plus 20 age-matched controls. Urinary NAG was measured using a colorimetric method (Roche) and α and πGST were assayed using the Biotrin Urinary Alpha and Pi GST EIA kits respectively (Biotrin International, Dublin Ireland). Urinary NAG levels expressed as University/mmol creatinine and α and πGST levels, expressed as μg / mmol creatinine were (median: range): group 1, NAG 0.54 (0.21-2.96), αGST 0.21 (0.03-4.60), πGST 2.27(0.13- 19.2); group 2, NAG 0.69(0.23-2.16), αGST 0.2(0.032.5), πGST 2.55(0.14-33.3) and group 3, NAG 1.12(0.42-2.60), αGST 0.18(0.03-1.55), πGST 5.34(1.37-11.98). Urinary NAG, α and πGST in the control group were 0.33 (0.11-0.53), 0.31(0.04-1.15) and 0.84(0.18-1.32) respectively. NAG and πGST levels in the diabetic subgroups were significantly different to controls ($p<0.05$), whereas no significant differences in αGST were seen ($p>0.05$). These results suggest that proximal tubular dysfunction or compensation (increased NAG release), rather than damage (no αGST release), occurs in diabetic renal injury and that distal tubular damage (πGST release) is a very early event in the development of renal injury. By the use of biomarkers released according to different mechanisms and from different sites, new mechanisms of renal injury were seen.

1256 EVALUATION OF A HIGH-THROUGHPUT ARRAYPLATE TEST PLATFORM FOR GENOMIC BIOMARKERS OF TOXICITY.

I. Botros[1], F. M. Goodsaid[2], B. Seligmann[1], J. W. Davis[2], M. Crawford[1], R. J. Smith[2], R. Martel[1] and I. Y. Rosenblum[2]. *[1]High-Throughput Genomics, Tucson, AZ and [2]Genetic and Molecular Toxicology, Schering-Plough Research Institute, Lafayette, NJ.*

An important milestone in the development of genomic biomarkers of toxicity is their application in a high-throughput screening format. The gold standard for testing limited numbers of genomic biomarkers of toxicity per compound is quantitative RT-PCR (qRT-PCR). This platform can detect single copies of mRNA per test well and has a dynamic range over at least 108 copies of mRNA per test well. However, the real-time measurements are constrained by limited throughput in the range of 1500-3000 test wells per day. Hybridization endpoint platforms have the potential for throughput in the range of 3000-30000 test wells per day. These platforms are limited due to a lower sensitivity and dynamic range compared to qRT-PCR. We compared the sensitivity and dynamic range of the high-throughput ArrayPlate genomic test platform from High-Throughput Genomics (HTG) with those of qRT-PCR for HAVcr-1 and clusterin, two genomic biomarkers of nephrotoxicity we have studied in Cynomolgus monkeys. The ArrayPlate format measures hybridization for 16 genes per well. We found the sensitivity of the HTG platform using standard ArrayPlate reagent sets to be in the qRT-PCR CT range of 20-25, corresponding to approximately 105 copies of mRNA per test well. Its dynamic range is over a minimum of 103 copies of mRNA per test well. Clusterin was readily measured, while the sensitivity for detection of HAVcr-1 was limited by sample size. These results suggest that the sensitivity of the ArrayPlate test platform is within an order of magnitude of that needed for an accurate measurement of gene expression of HAVcr-1 using small samples in a high-throughput screening format. However, this platform provides values of at least an order of magnitude higher than similar array platforms we have tested. We will show the results using high sensitivity ArrayPlate reagent sets to bridge the sensitivity gap remaining for this platform.

1257 EVIDENCE OF AN IMMUNE-MEDIATED MECHANISM FOR NEVIRAPINE-INDUCED SKIN RASH IN THE BROWN NORWAY RAT.

J. M. Shenton and J. P. Uetrecht. *Pharmaceutical Sciences, University of Toronto, Toronto, ON, Canada.*

Rationale: The characteristics of a potential animal model of nevirapine-induced idiosyncratic skin rash in the female Brown Norway rat revealed a possible immune-mediated mechanism for the reaction. Specifically, an inflammatory infiltrate composed of T cells and macrophages was present in the skin of treated animals, rats rechallenged with nevirapine developed the skin rash faster than on first exposure, and splenocytes from rechallenged rats could transfer rash susceptibility to naive recipients. Consequently, the role of the immune system in this reaction was further investigated. Methods and Results: Cotreatment of rats with nevirapine (150 mg/kg/day) and either the immunosuppressant tacrolimus (1 mg/kg/day) or cyclosporin (20 mg/kg/day) prevented the development of skin rash during nevirapine treatment. CD8 T cells were depleted in thymectomized rats by administering the monoclonal antibody OX-8 prior to treatment with nevirapine; however, the rats still developed a skin rash. Correspondingly, splenic T cells or CD4 T cells isolated from rats after nevirapine rechallenge transferred susceptibility to rash to naive recipients; however, CD8 T cells did not. Finally, splenocytes and lymph node cells (mesenteric and popliteal) were prepared from untreated rats, rats on initial nevirapine exposure, and rats on rechallenge and stained for flow cytometric analysis to characterize changes in cell populations related to nevirapine treatment. Paradoxically, the CD4 to CD8 T cell ratio was significantly decreased in lymph nodes from treated rats compared to control rats (due to an increase in CD8 T cells); whereas, in spleen the results were inconsistent. Conclusions: Nevirapine-induced skin rash has an immune-mediated mechanism; and thus the mechanism is likely to be similar to the mechanism of nevirapine-induced rash in humans. (Funded by the Canadian Institutes of Health Research).

1258 CONTRIBUTION OF MAJOR HISTOCOMPATIBILITY COMPLEX DIFFERENCE ON OCCURRENCE OF SYSTEMIC ANAPHYLAXIS IN MICE.

Y. Heo[1], H. Kim[2] and S. Hur[3]. *[1]Occupational Health, Catholic University of Daegu, Kyongsan si, Kyongbuk, South Korea, [2]Preventive Medicine, The Catholic University of Korea, College of Medicine, Seoul, South Korea and [3]Biological Products, Korea Food & Drug Administration, Seoul, South Korea.*

This study was undertaken to investigate contribution of major histocompatibility complex difference on systemic anaphylaxis occurrence following vaccination. Anaphylaxis has been induced in BALB/c (H-2d), DBA2 (H-2q), C3H (H-2k), and C57BL/6 (H-2b) mice through intraperitoneal sensitization with chicken egg ovalbumin (OVA) followed by intravenous challenge with OVA. The magnitude of anaphylaxis occurrences was determined by measuring levels of plasma histamine, IgE, OVA-specific IgG, total IgG1, IgG2a, cytokine production from splenocytes stimulated *in vitro*, and expression of CD23 low affinity IgE receptors, CD16 FcγIII/II IgG receptors. The highest histamine level was obtained in the DBA/2 mice and the lowest in the C57BL/6 mice. Comparing levels of IgE between the OVA sensitized mice and the mice administered to saline or adjuvant (cholera toxin plus alum), significant elevation was observed in the DBA/2 and BALB/c mice, but not in the C3H and C57BL/6 mice. Furthermore, level of plasma OVA-specific IgG1, another anaphylaxis marker previously reported by the authors, was higher in the DBA/2 and BALB/c than C3H mice, and lowest in the C57BL/6 mice. Order of IL-4 versus IFNγ ratio, which indicates skewing of helper T cell reactivities, was BALB/c > DBA/2 > C57BL/6 > C3H. Significant increase in expression of CD23 was resulted in B cells from the DBA/2 mice developing anaphylaxis compared with the control mice, but not in other strains. This study endows the scientific evidence on a hypothesis that differences in the immuno-genetic background could significantly contribute the occurrence of systemic anaphylaxis observed in children following various vaccinations.

1259 INVESTIGATING IMMUNOGENICITY OF 2-PHENYLPROPENAL, A REACTIVE METABOLITE OF FELBAMATE.

M. Popovic[1], S. Nierkens[2], W. Santos[3], R. Pieters[2] and J. Uetrecht[1]. *[1]Pharmaceutical Sciences, University of Toronto, Toronto, ON, Canada, [2]IRAS-IT, Utrecht, Netherlands and [3]Harvard University, Cambridge, MA.*

Felbamate is an anti-epileptic drug with unique efficacy against difficult cases of epilepsy. Within the first year of its widespread use, felbamate therapy was associated with an occurrence of aplastic anaemia and hepatotoxicity. The mechanism of felbamate-induced idiosyncratic reactions has not been elucidated; however, most such reactions appear immune-mediated. Furthermore, it has been proposed that the formation of the reactive metabolite 2-phenylpropenal is responsible for felbamate-induced adverse reactions. To investigate the immune stimulating potential of felbamate and its metabolites *in vivo*, we performed reporter antigen popliteal lymph node assay (RA-PLNA) using TNP-Ficoll antigen and Balb/C mice. Immunological effects of felbamate, 2-phenylpropenal and its precursor monocarbamate felbamate were investigated. Mice foot pads were s.c. injected with one of the compounds, and a week later the PLN was removed and presence of immune response analyzed. We found that neither felbamate nor monocarbamate felbamate induced an immune response in PLNs in mice *in vivo*. However, 2-phenylpropenal appeared immunogenic, causing inflammation of foot pads, followed by their hard-

ening, increase in thickness and crustiness. The PLN cell count showed a nine-fold increase in the 2-phenylpropenal-treated mice compared to the controls. Immunohistochemical analysis of the PLNs revealed germinal center formation, indicating B cell proliferation. Cytokine analysis suggested an increase in the production of IL-4 and to a lesser extent IFN-g. Flow cytometry on PLN confirmed previous findings; total B cell numbers increased, the majority of B cells being activated, co-expressing CD54 and CD19 cell surface markers. In addition, it appears as if B cells had a role as antigen presenting cells, because they co-expressed both CD86 and CD19. Our study supports the hypothesis that 2-phenylpropenal is responsible for the idiosyncratic reactions associated with felbamate.

1260 CHARACTERIZATION OF HARDWOOD AND SOFTWOOD DUST INDUCED EXPRESSION OF CYTOKINES AND CHEMOKINES IN MOUSE MACROPHAGE RAW 264.7 CELLS.

J. Maatta[1], M. Majuri[1], A. Lauerma[2], K. Husgafvel-Pursiainen[1], H. Alenius[1] and K. Savolainen[1]. *[1]Department of Industrial Hygiene and Toxicology, Finnish Institute of Occupational Health, Helsinki, Finland and [2]Department of Occupational Medicine, Finnish Institute of Occupational Health, Helsinki, Finland.*

In addition to nasal and sino-nasal adenocarcinomas, wood dust exposure can induce several nonmalignant, mainly respiratory diseases such as allergic rhinitis, chronic bronchitis, and asthma. To find out whether wood dust is able to influence to development of inflammatory process through macrophages, we have elucidated the effects of wood dust exposure on the cytokine and chemokine expression of mouse macrophage cell line cells (RAW 264.7). The cells were exposed to graded doses of selected hardwood (birch, beech, oak, and teak) and softwood dusts (pine, spruce, and heat-treated pine). TiO_2 and LPS were used as controls. The mRNA expression of major proinflammatory cytokines (IL-1β, TNF-α, and IL-6), an anti-inflammatory cytokine (IL-10), and several chemokines (CCL2, CCL3, CCL4, CCL5, and CCL24) were assessed by real time PCR at several time points after wood dust exposure. TNF-α, IL-6, and CCL2 expression was studied also at the protein level using the ELISA method. Wood dust had in general more effects on cyto- and chemokine expression than inorganic dust TiO_2. All wood dusts induced TNF-α, IL-6, CCL3, and CCL4 expression and inhibited IL-1β and CCL24 expression. However, many differences were detected in the strength of induction or inhibition between different wood dusts. In the case of CCL2, birch, beech, pine, spruce, and heat-treated pine induced CCL2 production, but oak and teak dusts had no effect. Oak dust, that has been previously shown to be carcinogenic, appears to be a weaker inducer of inflammatory response than the other wood dusts. Our results show that exposure to different wood dusts elicits dose-dependent changes in the levels of inflammatory mediators in mouse macrophage cells. These findings suggest that exposure to wood dust may significantly influence development of inflammatory process in the airways by modulating the expression of proinflammatory cytokines and chemokines.

1261 CAN THE POPLITEAL LYMPH NODE (PLN) ASSAY BE USED TO PREDICT SPECIFIC IgE ADJUVANT ACTIVITY OF AMBIENT AIR PARTICLES? RESULTS FROM THE EUROPEAN RAIAP PROJECT.

M. Lovik, T. Lovdal, E. Groeng and E. Dybing. *Environmental Medicine, NIPH, Oslo, Norway.*

For the project Respiratory allergy and inflammation due to ambient particles: a European-wide assessment (RAIAP), ambient particulate matter was collected with high-volume samplers during three seasons (spring, summer, and winter) in Oslo (Norway), Rome (Italy), Lodz (Poland) and Amsterdam (The Netherlands), and at the Dutch seaside background site de Zilk (winter only). Coarse ($PM_{2.5-10}$) and fine ($PM_{0.1-2.5}$) particles were collected, and altogether 26 different particulate matter (PM) preparations were obtained. The PM preparations were examined for allergy adjuvant activity in two different assays in BALB/c mice. In the PLN assay the cellular response in the draining popliteal lymph node was measured after injection into the footpad of PM alone or PM plus allergen (ovalbumin, OVA). In the specific antibody assay, serum anti-OVA IgE, IgG1 and IgG2a were measured after similar immunization with PM + OVA. In the PLN assay, the cellular response to particles without allergen reflects the inflammatory capacity of the PM, while after co-injection of PM and allergen the response may be dominated by the immune response to the allergen. Coarse particles were generally found to have stronger inflammatory capacity than fine particles. Few statistically significant differences between PM preparations of the same type (coarse or fine) from different cities were observed. Similarly, few differences between PM preparations of the same type were observed in the PLN assay after injection of PM + OVA. Fine particles generally had stronger adjuvant activity than coarse particles in the antibody assays. A high inflammatory capacity in the PLN assay did not predict high adjuvant activity after co-injection of PM and allergen, neither in the PLN nor in the antibody assays. The antibody assays appeared to discriminate better between different PM preparations than the PLN assay with regard to adjuvant activity. Further, the antibody response represents a more well-defined and relevant parameter in relation to allergy than the PLN response.

1262 SENSITIVITY AND SPECIFICITY OF A SEROLOGICAL TEST THAT DETECTS HUMAN IGE ANTIBODY TO THE BACILLUS ENZYME Y217L BPN'.

K. Sarlo[1], B. Schnell[1], R. J. Harbeck[2], D. Leto[2], E. Finn[1] and B. Kirchner[1]. *[1]Miami Valley Laboratories, Procter & Gamble Company, Cincinnati, OH and [2]National Jewish Medical and Research Center, Denver, CO.*

A fluorescence immunoassay (FEIA UniCAP®) was developed to detect Bacillus protease Y217L BPN' specific IgE antibody in serum from humans exposed *via* the work environment or *via* clinical tests. Receiver-operating characteristic analysis (ROC) was done to determine the sensitivity and specificity; concordance correlation coefficients (CCC) were calculated to measure reproducibility of the assay. Serum from workers that were exposed to the enzyme and defined as skin prick test (SPT) positive or negative for enzyme-specific IgE antibody were used to define true IgE positive and true IgE negative samples. In addition, serum from clinic test subjects SPT (-) to the enzyme were used to supplement the true IgE negative population. A total of 561 samples were tested. The World Health Organization (WHO) IgE/anti-IgE standard was used to define the threshold for positive response in the assay. Sensitivity was defined by the percentage of SPT (+) subjects with positive IgE in serum; specificity was defined by the percentage of SPT (-) subjects with negative IgE in serum. ROC curves were generated by plotting sensitivity (y-axis) vs. 1-specificity (x-axis). Area under the curve (AUC) was used to assess accuracy where 1.0 represents perfect accuracy. The AUC for the enzyme UniCAP assay was 0.944 showing that this assay has a high ability to distinguish between SPT (+) and (-) individuals. Using the WHO threshold for positive response in the UniCAP, the sensitivity from the ROC curve was 81.8% and the specificity was 95.2%. The false positive rate for the assay was 0.59%. The CCC that measured the agreement between replicate data generated on the same serum samples and a 45-degree line on a coordinate system was 0.999. Perfect agreement = 1.0. In summary, the UniCAP enzyme assay was highly specific, sensitive and reproducible for human enzyme-specific IgE serum antibody.

1263 DIFFERENTIAL GENE EXPRESSION IN OCCUPATIONAL ASTHMA.

J. F. Regal[1], A. L. Greene[1], R. R. Regal[2], M. S. Rutherford[3], G. H. Flickinger[3], J. A. Hendrickson[3] and M. E. Mohrman[1]. *[1]Pharmacology, University of Minnesota, Duluth, MN, [2]Mathematics and Statistics, University of Minnesota, Duluth, MN and [3]Veterinary Pathobiology, University of Minnesota, St. Paul, MN.*

Trimellitic anhydride (TMA) is a small molecular weight chemical, and ovalbumin (OA) is a reference protein allergen in immunology. Both are known causes of occupational asthma, and effector mechanisms for both allergens are presumed to be the same. To determine if unique genes were expressed for each allergen, gene expression in lungs of mice sensitized and challenged with either TMA or OA was examined. Balb/c mice were sensitized intradermally on days 1 and 3 with TMA (3%) or OA (0.3%) in corn oil vehicle, and intratracheally day 12 with 30 μg of either TMA conjugated to mouse serum albumin (TMA-MSA) or OA. To elicit the allergic response, mice were challenged intratracheally on day 19 with 400 μg TMA-MSA, OA or control MSA. 72 hours later, lung lobes were removed for RNA isolation and analysis of eosinophil peroxidase as a measure of eosinophilia. Challenge with either OA or TMA resulted in similar significant lung eosinophilia. To assess gene expression, separate Affymetrix U74Av2 microarrays were used for each of 6-8 animals in 4 treatment groups. Data were normalized using Robust Multichip Analysis in GeneTraffic software. ANOVA p values were calculated with SAS. The q values (false discovery rates) were calculated using R and considered significant if less than 0.12. OA and TMA sensitization and challenge resulted in differential expression of 79 genes and 44 genes, respectively. Fold changes in gene expression in the OA sensitized and challenged group were consistent with recent reports of others. Comparing the difference in gene expression in OA vs OA control to the difference in TMA vs TMA control revealed significant differences in 15 genes. These data demonstrate clear differences in gene expression depending on the allergen and suggest that unique effector mechanisms may exist for different causative agents of occupational asthma. (Support: Graduate School & Academic Health Center, University of Minnesota; US Army Med. Res Acquisition Activity DAMD 17-02-1-0191).

1264 PULMONARY HYPERRESPONSIVENESS FOLLOWING DERMAL EXPOSURE TO CERTAIN DIISOCYANATES.

M. K. Selgrade, E. H. Boykin, N. H. Coates, D. L. Doerfler and S. H. Gavett. *ORD/NHEERL, University.S.EPA, Research Triangle Pk, NC.*

Exposure to certain low molecular weight chemicals is associated with asthma. A simple test is needed to identify chemicals that pose this hazard. Increases in total serum IgE or Th2 cytokines in draining lymph nodes following dermal exposure have been pursued as means to screen potential asthmagens. It would be useful to relate these responses to a respiratory effect. We hypothesized that dermal exposure to certain isocyanates would result in enhanced pulmonary hyperresponsiveness to methacholine challenge. We assessed 4 treatment groups including 2 chemicals associated with asthma, 2% diphenylmethane-4, 4'-diisocyanate (MDI) and 2% dicyclohexylmethane-4, 4'-diisocyanate (HMDI); a contact sensitizer not associated with asthma, 1% dinitrochlorobenzene (DNCB); and a vehicle control (4 acetone:1 olive oil). BALB/c mice received 5 dermal exposures over a period of 2 wks. Mice were then challenged with increasing doses of methacholine and responsiveness was assessed using whole body plethysmography (Buxco). Half of each group was killed and serum, bronchoalveolar lavage fluid (BALF), and lymph nodes were collected for total IgE, cell counts, and cytokine profiling. The remaining mice were exposed for an additional 2 wks and reassessed. There were no changes in total or differential cell counts for any of the exposure groups at any time compared to vehicle. Significant increases in serum IgE were apparent for the 3 exposure groups at 2 and 4 wks, with differences greater at 4 wks. Mice exposed to HMDI showed significant hyperresponsiveness at 2 wks; these differences were greater at 4 wks. Neither MDI nor DNCB produced significant hyperresponsiveness at either time. This data suggests a disconnect between the total serum IgE response and hyperresponsiveness. Also, using the current protocol hyperresponsiveness occurred after dermal exposure for only one of the two asthmagens. (This abstract does not reflect EPA policy).

1265 SUITABILITY OF AFFYMETRIX HUMAN GENECHIPS FOR ANALYSIS OF RNA SAMPLES FROM NON-HUMAN PRIMATES.

M. Derbel, S. Szak, M. Rosenberg, M. Cooper, D. Enke, M. Subramanyam and J. Green. *Preclinical and Clinical Development Sciences Division, Biogen, Cambridge, MA.*

New drugs are screened for adverse reactions using a laborious, costly process and still some promising therapeutics are withdrawn from the marketplace because of unforeseen human toxicity. Gene expression profiling, through the use of microarray technology, is rapidly becoming a standard analysis in toxicology studies, and has the potential to play a pivotal role in all stages of drug safety evaluation. Non-Human primates are widely used in the development of biotechnology-derived products. Indeed, their close phylogenic distance to humans often makes them the only pharmacological responsive species. Our genomic sequence analysis revealed that the chimpanzee and human genomes are sufficiently similar to warrant cross-species use of human Affymetrix GeneChips. The genes of cynomolgus and rhesus monkeys, on the other hand, are too dissimilar to human genes to be reliably assayed on a human-specific chip. Data also suggest that rhesus and cynomolgus monkeys' genes are at least as similar to each other as those of human and chimpanzee. Results from this study will be useful in assessing biomarkers development strategies for biotechnology-derived products.

1266 DEVELOPMENT OF A PUBLIC TOXICOGENOMICS SOFTWARE FOR MICROARRAY DATA MANAGEMENT AND ANALYSIS.

W. Tong, S. Harris, X. Cao, H. Fang, L. Shi, H. Sun, J. Fuscoe, H. Hong, Q. Xie, R. Perkins and D. Casciano. *NCTR, Jefferson, AR.*

Robust bioinformatics capability is widely acknowledged as central to realizing the promises of toxicogenomics. Successful application of the toxicogenomics approach, such as DNA microarray, is inextricably relied on appropriate data management, the ability to extract knowledge from massive data and the availability of functional information for data interpretation. At the FDA's National Center for Toxicological Research (NCTR), we are developing public microarray data management and analysis software, named ArrayTrack. ArrayTrack is MIAME (Minimum Information About A Microarray Experiment) supportive to store both microarray data and experiment parameters associated with a toxicogenomics study. A quality control mechanism is implemented to assure the quality of entered expression data. ArrayTrack also provides a rich collection of functional information about genes, proteins and pathways enriched from various public biological databases for facilitating data interpretation. In addition, several data analysis and visualization tools are also developed in ArrayTrack and more tools will be available in the next released version. Importantly, gene expression data, functional information and analysis methods are fully integrated so that the data analysis and interpretation process is simplified and enhanced. ArrayTrack is publicly available online to non-commercial use and the prospective user can also request a local installation version by contacting the authors.

1267 DRUG SIGNATURES PREDICT CHRONIC RENAL TUBULE INJURY FOLLOWING SUB-ACUTE DRUG ADMINISTRATION.

M. R. Fielden, S. Baumhueter, G. Day, S. Dunlea, B. Eynon, S. Fujimoto, B. Ganter, R. Idury, K. Jarnagin, K. Kolaja, M. Lee, R. Nair, G. Natsoulis, S. Nicholson, C. Pearson, A. Roter, S. Thode, A. Tolley, S. Tugendreich, S. Tugendreich and S. Tugendreich. *Iconix Pharmaceuticals, Mountain View, CA.*

To improve compound selection during drug development we have developed a large library of gene biomarkers, Drug Signatures™, for important toxicities and mechanisms of action. These signatures are derived from a comprehensive database of ~13, 000 microarray expression profiles derived from treating rats with 580 drugs, in addition to traditional clinical pathology and pharmacology measurements. Using long-term low-dose drug-induced tubular nephrosis in the rat kidney as a model system, we demonstrate the utility of a large reference database to identify Drug Signatures™ predictive of chronic pathology after sub-acute administration. Male adult SD rats were dosed daily for 5, 14 or 28 days with one of 11 renal tubular toxicants including platinum drugs, aminoglycoside antibiotics and heavy metals. Clinical and histological pathology data confirmed that treatment with 10 of 11 compounds did not produce renal tubule injury on day 5, while histopathological evidence of progressive and degenerative tubular injury was subsequently observed for 9 of these 10 compounds on days 14 and 28. Using a linear discriminant-based SVM algorithm and a reference database of over 400 kidney expression profiles, representing >100 compounds, a Drug Signature™ predictive of tubular nephrosis was developed. Cross-validation of the signature demonstrates an accuracy of >98% for identifying tubular nephrosis based on 5-day gene expression data. Genes in the signature included GH receptor and the immediate early gene Fn14, suggestive of an early regenerative response prior to the appearance of injury. These results demonstrate the utility of a comprehensive database to identify gene biomarkers predictive of chronic pathology after sub-acute administration, thus saving time and expense during drug development decision making.

1268 DECISION FOREST FOR PREDICTING PROSTATE CANCER BASED ON SELDI-TOF MS DATA - SHOW ME THE CONFIDENCE.

W. Tong[1], Q. Xie[2], H. Hong[2], H. Fang[2], L. Shi[1], R. Perkins[2] and R. Kodell[1]. *[1]Nat. Ctr for Toxicology Research, Jefferson, AR and [2]Bioinformatics and Computational Science Group, Northup Grumman Information Technology, Jefferson, AR.* Sponsor: D. Casciano.

Class prediction using proteomics data from SELDI-TOF MS are playing an increasing role in diagnosis, prognosis and risk assessment. Usually, SELDI-TOF data has very many predictor variables (i.e. m/z peaks) over relatively few samples and, sometimes, is noisy. The nature of the data manifests that the challenge in class prediction is no longer in constructing a fitted model that is internally consistent; rather, it is how to quantify the predictivity of the fitted model for classifying unknown samples. Commonly, the predictivity of a fitted model is assessed by predicting a test set that contains samples not used in the model development. This external validation procedure offers a sense of real-world application, but the conclusion might not reflect the predictivity of the model due to the limitation of the sample representation of the test set. We developed a class prediction model for early detection of prostate cancer based on SELDI-TOF data using a novel ensemble method, Decision Forest. Decision Forest combines the results of multiple heterogeneous but comparable decision trees to produce a single prediction. Using an extensive leave-10-out (L10O) cross-validation procedure, we were able to assess two essential characteristics associated with the model's predictivity for predicting the samples not included in the model development, i.e. the level of prediction confidence and the degree of chance correlation. The results demonstrated that the proposed extensive L10O cross-validation provides an unbiased and rigorous way to validate the predictivity of a Decision Forest model for class prediction.

1269 INTEGRATION OF GENOMICS AND METABONOMICS DATA WITH ESTABLISHED TOXICOLOGICAL ENDPOINTS. A SYSTEMS BIOLOGY APPROACH.

K. Kramer[1], S. Patwardhan[3], K. A. Patel[3], S. T. estrem[2], J. M. Colet[1], R. A. Jolly[2], G. S. Ganji[3], N. J. Lewin-Koh[3], H. Gao[2], I. L. Smyej[1], S. Huang[2], H. R. Qian[2], P. Chen[2] and V. K. Kanjilal[3]. *[1]Lilly Development Centre, Mont Saint Guibert, Belgium, [2]Eli Lilly, Indeanapolis, IN and [3]Eli Lilly, Singapore, Singapore.*

Introduction: The purpose was to develop and evaluate methods to integrate genomics and metabonomics data with histopathological and clinical pathology data obtained in 5 *in vivo* single dose studies in the rat. An integrated approach

analysing parameters monitored in tissues and those accessible in biofluids is essential for the identification of markers that potentially can be transferred to clinical studies. It also facilitates a better understanding of mechanisms of toxicity. Study design: Rats were treated with a minimally toxic or toxic single dose of either Adriamycin, Bromobenzene, Clofibrate, Diethylhexyl Phthalate, Valproate or respective vehicle. 1H NMR data were collected in urine samples up to 168h after dosing. Gene expression profiles were determined in livers 4, 24, 48 and 168h, histopathological assessment was carried out 48 and 168h after dosing. Clinical chemistry parameters were measured at various time points up to 168h after dosing in urine and serum samples. Data Analysis: Methods employed, in addition to well described statistical approaches, included knowledge based integration based on pathway mapping and clustered correlation. Conclusions: Integrated transcriptome and metabolome pathway mapping is an effective approach for mechanistic investigations. For example: In the case of the PPAR agonists good correlation was found between ketone bodies monitored in urine samples and genes involved in ketogenesis. Phenotypical anchoring of the data based on histopathological assessment is important. Integrated analysis provides better separation between treatment groups compared to individual genomic or metabonomic analysis. For example: Whereas in the clofibrate study neither genomics nor metabonomics data alone were able to sufficiently separate low dose versus control, an integrated data analysis separated well both low and high dose groups from control samples.

1270 TARGETED PROTEOMICS PROFILING:USE OF LECTIN AFFINITY CHROMATOGRAPHY TO ISOLATE CANCER-RELATED FUCOSYLATED PROTEINS FROM SERUM.

C. R. Wilson[1], C. L. Feasley[2], F. E. Regnier[2] and S. B. Hooser[1]. *[1]Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN and [2]Chemistry, Purdue University, West Lafayette, IN.*

Differential expression of α(1, 6)-fucosylated proteins has been shown to occur in various types of neoplasia including lymphoma, hepatocarcinoma, and colorectal cancer. Using targeted proteomics profiling, a method has been developed and optimized for the isolation of α(1, 6)-fucosylated peptides from tryptically digested canine serum. Isolation of these peptides was accomplished using HPLC and an affinity chromatography column with L. tetragonolobous lectin (LTL), which binds α(1, 6)-fucosylated proteins and peptides with high specificity. The LTL column was synthesized by conjugating the lectin to diol-silica by reductive amidation following periodate oxidation. It was connected inline with HPLC/UV. Selecting fucosylated peptides from normal canine serum was accomplished using optimized sample preparation procedures and LTL affinity column selections. Briefly: 1) conditions for tryptic digestion of proteins from normal canine serum were optimized, 2) tryptically digested peptides from normal canine serum were injected onto an LTL affinity column to select for α(1, 6)-fucosylated peptides, 3) the selected α(1, 6)-fucosylated peptides were separated using reverse phase chromatography resulting in the isolation of peptide-containing fractions for identification by mass spectrometry. Procedures are being optimized for deglycosylation of the peptides followed by mass spectral analysis. MALDI-TOF and ESI-MS are being performed to characterize and identify the fucosylated serum proteins. Overall, this procedure is unique in that it selects for cancer-related proteins which, when coupled to Global Internal Standard Technology (GIST) labeling, will facilitate the identification and quantification of the differential expression of these proteins in normal serum versus serum from dogs with cancer. When applied to humans, this will allow diagnosis of some types of cancer and permit evaluation of treatment efficacy and prognosis following chemotherapy.

1271 GENE EXPRESSION CHANGES IN PRIMATES ARE DIFFERENT FROM THOSE IN RODENTS FOLLOWING EXPOSURE TO THE HEPATOTOXIN ACETAMINOPHEN.

M. S. Lawrence, D. Redmond, R. Roth, J. Elsworth, S. Tam, R. Jensen and S. Gullans. *RxGen, Hamden, CT.* Sponsor: Y. Dragon.

Early and sensitive detection of potential hepatotoxins is essential to assuring efficiency of drug development and minimizing post marketing health risks and liability. Toxicogenomics offers such predictive potential, but in determining possible human risk important attention must be paid to the animal models employed. Nonhuman primates offer the greatest promise of yielding truly predictive toxicogenomic modeling. To establish the feasibility of primate toxicogenomics we have used microarrays to evaluate the *in vivo* gene transcriptional changes in the liver of the African green monkey following the administration of acetaminophen, a classic and prevalent hepatotoxin. Acetaminophen was delivered to 8 monkeys *via* nasogastric tube in a single dose of either 200 or 400 mg/kg 48 hours prior to sacrifice. Blood and liver tissue were collected and compared to 4 control animals. Histopathology revealed microvascular change in acetaminophen exposed liver tissue relative to controls, and serum chemistries were consistent with early liver injury. Preliminary microarray analyses using Amersham CodeLink human arrays demonstrated hepatic transcriptional changes in response to acetaminophen that are different from those previously reported in rodents. Given these preliminary findings with an important hepatotoxin primate toxicogenomics may offer a more relevant, sensitive and ultimately cost effective means of improving predictive toxicology.

1272 GENOMIC CHARACTERIZATION OF IDIOPATHIC AND DRUG-INDUCED DILATED AND HYPERTROPHIC CARDIOMYOPATHY IN THE RAT HEART.

D. Donna[1], M. R. Fielden[2], K. Kolaja[2], J. Moehlencamp[3], M. Peden[3] and B. Car[1]. *[1]Bristol-Myers Squibb, Princeton, NJ, [2]Iconix Pharmaceuticals, Mountain View, CA and [3]Bristol-Myers Squibb, Evansville, IN.*

Over-expression of the Gαq-coupled serotonin 2B receptor (5-HT2BR) leads to non-pathological cardiac hypertrophy, whereas over-expression of Gαq leads to dilated cardiomyopathy (CM). Paradoxically, congenital deletion of 5-HT2BR also results in dilated CM. Thus, drug-induced activation or inhibition of 5-HT2BR signaling could cause CM. To understand and predict CM induced *via* diverse yet overlapping signaling pathways, we evaluated expression profiles derived from drug-induced models of CM in the rat in the context of a large chemogenomic reference database (DrugMatrix™). Adult male SD rats were dosed for 5 days with BW-723C86, a 5-HT2BR agonist, a 5-HT2BR antagonist, or tri-iodothyrinine (T3) as a model for cardiac hypertrophy. Aged rat heart was also profiled as a model of idiopathic CM. Utilizing Iconix proprietary analysis tools and Drug Signatures™, we were able to delineate the agonist vs antagonist effects of the 5-HT2BR ligands on cardiac gene expression. Further, expression profiles were consistent with BW-723C86 and the 5-HT2B antagonist to induce hypertrophic and dilated CM, respectively, consistent with genetic models of activation and deletion of 5-HT2BR signaling. These findings were also identified prior to the appearance of histopathological injury in the heart. T3 was identified as being cardiotoxic since it induced expression changes consistent with hyperthyroidism and remodeling of the extracellular matrix. Evaluation of other compounds in DrugMatrix™ with sub-micromolar affinity for 5-HT2BR reveals expression changes similar to anthracycline drugs, including suppression of cell-cycle genes (CDC2A, Cyclin A2) and induction of clock genes (Dbp, Tef). Collectively, these results show the value of expression profiling for identifying mechanisms of CM, and reveal novel insights into the mechanism of action of 5-HT2B ligands, as well as anthracycline drugs.

1273 FTICR-MS ACCURATE MASS AND TIME TAG ANALYSIS OF HUMAN PROTEOME ORGANIZATION REFERENCE SERA AND PLASMA.

J. N. Adkins, K. J. Auberry, N. Tolic, M. E. Monroe, R. Karin, R. D. Smith and J. G. Pounds. *Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA.*

Characterization of the human blood plasma proteome is important to discovery of useful biomarkers for disease diagnosis, susceptibility, and prognosis. The Human Proteome Organization (HUPO) has begun to evaluate the proteomes of human blood sera/plasmas in a systematic multi-pronged approach. Our contribution to this study uses a combination of powerful mass spectrometry technologies with improvements in sample preparation to perform a global proteomic analysis of the proteins found in sub-mL quantities of the HUPO reference sera/plasmas. We used an extensively characterized MS/MS database of serum-derived peptides as the basis for an AMT approach using Fourier-transform ion cyclotron mass spectrometry (FTICR-MS). Due to peptide sequence variability, immunoglobulins were depleted from serum/plasma using protein A/G. The IgG-depleted serum/plasma was then digested with modified-trypsin and analyzed by capillary-LC FTICR MS. Approximately 550 proteins are detected in serum and plasma with high confidence based on reproducibility of measurement; 150 proteins are detected with less confidence. Approximately 590 proteins were identified in both serum and plasma and about 120 were detected in only serum or the plasma samples to give a total of 850 proteins identified. Among the proteins identified are lower abundance serum/plasma proteins known to be in the ng/mL range. We also used the program, SEQUEST, to compare peptides identified from the NCBI vs. the IPI human protein databases. Analysis of LC-MS/MS data against the IPI database produced about 10% fewer protein identifications than the NCBI database. These analyses demonstrate the sensitivity of the AMT strategy and the high resolution of MS instruments such as the FTICR-MS to identify low abundance proteins. The data also underscore the importance of the database used for protein identifications. The AMT approach will improve sample throughput to improve characterization and understanding of how biological systems interact with their environment

1274 TRANSCRIPTIONAL PERTURBATION OF LYSYL OXIDASE BY CIGARETTE SMOKE CONDENSATE IN CULTURED LUNG FIBROBLASTS.

W. Li[1], K. Chen[1], Y. Zhao[2], L. Chen[1], P. Toselli[1], I. Chou[2] and P. Stone[1]. *[1]Biochemistry, Boston University School of Medicine, Boston, MA and [2]Microbiology, Boston University School of Medicine, Boston, MA.*

Lysyl oxidase (LO) catalyzes crosslinking of collage and elastin playing a central role in maintenance of the structural integrity of the lung extracellular matrix (ECM). To understand mechanisms of cigarette smoke (CS)-induced emphysema, we investigated the effects of cigarette smoke condensate (CSC) on LO mRNA expression in cultured rat fetal lung fibroblasts (RFL6). Exposure of RFL6 cells to CSC at 0-120 μg/ml for 24 h induced a dose-dependent downregulation of LO mRNA as exemplified by the reduction of LO mRNA levels in the presence of 80-120 μg CSC/ml to below 10% of the control. The trypan blue exclusion assay indicated no significant effects of CSC on cell *via*bility under the same conditions. Furthermore, identical CSC treatments did not change mRNA levels of internal controls, e.g., actin and GAPDH. Using actinomycin D, an inhibitor of mRNA synthesis, in a chase assay we showed that LO mRNA exhibited relative stability in control cells with t1/2 = 24 h. However, CSC exposure reduced the stability of LO transcripts as evidenced by a 50% decrease in the level of LO mRNA in cells treated with actinomycin D (5 μg/ml) in the presence of CSC (120 μg/ml for 24 h) in comparison to cells in the absence of CSC. Moreover, nuclear-run on assays indicated a 2.5-fold decrease in the amount of LO transcripts found in nuclei isolated from cells treated with CSC (120 μg/ml for >6 h) relative to that of nuclei from control cells. Thus, the reduction of LO mRNA stability combined with the suppression of transcriptional initiation both contributed to downregulation of LO mRNA in CSC-treated cells. Note that inhibition of LO mRNA expression by CSC was closely accompanied by markedly decreased levels of transcripts of collagen type I and tropoelastin, two substrates of LO. Thus, transcriptional perturbation of LO and its substrate may be a critical mechanism for ECM damage in CS-induced emphysema (supported in part by Philip Morris Foundation and NIH grant R01-ES 11340).

1275 INHIBITION OF LYSYL OXIDASE AT PROTEIN AND CATALYTIC LEVELS AND CELLULAR THIOL HOMEOSTASIS IN RAT LUNG FIBROBLASTS TREATED WITH CIGARETTE SMOKE CONDENSATE.

L. Chen[1], Y. Zhao[2], K. Chen[1], P. Toselli[1], I. Chou[2], P. Stone[1] and W. Li[1]. *[1]Biochemistry, Boston University School of Medicine, Boston, MA and [2]Microbiology, Boston University School of Medicine, Boston, MA.*

Lysyl oxidase (LO) plays a critical role in the formation and repair of the extracellular matrix (ECM) by oxidizing the epsilon-amino group of peptidyl lysine residues in elastin and collagen. To better understand the role of LO in cigarette smoke-induced emphysema, we examined changes in LO at the level of protein expression and LO catalytic activity, the levels of its substrates, elastin and collagen type I, as well as cellular thiols, e.g., glutathione (GSH), gamma glutamylcysteine synthetase (GCS) and metallothionein (MT) in cigarette smoke condensate (CSC)-treated rat fetal lung fibroblasts (RFL6). Exposure of RFL6 cells to CSC at 0-120 μg/ml for 24 h decreased the levels of catalytic activity and protein species of LO, i.e., 46 kDa preproenzyme, 50 kDa proenzyme and 32 kDa mature enzyme and its substrates, i.e., elastin and collagen type I, in a dose-dependent manner. Meanwhile, cellular thiols including GSH, GCS and MT were markedly upregulated in these CSC-treated cells. Since LO is a Cu-dependent enzyme and MT and GSH have high affinities for Cu ions, elevated levels of MT and GSH may interfere with Cu bioavailability for LO. To test this possibility, we further examined effects of glutathione monoethyl ester (GME), a GSH delivery system, on LO protein expression and catalytic activity. Interestingly, exposure of cells to GME at 1.0 mM, 2.0 mM and 4.0 mM, which effectively increased intracellular GSH levels, induced a dose-dependent decrease in LO protein species and catalytic activity. These results suggest that upregulation of cellular thiols by CSC has the potential to limit cellular availability of Cu for LO and may be important for downregulation of LO and destabilization of the ECM in CS-induced emphysema (supported in part by Philip Morris Foundation and NIH grant R01-ES 11340).

1276 NANOPARTICLES ARISING FROM COMBUSTION OF 1, 3-BUTADIENE TRANSPORT PAHS AND OXIDANTS TO RESPIRATORY EPITHELIAL CELLS.

A. penn, W. Henk, G. Murphy, W. Catallo and S. Barker. *comp biomed sci, lsu vet med, baton rouge, LA.*

1, 3-Butadiene (BD), is a high volume (3x109 lbs/yr, US) petro-chemical. BD that escapes industrial processing is burned. This generates butadiene soot (BDS), a homogeneous, PAH-rich particulate mixture. 90 percent of laboratory-generated BDS is <2.5um in size. BDS particulates are aggregates of 50-100 nm (diam) nanoparticles. When added to serum-free cultures of BEAS-2B cells, BDS elicits a time- and dose-dependent autofluorescence (AF) that progresses from diffuse (30-120 min) to punctiform by 4 hours. When freshly prepared BDS is extracted with organic solvents of widely differing polarity and the filtrates (0.45um) are added to the cultures, all elicit AF, while solvent-washed BDS, Buckminsterfullerene and graphite do not. Thus, fine carbon particles and graphitic lattices, which lack PAHs and a nanoparticle substructure, are inadequate to elicit AF. Benzoyl peroxide, H2O2, styrene, naphthalene, acetaldehyde and BD alone do not elicit AF. Thus, neither strong oxidants alone nor low molecular weight compounds adsorbed onto BDS are responsible for AF in this system. BDS-associated AF rarely co-localizes with a lysosomal marker (LysoTracker Red), an autophagic vacuole marker (monodansylcadaverine) or an endosomal compartment marker (dextran-rhodamine). Thus, the punctiform AF apparently develops neither from an endocytic process nor through lysosomal association. When BDS in medium is placed in transwells (0.4um) within a 24-well plate, cells in the plate display punctiform AF at 48 hr. Mass spectrometry of dichloromethane (DCM) extracts of these cells yields PAH-rich spectra that are indistinguishable from spectra of fresh BDS extracted with DCM. BEAS-2B cells exhibit a significant decrease in proliferative capacity while exposed to BDS. This is reversed by thorough washing, which attenuates the AF. These results suggest that PAHs adsorbed onto BDS nanoparticles are strongly associated with BEAS-2B AF and also suggest a general mechanism whereby combustion-generated particles of respirable size can deliver toxicants to target cells.

1277 CHANGES IN INFLAMMATORY MEDIATORS AND LUNG PERMEABILITY MARKERS IN RATS AFTER ACUTE EXPOSURE TO CIGARETTE MAINSTREAM SMOKE.

P. Vanscheeuwijck[2], E. Van Miert[2] and P. Kuhl[1]. *[1]PHILIP MORRIS Research Laboratories GmbH, Cologne, Germany and [2]PHILIP MORRIS Research Laboratories bvba, Leuven, Belgium.* Sponsor: H. Haussmann.

Exposure to cigarette mainstream smoke (MS) is known to induce inflammatory changes in the respiratory tract. While acute exposures (1 day) are often used to evaluate the toxicity of airborne pollutants such as dusts, fumes, and residual oil fly ash, the effects of MS are usually evaluated after longer exposure periods. Here we report on respiratory tract and systemic changes in Sprague-Dawley rats after a single 2-hour exposure to MS at concentrations of 250, 500, 750, 1000, and 1250 μg total particulate matter (TPM)/l. This MS-concentration range goes well beyond exposure levels representative for human smokers. In rats, the toxicity of such high concentrations was evidenced by a body temperature decrease of 8 °C observed immediately after exposure to 1250 μg TPM/l. Bronchoalveolar lavage fluid (BALF) and serum were collected at 2, 4, 15, and 24 h post-exposure and the concentrations of pro-inflammatory cytokines (IL-1, TNFα, GM-CSF, IL-6), chemokines (CINC-1, CINC-3, MIP-1α, MCP-1), and lung permeability markers (CCSP in serum and albumin in BALF) were determined. Cytokines in BALF were increased after exposure to the highest MS concentrations at 15h and 24h (up to 6-fold for IL-1β). Chemokines in BALF were increased after exposure to low concentrations of MS at 4h (2-fold for CINC-1) and, to a greater extent, after exposure to high concentrations of MS at 15h and 24h (up to 7-fold for MCP-1). Cytokines in serum were increased at 2h and 4h at 1250 μg TPM/l only. Chemokines in serum were increased at 2h and 4h in an MS concentration-dependent way. CCSP and albumin levels revealed an MS concentration-dependent increase in lung permeability, which was most prominent at 2h and 4h. Results indicate that acute inhalation exposure to MS increases the concentration of inflammatory mediators in BALF and serum as well as lung permeability markers, suggesting that this may be a valuable short-term assay to assess the activity of cigarette mainstream smoke.

1278 *IN VIVO* DETECTION OF LUNG TUMORS IN MICE BY HIGH-RESOLUTION X-RAY MICROTOMOGRAPHY.

K. Meurrens[1], H. Weiler[2], N. M. De Clerck[3] and A. A. Postnov[3]. *[1]PHILIP MORRIS Research Laboratories bvba, Leuven, [2]PHILIP MORRIS Research Laboratories GmbH, Cologne, Germany and [3]University of Antwerp, Antwerp, Belgium.* Sponsor: H. Haussmann.

The major application of micro-CT in biomedical research has been the imaging of calcified tissues. *In vivo* high-resolution X-ray microtomography (micro-CT) is a promising technique for the non-invasive imaging of soft tissues of small laboratory animals. For example, detection and evaluation of structural lung disorders has up to now required many animals to be killed at many timepoints, and for emerging lesions, such as small tumors, time-consuming step-serial histological sectioning is unavoidable. As a possible alternative, the application of X-ray micro-CT to detect lung tumors was investigated in live mice at an early and more advanced stage of tumor development. A/J mice were treated with urethane 1000 mg/kg bodyweight ip and examined at 3.5 and 9 months post treatment. Healthy age-matched groups of mice were used as control. The chest area of anesthetized mice was scanned for

lung tumors by X-ray micro-CT (Skyscan 1076, Aartselaar, Belgium) without gating for cardiac or respiratory motion. Positioning the mice on their backs in the X-ray micro-CT and banding the chest area reduced motion artifacts. Scans lasted 17 minutes per mouse: spatial resolution was 35 μm and radiation load was 0.4 Gy. All mice survived the procedure with no apparent ill effects. Reconstructed virtual CT cross sections of the lungs (Feldkampf cone beam algorithm) confirmed the macroscopic evaluation. Tumors in the hilar region or at the periphery of the lungs were more difficult to localize on the CT cross sections. In step-serial sections (every 100 μm) from one pair of lungs, the tumors detected by CT were seen plus a few additional, very small tumors (≤ 150 μm ϕ). The *in vivo* X-ray micro-CT method opens broad perspectives for non-invasive imaging. Continued improvements in resolution and detection in soft tissue imaging may make this a powerful research tool for monitoring tumor development and progression.

1279 NRF2 DEFICIENT MICE DEVELOPS PULMONARY EMPHYSEMA IN RESPONSE TO CIGARETTE SMOKE.

S. Biswal[1], T. Rangasamy[1], R. K. Thimmulappa[1], K. H. Mai[1], S. Srisuma[1], T. W. Kensler[1] and R. M. Tuder[2]. *[1]Environmental health Sciences, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD and [2]Deapartment of Pathology, Johns Hopkins Medical Institution, Baltimore, MD.*

Nrf2 is a CNC b-zip transcription factor involved in the transcriptional induction of an array of antioxidant enzymes and cytoprotective genes in response to oxidative stress. The role of Nrf2 in conferring protection against cigarette smoke (CS) induced pulmonary emphysema was investigated. Nrf2 +/+ and -/- mice were exposed to CS (7 h/day, 7 days/week) for various time periods (1.5, 3 and 6 months) using a smoke machine and emphysema in the lung was assessed by morphometric measurements. There was a 3 fold and 4 fold increase in mean linear intercept and lung alveolar diameter respectively, in the 6 months CS exposed Nrf2 -/- mice relative to +/+. Hydroxy proline, a marker of collagen destruction is increased in the lungs of CS exposed Nrf2 -/- mice. Fluorescent TUNEL and imunohistochemical staining revealed the greater number of apoptotic, caspase-3 and 8-Oxo-dG positive cells in the lungs of CS exposed Nrf2 -/- mice suggesting the occurrence of increased oxidative stress and apoptosis. Differential cell counts of the branchoalveolar lavage fluid showed increased infiltration of inflammatory cells into the lungs of CS exposed Nrf2 -/-. Oligonucleotide microarray analysis (Affymetrix) with the RNA from the lungs of CS exposed mice revealed the expression of more than 60 different antioxidant enzymes and cytoprotective genes containing antioxidant response elements in the lungs of CS exposed Nrf2+/+ mice which are uninduced in CS exposed Nrf2 -/- mice. Based on these observations we suggest that Nrf2 plays a critical role in the protection of lungs against CS induced emphysema in Nrf2 +/+ mice, by the positive regulation of various antioxidant enzymes and cytoprotective proteins. (This work was supported by grants: P50 CA058184-09 and NIEHS center grant P30 ES 03819)

1280 ACUTE RESPONSES IN MICE EXPOSED TO POLYMER AND TOBACCO COMBUSTION PRODUCTS USING A DIN FURNACE.

R. Lemus[1], K. M. Lee[2] and M. S. Werley[1]. *[1]Philip Morris USA, Richmond, VA and [2]Battelle Toxicology NW, Richland, WA.*

The acute toxicity of combustion products (CP) from polymers possibly found in manufacturing parts was evaluated using a previously validated system consisting of a Deutsches Institut für Normung (DIN) 53-436 furnace connected to an animal exposure unit. Polymers [wool, Nylon 6, 6/6, and 12, acrylonitrile-butadiene (AB), or AB-styrene] were individually mixed with tobacco blend (TB) at 0, 3, 10, & 30% (w/w). Male Swiss Webster (SW) mice were exposed to 150 μg of total particulate matter (TPM)/L for 30-min by nose-only inhalation to CP generated at ~750°C. CO/CO2, NO/NOx, TPM, hydrogen cyanide (HCN), formaldehyde, acetaldehyde, acrolein, and nicotine were measured in the test atmosphere. Respiratory function (Buxco Biosystems) during exposure, postexposure blood cyanide (CN^-) and carboxyhemoglobin (COHb) levels, respiratory tract histopathology (1 & 7 days postexposure), alveolar macrophages (AM), LDH and protein changes in BAL fluid (Day 1) were evaluated. Experimental replacement of tobacco mass with polymer generally increased TPM, HCN, some aldehydes and CO, while nicotine was decreased. Body weights were overall comparable among polymer, TB and sham groups. Postexposure COHb levels were not different among all exposed groups. Blood CN^- levels were below ELOQ (0.2 μg/g) for all groups. More respiratory irritation was observed in the groups with 30% Nylon 6/6 or AB based on delayed postexposure recovery of depressed respiratory function. Day-1 postexposure lesions in nasoturbinates, lateral wall, and maxilloturbinates in exposed groups were minimal to moderate and limited to the anterior nose area. Necrosis was observed with some of the 30% groups compared to TB or lower polymer ratio groups, but all lesions were resolved by day 7. The only change in BAL parameters was a slight decrease in the ratio of resting/activated AM in the exposed groups compared to control. These results show that nose lesions were the most distinct acute toxicity related to CP from these polymer-TB mixtures and from the polymers tested Nylon 6/6 and AB were potentially more toxic in SW mice.

1281 INVESTIGATION OF A METHOD OF PREPARING A SINGLE CELL SUSPENSION IN THE LOCAL LYMPH NODE ASSAY USING CHEMICAL DISSOLUTION.

D. Dreher and D. Everett. *Covance Laboratories Ltd., Harrogate, United Kingdom.*

In the murine local lymph node assay (LLNA), sensitising chemicals induce a primary proliferation of lymphocytes in the lymph nodes draining the site of chemical application. To assess this proliferation radioactive labelling is used, and to measure the degree of radioactive labelling a single cell suspension of lymph node cells is prepared. The method detailed in OECD Test Guideline 429 describes the use of mechanical disaggregation which is time consuming, and is also open to potentially large degrees of error if cells are lost during the disaggregation process. A comparison was performed of mechanical disaggregation and a chemical dissolution method using SolueneR-350 in a series of assay's using known sensitisers. The results obtained from chemically dissolved samples were generally lower than values obtained from lymph nodes processed by mechanical disaggregation. It was shown that dissolving lymph nodes in SolueneR-350 was not a suitable method for evaluating the proliferation of lymphocytes in the LLNA in our laboratory.

1282 RETROSPECTIVE ASSESSMENT OF THE RABBIT ENUCLEATED EYE TEST (REET) AS A SCREEN TO REFINE WORKER SAFETY STUDIES.

F. J. Guerriero[1], C. W. Seaman[2], M. J. Olson[3], R. Guest[4] and A. Whittingham[4]. *[1]GlaxoSmithKline, King of Prussia, PA, [2]GlaxoSmithKline, Ware, Herts, United Kingdom, [3]GlaxoSmithKline, Research Triangle Park, NC and [4]SafePharm Laboratories, Shardlow, Derbyshire, United Kingdom.*

The REET is an *ex vivo* alternative that has been studied as a screen to identify severe eye irritants in the workplace. GSK has incorporated the REET into a tiered protocol designed to reduce the use of the traditional Draize eye protocol. Corneal opacity and thickness, fluorescein dye uptake and the condition of the corneal surface are observed after *in vitro* exposure to test materials. Previously 30 pharmaceutical process materials which had been evaluated with the Draize method were assessed by the REET. An evaluation of the REET data suggested that a score for any one of the following parameters appeared highly predictive of the Draize response: corneal opacity/area >/= 3; corneal swelling >/= 25; fluorescein dye uptake/area >/= 4 or select gross morphological changes. The REET-positive criteria was met by 17/30 materials and included 13 labeled severe (R41) with 1 moderate and 3 mild by *in vivo* evaluation. Thirteen chemicals did not meet the REET criteria: 9 were minimal/mild eye irritants and 4 were moderate. An additional 14 materials have now been assessed using the REET with data for the *in vivo* comparison obtained from the published literature. The REET-positive criteria were met by 8/14 materials and included 2 labeled severe (R41) with 5 moderate and 1 mild. Six chemicals did not meet the REET criteria: 5 were non/minimal/mild eye irritants and 1 was a moderate. Pooling data resulted in REET positive criteria being met by 25/44 materials and included 15 labeled severe (R41) with 6 moderate and 4 mild. Nineteen chemicals did not meet the REET criteria: 14 were non/minimal/mild eye irritants and 5 were moderate. There were no false negatives in either dataset. Based on accumulated experience, those materials with REET scores meeting or exceeding the threshold values are labeled internally as severe eye irritants (R41). When used as a pre-screen in conjunction with the Draize assay, the REET contributes to significant reduction in the number of animals used to test for eye irritant potential.

1283 PRECISION-CUT RAT LIVER SLICE TECHNOLOGY: EFFECTS OF CARBON DIOXIDE VS. "RAT MIX" ANESTHESIA.

W. Y. Ho, M. H. French, J. Singh, A. V. Valles and J. B. Ulreich. *Surgery, University of Arizona, Tucson, AZ.*

Carbon dioxide or "rat mix" (2:5:8, acepromazine:ketamine:xylazine) are commonly used general anesthetics in survival and non-survival rat surgeries. High concentrations of carbon dioxide cause excitation, followed by depression, of the central nervous system resulting in loss of consciousness. The components of "rat mix" have the effects of a dissociative anesthetic by producing sedation and muscle relaxation. Ketamine/acepromazine produces medium to deep anesthesia sufficient for major surgery and peripheral vasodilation by acepromazine reduces blood pressure, inducing moderate respiratory depression. The addition of xylazine prolongs recovery but produces hyperglycemia. Our goal was to determine whether the choice of

anesthetic affected endpoints in assays commonly used in precision-cut liver slicing. Fischer F344 rats were anesthetized either in a carbon dioxide inhalation chamber until unconscious, or by intramuscular injection of 1.0ml/kg "rat mix". Total hepatectomies were performed and livers placed in V-7 cold preservation solution. Livers were cored (8mm) and precision-cut at 250µ. Slices were loaded on titanium carriers. Incubation was in a Vitron rolling incubation system under 95% oxygen / 5% carbon dioxide for 24, 48, or 72h in complete Waymouth's medium. At various timepoints, liver slices were harvested and weighed. The following indices of slice *via*bility were employed: 1) potassium content was measured by flame photometry, 2) lactate dehydrogenase (LDH) release was measured spectrophotometrically using a Sigma kit and 3) ATP reactions with luciferin-luciferase were measured using a luminometer. The only significant difference between the two anesthetics for these parameters was at 0h, where the carbon dioxide group significantly lowered ATP levels. However, there were no significant differences at 24, 48, and 72h. These results might be different for liver slices incubated with drugs and/or diluents. Supported by the Siegel Foundation and the UA Undergraduate Biology Research Program.

1284 IGE MEASUREMENTS : COMPARISON OF RBL AND PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) ASSAYS.

R. Skinner, N. Deakin, D. Shaw, R. J. Dearman and I. Kimber. *Syngenta CTL, Macclesfield, United Kingdom.*

Specific IgE antibody production is a critical event in immediate type hypersensitivity reactions, including asthma and food allergy. Due to the presence of much higher concentrations of other immunoglobulin isotypes, the accurate assessment of specific serum IgE antibody poses substantial technical challenges. We have examined the utility of the rat basophilic leukemia (RBL) cell line for the measurement of murine IgE responses *in vitro*. This cell expresses high affinity receptors specific for IgE. Receptor-bound IgE is cross-linked with protein allergen, releasing granules that have been prelabelled with 3H-serotonin. RBL cells were sensitized with mouse monoclonal anti-dinitrophenyl (DNP) IgE antibody (1-125ng/ml) and challenged with DNP-albumin conjugates (10-40ng/ml) with various hapten substitution ratios (SR). Polyclonal anti-OVA IgE antiserum of low and high titer (raised in BALB/c strain mice) were also assessed for activity in the RBL assay. Results were compared with titers measured in homologous PCA assay. Marked degranulation of RBL cells (40% to 60% specific release) was induced by conjugates with SRs of between 16 and 32 molecules of DNP per molecule of protein, whereas conjugates with lower SRs (of 10 or 3) failed to elicit significant serotonin release. In all cases where significant serotonin release was recorded, clear bell-shaped dose response curves were observed. All conjugates were able to induce mast cell degranulation *in vivo* in an homologous PCA assay. Anti-OVA antisera with titers of 1/32 to 1/64 (as measured by PCA) failed to stimulate RBL cell degranulation, whereas high titer antibody (1/2048 to 1/4096 by PCA) induced a positive RBL cell response. Successful stimulation of RBL cell degranulation requires not only appropriate epitope densities but also high affinity antibody. These data indicate that this assay is inappropriate for the routine analysis of specific polyclonal IgE antibody responses.

1285 THE IMPORTANCE OF MULTIPLE ENDPOINT ANALYSIS (MEA) USING RECONSTITUTED HUMAN TISSUE MODELS FOR IRRITATION AND COMPATIBILITY TESTING.

B. De Wever, M. Cappadoro and M. Rosdy. *SkinEthic Laboratories, Nice, France.* Sponsor: A. Goldberg.

The use of *in vitro* reconstituted human tissue models has become an established standard screening method in pharmaceutical and consumer-product/cosmetic product development. Because of the morphological and biochemical similarity with the *in vivo* tissues, these models are an unique tool to investigate different endpoints / aspects of irritation mechanism . Products are applied directly on the tissue, at the same doses and exposure time used *in vivo* and after topical exposure, the irritation potential of actives and finished products can be assessed in a very reproducible way by Multiple Endpoint Analysis (MEA) including tissue *via*bility, histological evaluation and the release of inflammatory mediators. MTT test is used for cell *via*bility assessment compared to negative and positive controls, but too often however, MTT is being used as a single endpoint to evaluate the biocompatibility of test products. Since MTT is mainly converted by the basal and suprabasal cells in 3D constructs, necrosis of the upper superficial layers of the tissue model is not detected, and requires histological tissue analysis. Furthermore a very common need in the safety assessment of the products is to discriminate between slightly irritant and very slightly irritant products : they can be detected because they induce the release of pro-inflammatory mediators (IL-1alpha, IL-8) without affecting tissue integrity measured by MTT and histology. Therefore, critical evaluation of each endpoint in the MEA strategy is equally important for accurate *in vitro* predictions whether in the field of cutaneous risk assessment phototoxicity, ocular toxicity or oral irritation, amongst others. In this work, different examples of MEA approach will be described and discussed.

1286 AN *IN VITRO* MODEL OF DRUG-INDUCED ARRHYTHMOGENESIS USING MYOCARDIAL REFRACTORINESS: COMPARISON TO hERG ELECTROPHYSIOLOGY AND RUBIDIUM EFFLUX.

R. G. Caccese, H. G. Barthlow, G. D. Yasay, J. S. Smith, C. W. Scott and R. A. Bialecki. *Neuroscience, AstraZeneca Pharmaceuticals, Wilmington, DE.* Sponsor: M. Dyroff.

Drug-induced QT interval prolongation on the electrocardiogram is associated with a potentially fatal arrhythmia, torsades de pointes. One mechanism for this effect is increased ventricular repolarization time *via* blockade of hERG (human ether-a-go-go-related gene) cardiac ion channels. However, several ion currents contribute to the cardiac action potential and together determine functional outcome. Therefore, it is important to evaluate the sum of all currents contributing to repolarization. Our aim was to validate the intact ferret papillary muscle as a functional predictor of drug-induced changes in refractoriness. Myocardial effective refractory period (ERP) was measured with 19 drugs clinically linked to QT prolongation. The drug concentration causing a 10% increase in ERP (EC_{10}) was correlated with IC_{50} values for hERG block in cells transfected with the channel and tested with either whole cell patch clamp electrophysiology or rubidium efflux measured by atomic absorption spectroscopy. Baseline ERP at 37 °C without drug was 145±3 msec. Significant increases in ERP were found for 16 of 19 drugs. The class III antiarrhythmic E4031 was most potent in all assays with an EC_{10} of 0.024 uM. Class I antiarrhythmics, antipsychotics, antimalarials and other agents demonstrated EC_{10} values from 1-105 uM. Calcium channel antagonists terodoline and bepridil had EC_{10} values of 12-14 uM. Surprisingly, astemizole and terfenadine had little effect on ERP with EC_{10} values of 100 uM and were similar to probucol and erythromycin. The ERP and Rb efflux assays correlated strongly (r=0.89) whereas the correlation between ERP and patch clamp data was less (r=0.66). We conclude that hERG, Na^+ and Ca^{2+} channel blockers can significantly change ERP of ferret papillary muscle. Because papillary muscle represents the integration of relevant ion channel contribution to repolarization, this assay may serve as a *via*ble adjunct to hERG ion channel tests to determine the potential for drug-induced cardiac anomalies.

1287 SPECIES AND TISSUE DIFFERENCES IN ENDOPLASMIC RETICULUM CA^{2+}-INDEPENDENT PHOSPHOLIPASE A_2 EXPRESSION.

G. R. Kinsey[1], B. S. Cummings[3, 1], J. Mchowat[2] and R. G. Schnellmann[1].
[1]*Pharmacology Sciences., Med. University of South Carolina, Charleston, SC,*
[2]*Pharmacology and Biomed. Sciences., University of Georgia, Athens, GA and*
[3]*Pathology, St. Louis University, St. Louis, MO.*

We recently demonstrated the presence of a novel Ca^{2+}-independent phospholipase A_2 in the endoplasmic reticulum (ER-$iPLA_2$) of rabbit renal proximal tubular cells. Further, inhibition of ER-$iPLA_2$ activity potentiated oxidant-induced lipid peroxidation and oncotic cell death, suggesting that ER-$iPLA_2$ acts to protect these cells during oxidative stress. Since oxidants play a role in the injury of numerous tissues, the goals of this study were to determine if ER-$iPLA_2$ is present, 1) in other rabbit tissues, 2) in corresponding rat tissues and 3) in several cancer cell lines. Using plasmenylcholine and diacylcholine as substrates in the presence of EGTA, ER-$iPLA_2$ activity was observed in rabbit and rat kidney, brain and heart microsomes, and in microsomes from all five cancer lines tested (Caki-1, A172, A549, PC-3 and L1210). ER-$iPLA_2$ activities in rabbit and rat kidney and heart were sensitive to the $iPLA_2$ inhibitor bromoenol lactone (BEL) but not the $iPLA_2$ inhibitor methyl arachidonyl fluorophosphonate (MAFP). In contrast, rat brain ER-$iPLA_2$ activity was inhibited by both BEL and MAFP, while rabbit brain was BEL insensitive and MAFP sensitive. Reverse transcriptase polymerase chain reaction and/or immunoblot analysis revealed $iPLA_2\gamma$ expression in rabbit kidney and heart, rat kidney, heart and brain and in all five cancer cell lines tested. Since BEL-sensitive $iPLA_2\gamma$ and MAFP-sensitive $cPLA_2\gamma$ are the only PLA_2 family members known to reside in the ER, these data suggest that ER-$iPLA_2$ activity in rabbit and rat kidney and heart is mediated by $iPLA_2\gamma$, while ER-$iPLA_2$ activity in rabbit brain is mediated primarily by $cPLA_2\gamma$. Rat brain microsomes appear to contain both $iPLA_2\gamma$ and $cPLA_2\gamma$. These results demonstrate broad expression of ER-$iPLA_2$ activity across species and tissues, and in cancer cell lines, and differential tissue expression of $iPLA_2\gamma$ and $cPLA_2\gamma$.

1288 A PROTYPE *IN VITRO* NEUROTOXICITY DATABASE.

A. D. Weissman. *NovaScreen Biosciences Corp, Hanover, MD.* Sponsor: J. Sina.

There is a current need to develop credible *in vitro* endpoints that parallel the toxic effects of chemical compounds in humans. This information, incorporated into a relational database. would be useful for the virtual screening of compounds at an early stage in their development. Improving the predictive capabilities of current *in vitro* testing requires a battery of endpoints that include mechanism-based assays as well as general cxytotoxicity in cells appropriate to the target organ of interest. Towards this end we have tested a library of 204 compounds thought to be relevant to neurotoxicity in both a proliferative and differentiated human neuronal cell line. We examined seven measures of cellular toxicity with these compounds at six concentrations in duplicate across seven days of treatment. This has resulted in an initial *in vitro* toxicity database annotated with chemical data and LD50 values from *in vivo* studies. The database has shown internal consistency between *in vitro* toxicity measures, as well significant correlations with animal and human toxicity data.

1289 LYSIS OF ADHERENT HUMAN EPIDERMAL KERATINOCYTES IN SITU BY A MISONIX TISSUE CULTURE PLATE SONICATOR.

C. L. Gross, O. E. Clark, E. W. Nealley, M. T. Nipwoda and W. J. Smith. *USAMRICD, APG-EA, MD.* Sponsor: A. Sciuto.

Human epidermal keratinocytes (HEK) are used extensively in our laboratories as an *in vitro* model of human epidermis to study the vesicant action of sulfur mustard (HD). These adherent cells are released from their tissue culture vessels by proteolytic treatment that detaches the HEK from their plastic supports. However, this common procedure may compromise cells that are struggling to survive and influence the interpretation of damage from these moribund cells. Biomarkers of damage are isolated by lysing cells using specialized buffers or by subjecting the cellular pellet to sonication or other means of disruption. It is especially critical to be able to lyse cells in the desired buffer in situ to minimize losses of biological material used in analysis. Using a Misonix tissue culture plate sonicator, both 24- and 96-well plates of HEK were subjected to 30-sec bursts in a 4oC tray horn ultrasonic bath at the maximum setting of 10. There was 1 min of cooling between bursts for total sonication times of 2 to 5 minutes. The plates were inspected by microscopy, and wells were photographed to give a rough measurement of HEK lysis. Sonicated plates were also checked for the number of *via*ble cells remaining on the plate or in the supernatant by an MTS-PMS chromogenic *via*bility assay. The supernatant fluid was removed from the well and wells were again checked for the percentage of remaining adherent cells by microscopy. Glutathione levels in the supernatant fluid by both sonicated HEK and acid-extracted HEK were measured and appeared identical. From these results, it appears that the sonication technique may be a useful alternative for the proteolytic method commonly used for the isolation of specific biochemical markers.

1290 DIFFERENTIATION OF THE ABSORPTION KINETICS OF JET FUEL HYDROCARBONS WITH AN ETHANOL/WATER SYSTEM AND A MEMBRANE-COATED FIBER TECHNIQUE.

X. Xia and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

After dermal exposure to jet fuel, hydrocarbon components will penetrate the stratum corneum, transport and deposition through the epidermis and dermis into the vascular system. The distribution and transport mechanisms of different hydrocarbons through the lipophilic and aqueous routes are unknown. We have used an ethanol/water system to simulate the lipophilic and aqueous routes and studied their absorption kinetics with a membrane-coated fiber technique. The major aromatic and aliphatic components in jet fuel were selected as study compounds. The equilibrium absorption amounts of the aromatics in log scale decreased linearly with increasing ethanol/water ratio, while their absorption rates among different aromatics were not significantly different. Aliphatics showed quite complicated absorption kinetics as all of the jet fuel aliphatics are hydrophobic compounds with very small water solubility. The absorption rate of the aliphatics in water could be linearly predicted with their carbon number. When the carbon number was larger than 14 (tetradecane), the water solubility was so low that the absorption rate approached zero. Increasing the ethanol/water ratio will increase the hydrocarbon solubility. At a given ethanol/water ratio, the absorption rates were higher for smaller aliphatics, while the equilibrium absorption amounts were higher for larger aliphatics. When the ethanol/water ratio reached 50%, all of the aliphatics reached their highest absorption rates and could reach absorption equilibrium within 200 min. Further increasing the ethanol/water ratio, the equilibrium absorption amounts were decreased, while the absorption rates did not change significantly. These experimental results could aid in the understanding of the absorption and transport mechanisms of hydrocarbons in a human body after jet fuel exposure.

1291 THE PERFORMANCE OF *IN VITRO* TEST BATTERIES AS PRE-SCREENS TO *IN VIVO* SKIN AND EYE IRRITATION TESTS.

D. I. Lees and R. W. Lewis. *CTL, Syngenta, Cheshire, United Kingdom.* Sponsor: I. Kimber.

Our approach to rabbit skin and eye irritation tests has been refined to include elective *in vitro* pre-screens. For the detection of severe eye irritants, a simple cytotoxicity test is used followed, where necessary by the isolated rabbit eye test. The outcome of this test strategy is used to guide subsequent *in vivo* testing, and represents a refinement and potential reduction in the use of animals. Materials predicted to be severe irritants are initially tested at low volume in a single animal. Only if less that severe *in vivo* results are obtained is the full group size of animals completed. Test battery performance over a number of years shows an 86% ability of this approach to correctly predict materials that are less than severe ocular irritants *in vivo.* The ability of this battery of tests to correctly predict severe eye irritants is lower at 67%. For the assessment of skin corrosion, the Transcutaneous Electrical Resistance (TER) test followed by a dye binding step is used as the pre-screen. Materials identified as potentially corrosive *in vitro* may not need to be tested in the live animal. The ability to correctly predict materials as non-corrosive *in vivo* by this approach is high at 94%. The ability to correctly predict materials as corrosive is lower at 40%. However, this outcome may be influenced by the low prevalence of corrosive materials assessed. Data from over 300 tests covering a wide range of chemical classes are presented in this analysis.

1292 UPTAKE KINETICS OF JET FUEL AROMATIC HYDROCARBONS FROM AQUEOUS SOLUTIONS STUDIED BY A MEMBRANE-COATED FIBER TECHNIQUE.

J. E. Riviere and X. Xia. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

The absorption of aromatic hydrocarbons from aqueous media is a critical step involved in their transport and deposition in a human body after jet fuel exposure. We have developed a membrane-coated fiber (MCF) technique to study this process, in which a polymer membrane is coated on an inert fiber as a permeation membrane. A polydimethylsiloxane (PDMS) membrane intending to simulate human stratum corneum and a polyacylate (PA) membrane intending to simulate cell membrane were used in this study. The uptake kinetics of the major aromatic components in jet fuel were studied in a flow system, which provided a constant concentration for the prolonged permeation experiments. The absorption profiles of the aromatic compounds were regressed with a mathematical model of the MCF technique. The equilibrium absorption amount and a kinetic parameter that determined the absorption kinetics were obtained from the regression for each compound. The uptake and elimination rate constants of 6 benzene derivatives and 3 naphthalene derivatives were determined with PDMS and PA MCFs. An equilibration method and a regression method were developed to determine membrane/water partition coefficients. The PDMS/water partition coefficients of the benzene and naphthalene derivatives were linearly correlated with their octanol/water partition coefficients (LogKpdms/w=0.871LogKo/w-0.241, R2=0.995). The PA/water partition coefficients of the benzene derivatives and the naphthalene derivatives were correlated differently with their octanol/water partition coefficients. The correlation equations for benzene and naphthalene derivatives were LogKpa/w=0.865LogKo/w+0.0045, R2=0.997 and LogKpa/w=0.763LogKo/w+0.911, R2=1.00, respectively. This shows that the MCF technique can detect the subtle differences in intermolecular interactions of the two group derivatives between the two-membrane/water systems.

1293 DEVELOPMENTAL TOXICITY OF TRIETHYLENE GLYCOL, TRIETHYLENE GLYCOL MONOMETHYL ETHER AND TRIETHYLENE GLYCOL DIMETHYL ETHER IN INTACT *DROSOPHILA MELANOGASTER.*

D. Lynch. *Biomonitoring and Health Assessment Branch, NIOSH, Cincinnati, OH.*

To further characterize the *Drosophila*-based prescreen to detect developmental toxicants, triethylene glycol (TEG), triethylene glycol monomethyl ether (TEGMME) and triethylene glycol dimethyl ether (TEGDME) were evaluated using our published protocol (*Teratogenesis, Carcinogenesis, and Mutagenesis* 11:147-173, 1991). One or two experiments, each employing 10 equimolar concentrations (.0013-1.64mM/*via*l) of test chemical and including a concurrent control, were conducted per chemical. *Drosophila* were exposed throughout development (egg through third instar larva) in culture *via*ls to medium containing the test chemical. Each *via*l contained 1g of powdered medium and 5ml of distilled deionized water or a solution of test chemical in water. A mated, untreated, Oregon-R wild-type female (Mid-American Drosophila Stock Center, BGSU, Ohio) was added to each culture *via*l

and allowed to oviposit for 20 hours, then removed. Emerging offspring were collected over 10 days and examined microscopically (25x) for bent humeral bristles - a morphological defect shown to occur with an increased incidence in fruit flies exposed to developmental toxicants. The incidence of bent bristles was statistically increased compared to concurrent controls (chi-square) at the two highest TEG concentrations - 1.32mM, 38/181, p<0.001 and 1.64mM, 29/99, p<0.001. For TEGMME, the incidence of bent bristles was statistically increased at the four highest concentrations - 0.66mM, 8/155, p<0.01; 0.99mM, 12/134, p<0.001; 1.32mM, 11/75, p<0.001; and 1.64mM, 3/5, p<0.001. For TEGDME, the incidence of bent bristles was statistically increased at three concentrations - 0.34mM, 10/159, p<0.01; 0.66mM, 28/171, p<0.001; and 0.99mM, 6/25, p<0.001) - no flies emerged at 1.32 and 1.64mM. Based on these results, TEGDME > TEGMME > TEG in terms of developmental toxicity. Results with TEG, TEGMME and TEGDME parallel the developmental toxicity seen in mammals and support further utilization of this *Drosophila*-based assay as a prescreen for developmental toxicants.

1294 THE IMPACT OF ETHANOL ON THE *IN VITRO* SKIN PENETRATION RATES OF CAFFEINE IN ENGINEERED SKIN CONSTRUCTS.

G. Pugh[1], G. O. Moyer[2], H. A. Raabe[2], J. W. Harbell[2] and D. M. Bagley[1]. *[1]Colgate-Palmolive Co., Piscataway, NJ and [2]Institute for In Vitro Sciences, Inc., Gaithersburg, MD.*

The reference material, caffeine (prepared in ethanol), was evaluated in 3 *in vitro* models to compare the rates of skin penetration in each of the models. The models were human donor skin, an engineered skin construct (MatTek Corporation, Model EPI-606X) and slaughterhouse-derived pig skin. The tissues were mounted in flow-through diffusion cells (PermeGear, Inc., 0.64 cm^2 surface area), qualified for barrier function by $^3H_20$ passage, followed by the application of a 9 µL dose of ^{14}C-caffeine in ethanol (~4 µg/cm^2). The study duration was 24 hours. Total recovery of caffeine was acceptable (typically 95 to 100%) in each model. In human and pig skin, the rates of skin penetration were uniform and continuous throughout the 24-hour period. The mean amounts of caffeine that had been absorbed after 24 hours were 11% and 15% of applied dose, respectively (n=2 trials each). However, in the engineered skin model, the rate of penetration was remarkably high in the first 3 hours, followed by an abrupt decrease in the penetration rate thereafter. The amount absorbed after 3 hours was approximately 60% of applied dose. After 24 hours, the amount had increased to only 62% of applied dose. Subsequent experiments were conducted to evaluate the impact of the vehicle on caffeine's penetration rate in engineered skin. In the first experiment, caffeine prepared in water was tested in parallel with caffeine prepared in ethanol. A notable penetration rate lag phase was observed in the water-based preparation as compared to the ethanol-based preparation, but totals absorbed were 87% vs 66% of the applied dose, respectively. In the second experiment, engineered skin was pre-treated with ethanol followed by topical application of caffeine. Caffeine penetration rates and total caffeine absorption were similar in ethanol pre-treated and non-treated engineered skin. These results suggest that ethanol may have enhanced skin penetration upon initial exposure, but the solvent effect may have been rapidly modulated.

1295 PRECISION-CUT TISSUE CHIPS AS A TOXICOLOGICAL TOOL.

J. M. Catania, A. Fernandez and A. Gandolfi. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

The premise of these studies is to use precision-cut tissue chips from multiple organs from mice to simultaneously examine the *in vitro* toxicity of chemical agents. The use of the smaller tissue chips allows more samples since the small organs from the mouse only provide a limited amount of slices while 4-10 fold more tissue chips can be prepared from an organ. Tissue chips are produced by using a biopsy punch to make multiple 4 mm tissue chips from one 8 mm, 250 micron-thick tissue slice. Additionally, larger-sized diameter coring tools were explored to expedite and maximize chip production and quantity. Alterations to the Vitron Tissue Slicer have allowed more consistent slices, while some modifications allow the potential to generate multiple tissue slices at once. Tissue chips are incubated in 24 well plates, placed into a rotating 37 C shaking incubator and oxygenated with 95:5 O2:CO2. Due to the smaller amount of biomass in the tissue chip, fluorescent biomarkers have been used to assess *via*bility. TMRE, Mitotracker Far Red, 6-carboxy fluorescein, and NBD-TMA have been used for their permeability and detection characteristics, and for the assessment of cellular processes. Neutral red and MTT assays have been employed to expedite processing of tissue chips, and have been compared to our previous, more time-consuming *via*bility indicators (e.g. intracellular K+ content/DNA). Iodoacetemide (IAM) treatment (0-1000 uM), a fast-acting alkylator, has shown increases in TMRE fluorescence (500 uM IAM, 45 min), indicating increased mitochondrial activity, with a subsequent decrease in *via*bility in liver and kidney tissue chips as determined by both neutral red uptake and MTT assays. IAM treatment of 10 uM in liver and kidney tissue chips showed more than 50% decrease in *via*bility with a 12 h incubation. Kidney chips exhibit decreases in *via*bility in 2 h incubations with 1000 uM IAM treatment. This system will allow for a rapid examination of toxic effects in multiple target tissues to expedite toxicity profiling of potential new agents. (NIH R33 CA97449)

1296 EVALUATION OF THE BCOP ASSAY AS A PREDICTOR OF OCULAR IRRITATION OF PETROCHEMICAL PRODUCTS.

P. T. Bailey[1], J. J. Freeman[1], R. D. Phillips[1] and J. C. Merrill[2]. *[1]Toxicology and Environmental Sciences Division, ExxonMobil Biomedical Sciences, Inc., Annandale, NJ and [2]Institute for In Vitro Sciences, Inc., Gaithersburg, MD.*

Many initiatives have been undertaken in recent decades to reduce, refine, and replace the use of animals in evaluating toxicity, however, relatively few alternative studies have been conducted with petrochemical products. Therefore, a program was implemented to facilitate the development and validation of alternative *in vitro* test methodologies that could predict ocular irritation and acute systemic toxicity of petrochemicals. The Bovine Corneal Opacity and Permeability (BCOP) assay, a test that measures opacity and epithelial integrity to corneas, was used to evaluate 16 petrochemical products (e.g., lubricant additives, cutting fluids, solvents). Based on *in vivo* ocular irritation data and risk phrases, test articles were subdivided into the following three main categories: Category A (risk of serious damage to eyes), Category B (irritating to eyes), and Category C (non-irritating). Test article treated corneas were incubated at 32±1°C for two exposure times of 3- and 10-minutes. BCOP assay correctly classified the products in >95% of cases with the 10-minute exposure data, suggesting that it is valid for certain petrochemicals as a screening tool before *in vivo* testing (refinement) and as an alternative stand alone assay (replacement) for assessing hazard. The same 16 test articles were examined in the 3T3 Neutral Red Uptake (NRU) Bioassay as a predictor of dose selection and for assessing potential acute systemic toxicity. Poor predictability (<60%)was observed in the NRU assay, which was likely associated with their low solubility in water and the inability of cells being exposed to concentrations below desired concentrations. Although poor predictability was observed, results did indicate the possible use of the NRU assay as a refinement-screening tool for specific chemicals. Based on this body of data, these results could facilitate the validation and acceptance of alternative testing methodologies that will ultimately benefit animal welfare.

1297 IMPROVED 'ACCOUNTABILITY' OF *IN VITRO* METHODS USED FOR ASSESSING DERMAL ABSORPTION OF ENVIRONMENTAL CONTAMINANTS.

R. P. Moody, M. Akram and I. Chu. *Health Canada, Federal Government, Ottawa, ON, Canada.*

As part of our research to examine *in vitro* methods for quantifying dermal absorption, we have refined the method used to evaluate this process. Since both the amount and persistence of chemical remaining in the skin and of that more completely absorbed into the receivor blood simulant solution is important to measure for hazard risk assessment, the method used must provide complete 'accountability' of the chemical dose used. Human skin permeation data for the lipophilic compound, nonyl phenol, obtained using our in-house developed Automated *In vitro* Dermal Absorption (AIDA) method will be presented to show the importance of having a complete mass balance for such 'accountability' purposes. This will include simple considerations such as loss of the applied test 14C-radiolabeled chemical to the test apparatus including the plastic pump tubing used to circulate the donor chemical treatment solution. We have previously reported very significant loss of this lipophilic compound (Log octanol/water partition coefficient = 6) to peristaltic pump tubing (Akram et al., 2003). We expand this here to show 14C-nonyl phenol data for several soap water and solvent apparatus washes obtained from human skin absorption tests. The results showed loss of as much as 60 percent of the applied radiolabel test chemical and that most of this loss was due to adsorption into the test apparatus, particularly the peristaltic pump tubing used. In order to provide a possible solution to this problem, tests with 1 percent soap added to the donor solution were conducted. Although tests with 1 percent soap added prevented adsorption to pump tubing and full mass balance was obtained, further tests are needed to determine the suitability of this 'quick-fix' since the presence of soap in the donor solution, even at low concentration, could affect skin permeability and/or chemical depot storage/bioavailability and so adversely influence the data used for regulatory toxicology risk assessment.

1298 ESTIMATE OF FALSE NEGATIVE RATES FOR THE *IN VIVO* RABBIT DERMAL IRRITATION ASSAY.

N. Choksi[1], J. Haseman[2], R. Tice[1] and W. Stokes[3]. *[1]ILS, Inc., Research Triangle Park, NC, [2]NIEHS, Research Triangle Park, NC and [3]NICEATM, NIEHS, Research Triangle Park, NC.*

Alternative *in vitro* test methods proposed to substitute or replace an *in vivo* test method should provide equivalent or improved protection of human or animal health in order to gain regulatory and general acceptance. The ICCVAM and NICEATM are collaborating with the ECVAM to conduct a validation study of three *in vitro* dermal irritation assays. To assess the acceptability of these *in vitro* assays, an effort was undertaken to estimate the false negative rate of the *in vivo* test as defined by its ability to consistently identify irritants, mild irritants, and non-irritants according to the Globally Harmonized Classification Scheme. Data for 187 substances was obtained from the ECETOC database for skin irritation and corrosion. The distribution of rabbits with mean erythema or oedema scores of <1.5, between 1.5 and 2.3, or >2.3 was determined for each of the substances classified as negative, mild irritant or irritant. Since the true classification of each substance is unknown, a simplifying assumption was made that the results are correct for substances tested once only. For multiple-tested substances, the classification obtained from a majority of the studies was used. The analysis indicated: (1) the likelihood of a mild irritant being under-classified as a non-irritant was <5 percent when based on all substances and <10percent when based on multiple-tested substances, (2) the under-classification rate of irritants as non-irritants was <1 percent, and (3) the under-classification rate of irritants as mild irritants ranged from 9-30percent, depending on whether all substances or only multiple-tested substances were considered. Additional *in vivo* irritation data for studies using currently accepted procedures was requested from US federal agencies and industry. Appropriate data received will be added to the database and the false negative analysis refined. This evaluation emphasizes the need for high quality *in vivo* dermal irritation data that can be used to assess the performance of proposed new alternative test methods. ILS staff supported by NIEHS contract N01-ES-35504.

1299 ESTIMATE OF FALSE NEGATIVE RATES FOR THE *IN VIVO* RABBIT DERMAL CORROSION ASSAY.

R. Tice[1, 2], N. Choksi[1, 2], J. Haseman[3], R. Hill[4], M. Lewis[4], D. Lowther[4] and W. Stokes[2]. *[1]ILS, Inc., Research Triangle Park, NC, [2]NICEATM, NIEHS, Research Triangle Park, NC, [3]NIEHS, Research Triangle Park, NC, [4]EPA, Washington, DC and [5]FDA, College Park, MD.*

Alternative *in vitro* test methods proposed to substitute or replace an *in vivo* assay should provide equivalent or improved protection of human or animal health to gain regulatory and general acceptance. ICCVAM evaluated four *in vitro* dermal corrosivity assays as potential replacements for the *in vivo* dermal corrosivity assay. ICCVAM recommended that these assays be used in accordance with the globally harmonized tiered testing scheme in a weight-of-evidence approach. In this approach positive substances could be classified and labeled as corrosives and negative substances are further evaluated in accordance with an internationally accepted testing scheme. This recommendation was based largely on the 12-17 percent false negative rates of the *in vitro* assays in identifying corrosive substances. ICCVAM concluded that these false negative rates likely exceeded that of the currently used *in vivo* assay and would not provide adequate public health protection. To estimate the likelihood of a false negative result in the *in vivo* assay, the available data was reviewed. Relevant *in vivo* dermal corrosivity data were obtained from federal agencies and the published literature. The database consisted of 50 corrosive substances. Since the true likelihood of a corrosive response for each of the substances in the database was unknown, the sample rate was considered the best estimate of the true positive response rate. Initial analysis of the database indicated that the current *in vivo* dermal corrosivity test has an estimated false negative rate of 5.5 percent. The analysis also suggests that under-classification of a substance would most likely occur only for weak corrosives. NICEATM continues to seek additional high-quality *in vivo* corrosivity data to refine the estimated *in vivo* assay false negative rate. This evaluation emphasizes the need for high quality *in vivo* dermal corrosivity data that can be used to evaluate the performance of proposed alternative assays. ILS staff supported by NIEHS contract N01-ES-35504.

1300 CHARACTERIZATION OF BPA GLUCURONIDATION BY RAT AND HUMAN HEPATOCYTES.

R. K. Kuester, D. Castro, A. M. Solyom and S. Glenn. *Pharmacology, The University of Arizona, Tucson, AZ.*

Bisphenol A is monomer of polycarbonate plastics with weak estrogenic activity. Since the predominate metabolite of BPA, BPA-monoglucuronide, is devoid of estrogenicity, it is important to examine its rate of glucuronidation across species. This study compared the *in vitro* kinetic constants for the glucuronidation of BPA by male Sprague-Dawley rat and human hepatocytes obtained from various sources. When hepatocytes from both species were incubated for 20 min as suspensions in the presence of 14C-BPA (1.3-52 μM) BPA-monoglucuronide was the predominate metabolite. Metabolic constants (Vmax and Km) from freshly isolated rat hepatocytes were compared to fresh or cryopreserved rat hepatocytes obtained from commercial sources to evaluate if cryopreservation and/or delivery time affects the ability of hepatocytes to glucuronidate BPA. When rat hepatocytes were isolated and shipped on ice (18-24 h) prior to incubation with BPA, metabolic clearance (Vmax/Km) decreased from 0.015 to 0.01 ml/min compared to freshly isolated hepatocytes. Shipped cryopreserved rat hepatocytes incubated in suspensions produced BPA-glucuronide at rates comparable to those obtained with suspensions of freshly isolated rat hepatocytes (clearance rates of 0.016 and 0.015 ml/min per 375, 000 cells, respectively). Preliminary results demonstrate that suspensions of cryopreserved human hepatocytes conjugate BPA at rates similar to those obtained from recently isolated rat hepatocytes. Thus, cryopreserved human hepatocytes may be a useful model to assess variability among humans in rates of BPA glucuronidation. This research was supported in part by the Southwest Environmental Health Science Center (ES 06694) and the American Plastics Council.

1301 FURTHER DEVELOPMENT OF A FLOW CYTOMETRY-BASED LOCAL LYMPH NODE ASSAY WITH EAR SWELLING AND IMMUNOPHENOTYPIC ENDPOINTS.

S. Young, D. R. Cerven, T. L. Ripper and G. L. DeGeorge. *MB Research Laboratories, Spinnerstown, PA.*

The local lymph node assay (LLNA) is an alternative to the guinea pig sensitization test used to identify and characterize dermal sensitizers. We have applied flow cytometric techniques to the ICCVAM-validated LLNA protocol to enhance the basic LLNA and to increase sensitivity/specificity. Lymph node cell (LNC) proliferation is measured by BrdU incorporation, instead of 3H-thymidine uptake. In addition, dermal irritation (% ear swelling) and immunophenotypic markers such as B220 (CD45R), CD69, CD44, CD62L, CD3, CD4, CD8, I-Ak and CD25, were evaluated for predictivity of sensitization potential and discrimination of false-positive irritants. The most useful endpoints in the Enhanced LLNA (E-LLNA) were the number of proliferating LNC (BrdU +cells), B:T cell ratio, %B220+, %CD69+ and %IAk+ cells. Several key human sensitizers including oxazolone, DNCB, HCA, mercaptobenzothiazole (MBT) and chlorpromazine+UVA were correctly classified. SLS, which is a false positive in the standard radiometric LLNA, was correctly classified as a non-sensitizing irritant by the E-LLNA. SLS failed to induce changes that are characteristic of true sensitizers which include increases in %B220+, %CD69+, %IAk+ and the B:T cell ratio. The flow cytometry-based LLNA yields Stimulation Indices (SI) similar to those in the ICCVAM validation report for DNCB, HCA and many other chemicals. In the E-LLNA, the EC3 (concentration which induces a SI > 3) for HCA ranges from 4.2% to 9.8%, and for DNCB it ranges from 0.01% to 0.05%, consistent with ICCVAM LLNA test results using 3H-thymidine. In summary, we have developed an enhanced, flow cytometry-based LLNA which 1) can sensitively and specifically identify and characterize dermal contact sensitizers; 2) does not require radioactivity; and 3) allows better discrimination of false-positive irritants from true sensitizers when compared to the basic radiometric LLNA.

1302 DEVELOPMENT OF AN *IN VITRO* MODEL FOR ASSESSING PULMONARY INFLAMMATION.

J. M. Kennedy and J. M. Carter. *Central Product Safety, Procter & Gamble, Cincinnati, OH.*

Because of the complexity of the biology of the lung, *in vitro* tools to investigate the mechanisms of inhaled toxicants have not been fully explored as they have been in other fields. There exists an increasing need to develop new tools that are mechanistically based, less expensive and less time consuming than traditional assays that allow for the reduction of animal use whenever possible. Here we describe work done in our lab to develop tools to assess the inflammatory potential of inhaled particles. Briefly, *in vivo* particle-induced rat inflammatory studies involving the use of genomic/proteomics in our lab have identified key pro- and anti- inflammatory markers we are exploiting for use in developing a rat inflammatory *in vitro* assay system. The use of an immortalized rat alveolar macrophage cell-line has considerably advanced our *in vitro* mechanistic research and is aiding in the development of an *in vitro* screening assay. However, the macrophage alone cannot tell the entire story of the inflammatory process. We are also developing techniques to isolate and culture rat blood neutrophils that mimic *in vitro* the egression and maturation process of the neutrophils. We have used genomic data from *in vivo* studies to develop a cytokine cocktail similar to those used for dendritic cell maturation assays. This has facilitated *in vitro* mechanistic studies pertaining to particle-induced inflammation. This work correlates well with studies performed *in vivo* relating particle-induced inflammation to the pro-and anti-inflammatory markers used in this assay. We have seen good correlation between the measurement of release of reactive

oxygen and nitrogen species after exposure of neutrophils to particles *in vitro* and to *ex vivo* measurements of particle-induced reactive oxygen and nitrogen release of *in vivo* inflammatory cells. The development of these assay systems will lead to improvement in *in vitro* screening assays which ultimately reduce animal usage and provide less expensive assays for screening potential toxicants.

1303 PERFORMANCE OF THE PH 6.7 SYRIAN HAMSTER EMBRYO (SHE) CELL TRANSFORMATION ASSAY IN PREDICTING THE CARCINOGENIC POTENTIAL OF CHEMICALS.

H. Zhang and B. C. Myhr. *Genetic and Molecular Toxicology, Covance Laboratories Inc., Vienna, MD.*

The Syrian hamster embryo (SHE) cell transformation system is a well-developed *in vitro* short-term testing system for evaluating the carcinogenic potential of chemicals. Using morphological transformation as the end point, the SHE cell transformation assay has been used to study mechanisms of chemical carcinogenesis *in vitro*, as well as to predict rodent carcinogens. Early applications of the SHE cell transformation assay suffered from some operational difficulties, most of which have been overcome by culturing the SHE cells in a slightly acidic (pH 6.7) environment. From studies beginning in the 1980s, approximately 107 chemicals have been tested using the reduced pH method. Among these, rodent carcinogenicity data has been reported for 100 chemicals (68 carcinogens and 32 noncarcinogens). SHE cell transformation assays *in vitro* yielded an overall concordance rate of 81% (81/100) with the results of the rodent bioassay, a sensitivity of 85% (58/68), and a specificity of 72% (23/32). In addition, the SHE cell transformation assay correctly evaluated all but one (phenacetin) of the 30 known or suspected human carcinogens classified by the NTP/IARC, which indicated a high sensitivity of 97%. Assay evaluations are compiled for each tested chemical. Collectively, these data suggest that the SHE cell transformation assay out-performed other commonly used short-term tests for assessing the carcinogenic potential of chemicals. The SHE cell assay clearly provides a valuable short-term test for screening new chemicals for carcinogenic potential.

1304 DEVELOPMENT OF A CELLOMICS-BASED *IN VITRO* SCREEN FOR PHOSPHOLIPIDOSIS.

J. K. Morelli, M. Buehrle, F. Pognan and P. Ciaccio. *Safety Assessment, AstraZeneca Pharmaceuticals, Wilmington, DE.*

Several cationic, amphiphilic drugs induce local or systemic phospholipidosis (PLD), generally after chronic exposure in preclinical species and humans. PLD is characterized by the accumulation of drug, phospholipid and concentric lamellar bodies in lysosomes. We developed a fluorescence-based *in vitro* screen that is predictive of PLD using the Cellomics Arrayscan high content screening platform, which captures images from 96-well cell culture plates using multichannel fluorescence microscopy. I-13.35 adherent mouse spleen macrophage cells were plated and cultured with fluorescent phospholipid NBD-PE, and drug. Concentrations were in a range from 1 to 100 uM. After 24 h incubations, the cells were fixed and nuclei were identified by Hoechst staining and dead cells by ethidium homodimer-2 (EthD-2) incorporation. NBD-PE is taken up into the lysosomes passively and the effect of drug on its normal breakdown was monitored using the Arrayscan by quantification of fluorescence intensity in the FITC channel. Several parameters were tested for their effect on both relative and absolute NBD-PE fluorescence, including cell number (10, 000/well), matrix (collagen), cell exposure (24 h), NBD-PE concentration, and image analysis algorithm. For validation efforts, amiodarone, erythromycin, amitriptyline, chloroquine, chlorpromazine, memantine, chlormipramine & imipramine were used as positive controls. Chloramphenicol, tetracycline, & Zyvox were used as negative controls. Positive samples responded in a dynamic range of up to 5-7 fold-increases over vehicle controls; meaningful dose-responses over at least 2 concentrations; day-to-day, reproducible differences in NBD-PE accumulation between top concentrations and vehicle controls; and statistical significance. In addition, artifact of NBD-PE accumulation coming from excess cytotoxicity (30-100 uM) could be eliminated by ethidium staining and employing cell gating (dead cell rejection). Thus, in following these criteria, the assay was found to be both sensitive and selective in that 6/7 positive controls and 3/3 negative controls were correctly called.

1305 COMPARATIVE MICROARRAY ANALYSIS OF BASAL GENE EXPRESSION IN MOUSE HEPA-1C1C7 WILD-TYPE AND MUTANT CELL LINES.

C. J. Fong, L. D. Burgoon, M. D. Ramer and T. R. Zacharewski. *Department of Biochemistry & Molecular Biology, Department of Pharmacology & Toxicology, Institute of Environmental Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI.*

cDNA microarrays containing 4858 unique genes were used to examine differences in basal gene expression between mouse Hepa-1c1c7 wild-type and mutant (C1 - aryl hydrocarbon hydroxylase activity deficient, C4 - Ah receptor nuclear translocator deficient and C12 - 10% Ah receptor levels) cell lines. Using a modeled t-test approach, 728 unique genes were identified in the mutant cell lines that exhibited a significant ($p < 0.01$) difference in basal expression when compared to wild-type. Surprisingly C1 cells exhibited the greatest number (260 unique genes) of expression differences while 186 and 92 differences where identified in C4 and C12 cell lines, respectively, when compared to wild-type cells. Up-regulated functions unique to the C1 line include glutathione S-transferases and tRNA synthetases while genes associated with oxidative stress (Prdx) and lipid metabolism (Apobec1, Fasn) were down-regulated. C4 up-regulated genes are primarily associated with the mitochondrial function (Atp5g1, Atp5o) while down-regulated pathways include glucose metabolism (Gapd, Gpi1). Significant up-regulation of cell cycle (cyclins) and structural (Vcl) genes in the C12 line may be compensatory and related to their slow growth and unique cellular morphology compared to the wild-type cells. Quantitative RT-PCR has verified microarray data for 6 uniquely (C1 - Xcl1, Prdx3; C4 - Cldn3, Fosb; C12 - Cyp1a1, Tmem9) and 3 (Esr1, Rdbp, Rpl6) commonly expressed genes. These studies further characterize Hepa-1c1c7 wild-type and mutant cell line characteristics and provide further insights into the endogenous role of the AhR as well as potential mechanisms of toxicity of TCDD and related compounds. Supported by ES 11271.

1306 PHASE TWO: EVALUATING THE EYE IRRITANCY OF SOLVENTS IN A SIMPLE FRAGRANCE MIXTURE WITH THE BOVINE CORNEAL OPACITY AND PERMEABLITY (BCOP) ASSAY.

N. Cuellar[1], P. H. Lloyd[2], J. E. Swanson[1], J. C. Merrill[3], G. Mun[3], J. W. Harbell[3] and K. L. Bonnette[4]. *[1]S.C. Johnson & Son, Inc., Racine, WI, [2]SCJ EURAFNE Ltd., Egham, Surrey, United Kingdom, [3]Institute for In Vitro Sciences, Inc., Gaithersburg, MD and [4]Charles River Laboratories Inc., Spencerville, OH.*

Fragrances are complex mixtures used in many consumer products. Organic solvents, such as ethanol, are major components of fragrance formulations functioning mainly as solubilizers and fragrance delivery mechanisms. In Phase One (Cuellar et al, 2002), the BCOP assay and primary eye irritation study (EPA-OPPTS 870.2400) were conducted using simple fragrance mixtures containing six commonly used solvents. The corneal depth of injury was assessed histologically both *in vitro* and *in vivo*. In the BCOP assay, corneas were exposed for 1 and 3 minutes, rinsed and incubated for 20 hours before the opacity and permeability endpoints were assessed. *In vivo*, animals were scored at 1, 4, and 24 hours. Individual solvents impacted the level of irritation of these formulations. Phase Two evaluated the time course of lesion development after exposure in the BCOP assay and determined those early lesion that would be predictive of damage observed after 20+ hours *in vitro* and *in vivo*. Bovine corneas were exposed for 3 minutes, rinsed, and incubated for 2 or 4 hours before the endpoints were assessed and tissue taken for histology. *In vivo*, certain solvents increased the rate of lesion development but not the overall intensity or duration compared to the fragrance alone. Other solvents decreased the overall intensity and duration. The BCOP assay showed a generally similar pattern of lesion development. Those combinations that showed opacity at 4 hours *in vivo*, showed epithelial and stromal lesion in the BCOP by 4 hours post-exposure. Fragrance alone was slower to develop opacity *in vivo* and required the 20-hour post-exposure to produce appreciable lesions *in vitro*. These data suggest that the standard post exposure can be predictive of irritation potential of fragrance/solvent mixtures.

1307 COMPARISON OF *IN VITRO* EYE IRRITATION POTENTIAL BY BCOP ASSAY TO ERYTHEMA SCORES IN HUMAN EYE STING TEST OF SURFACTANT-BASED FORMULATIONS.

K. C. Cater[1], E. Patrick[2], J. W. Harbell[3], J. C. Merrill[3] and S. L. Schilcher[1]. *[1]The Dial Corporation, Scottsdale, AZ, [2]Consultant, Westfield, NJ and [3]Institute for In Vitro Sciences, Gaithersburg, MD.*

The BCOP assay can be used to predict relative eye irritation potential of surfactant-based personal care formulations relative to a corporate benchmark (*Toxicologist*, March 2001). The Human Eye Sting test is typically used to evaluate product claims of no tears/no stinging for children's bath products. A preliminary investigation was conducted to test a hypothesis that the BCOP assay could be used as a prediction model for relative ranking of human eye irritation responses under conditions of a standard Human Eye Sting test to surfactant-based formulations. BCOP assays and Human Eye Sting tests were conducted on four commercial body washes (BW) and one prototype body wash developed specifically for children or as mild bath products. In the Human Eye Sting assay 10 ul of a 10% dosing solution is instilled into one eye of each panelist (n=20), and the contra-lateral eye is dosed with sterile water as a control. Bulbar conjunctival erythema responses of each eye are graded at 30 seconds by an ophthalmologist. The BCOP assay permeability values (OD_{490}) for the five BWs ranged from 0.438 to 1.252 (i.e., least to most irritat-

ing). By comparison, the number of panelists exhibiting erythema responses (mild to moderately pink) ranged from 3 out of 20 panelists for the least irritating BW to 10 out of 20 panelists for the most irritating BW tested. The relative ranking of eye irritation potential of the five BWs in the BCOP assay compares favorably with the relative ranking of the BWs in the Human Eye Sting test. Based on these findings, the BCOP assay as described for surfactant-based formulations shows promise as a prediction model for relative ranking of conjunctival erythema responses in the human eye. Consequently, screening of prototype formulations in the BCOP assay allows for formula optimization of mild bath products prior to investment in a Human Eye Sting test.

1308 THE USE OF A HUMAN RECONSTITUTED EPIDERMAL MODEL FOR THE OCCUPATIONAL HAZARD ASSESSMENT OF PHARMACEUTICAL PROCESS MATERIALS.

C. W. Seaman[1], B. De Wever[2], M. Cappadoro[2], A. Whittingham[3], R. Guest[3] and C. Prusiewicz[1]. *[1]c/o GlaxoSmithKline, Ware, Herts, United Kingdom, [2]SkinEthic Laboratories, Nice, France and [3]SafePharm Laboratories, Derby, United Kingdom.*

Irritant dermatitis is a continuing problem arising from occupational exposure to chemicals. Traditionally, evaluation of the irritant potential of novel chemicals has relied on the use of *in vivo* studies . The requirement for ethical testing strategies and availability of new technologies has recently driven the assessment of alternative methods. This presentation describes the preliminary evaluation of a reconstituted epidermal culture system (SkinEthic) for determining the dermal irritant potential of pharmaceutical process materials. Briefly, epidermal cultures were treated with test materials for periods of 4 and 24 hours after which they were assessed for *via*bility (MTT), histopathology, and cytokine release (IL-1 alpha and IL-8). Positive controls (0.1% Triton and 20% SDS) induced severe tissue destruction as expected. Untreated controls showed normal morphology and *via*bility throughout the experiment. Test materials V-006, V-007 and V-021 showed minimal to no effects, dead tissue and moderate tissue damage, respectively. Increases in cytokine generation were also seen with V-007 and V-021. These results were consistent with known *in vivo* dermal irritant potential of these materials. The assay was readily transferred between laboratories and results between facilities generally consistent. In conclusion, results of this preliminary study indicate this system merits further evaluation as a model of the dermal irritant potential of pharmaceutical process materials. The results of this study also demonstrate the utility of multi-end point analysis in improving the predictivity of this model.

1309 THE USE OF A HUMAN RECONSTITUTED CORNEAL EPITHELIUM MODEL FOR THE OCCUPATIONAL HAZARD ASSESSMENT OF PHARMACEUTICAL PROCESS MATERIALS.

R. Guest[3], C. W. Seaman[1], B. De Wever[2], M. Cappadoro[2], A. Whittingham[3] and C. Prusiewicz[1]. *[1]c/o GlaxoSmithKline, Ware, Herts, United Kingdom, [2]SkinEthic Laboratories, Nice, France and [3]SafePharm Laboratories, Derby, United Kingdom.*

Ocular irritation is a potentially serious problem arising from contact with workplace chemicals. Prospective identification of the irritant potential of novel chemicals has traditionally relied on the use of *in vivo* studies. However, a growing requirement for ethical testing strategies and availability of new technologies has led to an increased focus on the need for alternative methods. This presentation describes a preliminary assessment of a reconstituted corneal epithelial culture system produced by SkinEthic for determining the ocular irritant potential of pharmaceutical process materials. Briefly, reconstituted corneal epithelial cultures were treated with test materials for periods of 5, 10 and 60 minutes after which they were assessed for *via*bility (MTT evolution), histopathology and cytokine release (IL-1 alpha IL-6 and IL-8). Positive controls (0.5 % and 1% SDS) showed severe tissue destruction as expected. Negative controls showed normal morphology and *via*bility throughout the experiment. Test materials V-006, V-007 and V-021 showed minimal to no damage, dead tissue and moderate tissue damage respectively. Although increases in Interleukin-1 alpha were observed, modified treatment regimes will be necessary to obtain useful results with IL-6 and IL-8. Test results reported by two laboratories were generally consistent with each other and with the known *in vivo* ocular irritant potential of the test materials. In conclusion, the results of this preliminary study show that the reconstituted corneal epithelial model merits further evaluation as an alternative to animal assays, for identifying the ocular irritation potential of pharmaceutical process materials. The results of this study also demonstrate the utility of multi-end point analysis in improving the predictivity of these models.

1310 IN-VITRO FIBER DISSOLUTION RATE AS A PREDICTOR OF FIBER CLEARANCE FROM THE LUNG.

R. M. Potter and J. G. Hadley. *Product Stewardship, Owens Corning Science and Technology Center, Granville, OH.*

Dissolution rates of silicate glass fibers from a wide compositional range have been measured in the laboratory in a modified pH 7.4 Gamble's solution. The dissolution was followed by three methods: mass loss, solution analysis for dissolved components, and fiber diameter decrease. The repeatability of each test method was within 10% of the measured value with good agreement among the three methods. This study includes 28 fiber compositions for which in-vivo long-fiber clearance rates had been determined after intratracheal instillation or short-term inhalation. In-vivo dissolution rates had been calculated for these fibers assuming uniform dissolution as the clearance mechanism. There is good agreement between the dissolution rates measured *in vitro* and those calculated from the in-vivo data except for a class of fibers characterized by low silica and high alumina contents. Fibers in this class are distinct in that they dissolve more rapidly under acid conditions than under the near-neutral conditions of the extracellular lung fluid. Their clearance mechanism is thought to involve breakage due to acidic conditions within phagocytic cells.

1311 COCAETHYLENE-INDUCED CHANGES IN ENDOTHELIAL PERMEABILITY AND CATION FLUX.

D. H. Tacker and A. O. Okorodudu. *Department of Pathology, The University of Texas Medical Branch at Galveston, Galveston, TX.*

Cocaethylene (CE) is a metabolite of cocaine and ethanol that has been implicated in ischemic tissue pathology associated with abuse, thus suggesting a role of the microvascular endothelium in pathogenesis. We previously used a CE exposure model in human microvascular endothelial cells (HMEC-1) to demonstrate that exposure to 1mM CE shifts the metabolic activity of HMEC-1 to an anaerobic state without causing lethality. Our current hypothesis is that CE induces vasculotoxicity by affecting endothelial permeability *via* alteration of intracellular calcium flux. For permeability studies, HMEC-1 grown on glass slips were treated with 1mM CE for 0, 1, 4, 12, and 24 hours, respectively, and fixed. Slips were then soaked in a silver nitrate solution, exposed to light, and visualized for intercellular gaps. Monitoring of intracellular calcium levels was performed using the indicator Fura-2 AM. HMEC-1 monolayers loaded with indicator were exposed to 1mM CE during kinetic measurement of ion levels or after a 1-hour pre-treatment with CE. After obtaining maximum intracellular calcium with digitonin stimulation, and minimum intracellular calcium with EGTA chelation, calculation of the intracellular calcium concentration was performed and compared between groups. Ion experiments were repeated using Mag-Fura-2 AM for determination of intracellular magnesium. We observed intercellular gap formations in CE-treated HMEC-1 that were not observed in the control monolayers. This occurred as soon as 1 hour of CE exposure. Intracellular calcium levels showed no immediate change in the treated group relative to controls, but pre-treatment with CE significantly ($p<0.05$, $n=4$) increased the maximum levels of intracellular calcium after stimulation. Baseline and minimal calcium were not affected by CE treatment. Maximal intracellular magnesium levels were also increased after pre-treatment ($p<0.05$, $n=3$). We conclude that the alteration in permeability was due to the alterations in calcium flux, since calcium is closely linked to cytoskeletal stability.

1312 A NON-ANIMAL PHOTOTOXICITY TEST USING EPIDERMAL TISSUE MODELS AND CYTOKINE ENDPOINTS.

D. R. Cerven, A. C. Gilotti, M. K. Reeder, C. A. Kirk, T. L. Ripper and G. L. DeGeorge. *MB Research Laboratories, Spinnerstown, PA.*

The phototoxic potential of chemicals, cosmetics, consumer products and pharmaceuticals are of increasing concern to regulatory agencies and industry. Currently in the US, there are no regulatory agency-accepted *in vitro* assays or alternatives to costly, low-throughput animal phototoxicity tests. To address this need, we have developed and pre-validated a high-throughput *in vitro* screening test for phototoxicity, designated the Enhanced Phototoxicity Assay in Reconstituted Skin (EPARS). EPARS overcomes many of the limitations of the 3T3 NRU Viability test, which has been validated in Europe by ECVAM. Specifically: 1) EPARS is based upon a differentiated tissue model that closely parallels human skin morphology, instead of a fibroblast monolayer; 2) the tissues are composed of primary human keratinocytes, a more relevant model than a mouse tumor cell line; 3) test substances can be applied directly, avoiding the often problematic solubilization of formulations into culture media. In EPARS, the test substance is applied topically to the reconstituted human skin models, +/- UV irradiation. Phototoxic effects are determined by measuring MTT uptake of irradiated vs. non-irradiated tissues. In

addition, to increase the sensitivity and specificity of the test, we measured the release of cytokines into the culture media *via* ELISA. Overall, EPARS proved to be an accurate and sensitive test for detecting phototoxic (photo-irritating) substances. PGE2 release was shown to be an early predictor of the toxic effects demonstrated in the *via*bility assay. Release of IL-1 alpha, IL-1ra, IL-8 and TNF-alpha supported the results of the cell *via*bility endpoint. Microarray analysis of gene expression showed that chlorpromazine treatment with UVA irradiation caused changes in gene expression that were not observed in untreated control tissues, tissues treated with UVA only, or chlorpromazine without UVA.

1313 ALTERNATIVE PHOTOSENSITIZATION ASSAY IN THE MOUSE (PHOTO-LLNA).

G. L. DeGeorge, T. L. Ripper, S. Young and D. R. Cerven. *MB Research Laboratories, Spinnerstown, PA.*

Although the Local Lymph Node Assay protocol has been validated as a sensitization test alternative for several years, several factors have limited its widespread application and development. These include the propensity for irritants to cause false-positive results in the assay, and the need to use radioactive chemicals. We have developed an alternative photosensitization test deemed the "PHOTO-LLNA" test to overcome some of these limitations. This test uses a non-radiometric method to assess lymph node cell (LNC) proliferation, and irradiation *via* a solar simulator to photo-activate test chemicals. The PHOTO-LLNA measures bromodeoxyuridine uptake into the DNA of proliferating cells using a BrdU-specific antibody and flow cytometry. Other immunophenotypic parameters including the B:T cell ratio, and the % B220+, CD69+, and I-A+ cells allow the discrimination of false-positive irritants from true photosensitizers. The PHOTO-LLNA correctly classified several photosensitizers including chlorpromazine (EC3UV = 0.095%), promethazine, bithionol, sodium omadine and tetrachlorosalicylanilide (TSCA), as well as the sensitizers DNCB and oxazalone, and the irritants benzalkonium chloride and SLS. Ear swelling measurements and theimmunophenotypic markers CD69, I-AK, B220 (CD45R) and the B:T cell ratio were the most useful immunophenotypic endpoints for discriminating true sensitizers and photosensitizers from false positive irritants such as SLS. In summary, we have developed a modified non-radiometric PHOTO-LLNA test to identify and characterize photosensitizers, which is sensitive enough to detect weak phototoxins. In addition, the enhanced PHOTO-LLNA can employ multiple immunophenotypic markers to discriminate between true photosensitizers and false-positive irritants which interfere with the interpretation of the results in standard sensitization tests.

1314 MULTI-CENTER PREVALIDATION USING *IN VITRO* RECONSTITUTED HUMAN CORNEAL EPITHELIAL MODEL TO ASSESS THE EYE IRRITATING POTENTIAL OF CHEMICALS.

B. De Wever[1], M. Cappadoro[1], F. Straube[2], N. Alepee[4], F. Van Goethem[3], P. Vanparys[3] and E. Adriaens[5]. *[1]SkinEthic Laboratories, Nice, France, [2]Novartis Pharmacology, Basel, Switzerland, [3]Johnson & Johnson Pharmaceutical R&D, Beerse, Belgium, [4]Pfizer Global R&D, Amboise, France and [5]University of Ghent, Ghent, Belgium.* Sponsor: A. Goldberg.

The development of *in vitro* alternatives to *in vivo* Draize eye testing is stimulated by scientific and ethical considerations. This multi-center study aimed at evaluating the predictive power of a new commercially available human cornea model (SkinEthic, Nice, France) to assess acute ocular irritation. When cultivated *in vitro* on filter inserts at the air-liquid interface using in chemically defined medium, the Human Corneal Epithelial cells of the cell line HCE form a corneal epithelial tissue, resembling histologically the outer epithelial cell layers of the human cornea. For this interlaboratory evaluation, a prevalidation approach (protocol optimization, transfer and performance) was used in order to provide sufficient evidence of the relevance (predictivity) and reliability (reproducibility) of the human corneal(HCE) model. At each of the 4 participating laboratories, 25 reference chemicals, ranking from non to very severe eye irritating, were applied topically to the HCE cultures and different treatment times were applied. The rate of cytotoxicity (tissue *via*bility and histological analysis) was compared to published *in vivo* acute eye irritation as well as existing data obtained in the Bovine Corneal Opacity Permeability (BCOP) test. A very good inter-laboratory reproducibility was obtained (correlation coefficient r > 0.82). Additionally, the *in vitro* data correlated well with BCOP scores, however correlation analysis with available Draize eye data resulted in lower values due to the often conflicting existing Draize eye test information. The final outcome of this study will establish whether this *in vitro* model is adequate to enter formal validation. In addition to reducing the number of animals required for safety testing, this *in vitro* eye irritation model is rapid and requires less compound than other *in vitro* assays.

1315 COMPUTATIONAL MODEL FOR RADIATION-INDUCED CELL DEATH AT LOW DOSES IN THE DEVELOPING NEOCORTEX.

N. M. DeFrank, W. C. Griffith, J. M. Gohlke and E. M. Faustman. *Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.*

Substantial evidence has demonstrated that low dose radiation exposures (10 to 50cGy) during particular gestational periods can result in permanent neuronal perturbations and eventual abnormalities in behavior and mental activity. It has been hypothesized that the mechanism underlying these effects includes radiation-induced cell death among neuronal precursors in the proliferative ventricular zone, thus disrupting the normal processes and timing of neurogenesis and leading to insufficient neuron production. Existing data sets were used in the construction of a computational model to describe the extent and pattern of cell death in the neocortex and the subsequent effects on neuron number at the end of neurogenesis. Results indicate that the induced death of a small fraction of neuronal precursors, expressed as a function of time and dose, leads to a significant decrease in the final neuron count compared to control embryos. The most drastic effects correspond to times of rapid proliferation and differentiation. For example, a single maternal dose of 24cGy on the 13th day of gestation induces cell death in a quarter of neuronal precursors of the fetal mouse. The model provides a means for comparing differences in absorbed dose, time of exposure, and duration of exposure. The model predicts about a 1% decrease of neuronal precursor cells per cGy during the most sensitive time window of neurogenesis and about a 0.5% decrease per cGy during other times of neurogenesis. The model is of risk assessment utility as it includes evaluation of alterations in specific developmental dynamic processes and permits comparisons of inter-species susceptibility, including mice, rats, and humans, across time and dose. Supported by the Center for Child Health Risk Research (R 826886-01-0 and P01 ES09601) and the Department of Energy (DE-FG02-03ER63674).

1316 CONTRIBUTION OF EXPERIMENTAL AND INTER AND INTRASPECIES VARIABILITY IN A COMPUTATIONAL MODEL FOR ETHANOL-INDUCED PERTURBATIONS OF NEOCORTICAL DEVELOPMENT.

J. M. Gohlke, W. C. Griffith and E. M. Faustman. *Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.*

We are quantitatively evaluating normal and chemically perturbed neocortical neurogenesis, migration and synaptogenesis with a biologically based model that allows for evaluation of sources of experimental and biological uncertainty. Data used to establish key parameters include the growth fraction, the founder cell population, the cell death rate during neurogenesis and synaptogenesis and the clearance time of cell death labeled neurons. This new model construct compares intra and interspecies variability in model parameters and model output based on independent, stereologically determined neocortical parameters in the rat, mouse, and monkey. Consideration of programmed cell death (PCD) during neocortical neurogenesis can reduce neuron output by as much as 24% in the rat. Higher PCD rates would deplete the progenitor population, not allowing for the vast proliferation that is the hallmark of neurogenesis. Furthermore, our simulations suggest the growth fraction may be less than 100% for most of the neurogenesis period and the clearance time of dying neurons labeled by TUNEL or pyknosis is relatively short, between 1 and 4 hrs. Based on the variability in estimates of adult neocortical neuron number (coefficient of variation (CV) between 7- 25%), true biological variation in rates of proliferation, transformation and death must be low (CV ≤ 2.5%), however variation estimates in experimental studies for parameter estimation are much larger (CV 10-30%). These results suggest experimental error can account for the majority of the observed variability in parameter estimation data, thus highlighting the importance of reduced experimental variability to discover subtle toxicant-induced effects. Our model framework allows for in silico evaluation of the variability in normal and perturbed states of neocortical development, providing a scientifically-framed, quantitative construct to inform inter and intra species variability considerations for risk assessments.

1317 PREDICTIVE MODELING OF *IN VITRO* BRAIN CHOLINESTERASE INHIBITION FOLLOWING SIMULTANEOUS OR SEQUENTIAL EXPOSURES TO BINARY MIXTURES OF ORGANOPHOSPHATES.

J. E. Chambers[1], R. L. Carr[1], H. W. Chambers[2], J. A. Kamykowski[1], E. C. Meek[1], S. F. Oppenheimer[3] and J. R. Richardson[1]. *[1]Coll Veterinary Med., Mississippi State University, Mississippi State, MS, [2]Department Entomology, Mississippi State University, Mississippi State, MS and [3]Department Mathematics, Mississippi State University, Mississippi State, MS.*

The active metabolites (oxons) of many common organophosphorus insecticides persistently inhibit nervous system cholinesterase as the primary mechanism of toxicity. Individuals are likely to encounter more than one organophosphate. These ex-

periments exposed rat brain homogenates to binary mixtures of organophosphates, either simultaneously for a 15 minute incubation period or sequentially (second oxon added 15 minutes after the first, with a subsequent 15 minute incubation period). Levels of inhibition of individual oxons did not exceed 30%. The cholinesterase activity was then determined spectrophotometrically using acetylthiocholine as a substrate, and the cholinesterase inhibition was calculated. Mass action models were developed for the resultant inhibition in the binary mixture, with model calibration occurring from the inhibition of the individual compounds with a 15 minute exposure. Subsequently, the mass action models were adapted to partial order models (using guidance of existing reaction models present in the agronomy literature). The partial order models were more effective in prediction with the simultaneous exposures to the binary mixtures than were the mass action models. The partial order models were also more effective in prediction of the sequential exposures to binary mixtures within the calibration range (i.e., 15 minute exposures and lower levels of inhibition), but the mass action models are more robust for the longer time of incubation and the higher levels of inhibition. However, both types of models predict greater inhibition than the experimental data, indicating that there are factors influencing the levels of cholinesterase inhibition with binary mixtures that we have not yet identified for the mathematical models. (Supported by American Chemistry Council 0161).

1318 STOCHASTIC MATHEMATICAL MODELING OF TUMOR GROWTH AND DIFFERENTIATION IN HUMAN CARCINOGENESIS.

S. Y. Whitaker[1], A. Kopp-Schneider[2] and C. J. Portier[1]. *[1]LCBRA, NIEHS, Research Triangle Park, NC and [2]Biostatistics, DKFZ, Heidelberg, Germany.*

Researchers generally agree that cancer is a multistage process where abnormal cells mutate to become cancerous cells. However, current research explores quantitative methods to describe the path and mechanisms that lead to the malignant state. As cancer is the unregulated growth of cells, there is a need for models that incorporate more biological realism while maintaining mathematical tractability. A stochastic multistage model with the expected number, variance and co-variance formulae expressed as ordinary differential equations is presented as a novel approach for characterizing possible carcinogenic mechanisms. The controlled growth and differentiation (CGD) model (Whitaker et al., 2003) was originally proposed to describe mechanisms in developmental toxicology. However, the model construct also allows for the implementation of a host of possibilities when modeling cancer. This model has a highly controlled birth and death process for cells at any stage of maturity. The formulation of the CGD model easily allows for the inclusion of an unspecified number of states in any biological process. The number of replications allowed in the progression of a cell colony is controllable and is able to more closely reflect the presence of a true stem cell population. The CGD model is able to provide a mathematical approximation to the number (and hence the size) of cells at each stage of the process. Expanding the model to calculate the size distribution of a cell colony can be incorporated and then compared to the observed size distribution. Here, we propose to fit the CGD model to the liver foci data set described in Kopp-Schneider et al. and compare the results of the CGD model, the existing one-stage clonal expansion model3, and the color-shift model (Kopp-Schneider et al., 1998).

1319 PHARMACOKINETIC (PK)/PHARMACODYNAMIC (PD) RELATIONSHIP OF PCB126 UNDER THE CONDITIONS OF MODIFIED ITO MEDIUM-TERM LIVER BIOASSAY.

M. Lohitnavy, L. Chubb, O. S. Lohitnavy, C. C. Yang, J. Homburg, J. A. Campain and R. S. Yang. *Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO.*

PCB126 is the most toxic congener of PCBs with carcinogenic potential. By using the modified Ito medium-term bioassay protocol, male F344 rats were given a single ip dose of 200 mg/kg of diethylnitrosamine (DEN) as an initiator. Daily oral PCB126 administration (3.3 and 9.8 μg/kg/day) was started at week 2 after DEN injection. One week after PCB126 dosing, a 2/3rd partial hepatectomy was conducted. Rats were sacrificed at 20, 24, 28, 47 and 56 days post-DEN injection. Tissue concentrations in the liver, fat, whole blood, kidney and muscle were measured by using GC/ECD following liquid extraction and clean up. Morphometric analysis of glutathione-S-transferase (GST)- P foci formation in the liver slices was performed as a PD endpoint to determine numbers and sizes of preneoplastic foci. Concentration-time courses of PCB126 in the liver, fat, kidney, muscle and whole blood were obtained. Despite high lipophilicity of PCB126, liver concentrations were the highest with the ratio between liver and fat concentrations of about 110-400 to 1. Assuming this is protein binding in the liver, our results revealed that PCB126 binds to liver about 11 to 40 times more strongly than TCDD. Proteomic analyses will shed further light on this interesting binding phenomenon. Dose- and time-dependence of GST-P foci formation were studied. Correlation analyses between area under the curve of PCB126 in the liver (AUC_{Liver}) as an internal dose and PD endpoints (foci numbers, area, and relative foci area) demonstrate linear relationship at low AUCs and nonlinear response at higher AUCs . The linkage of PBPK and clonal growth models will enable us to predict target tissue dosimetry as well as preneoplastic foci formation in F344 rats upon exposure to PCB126 in an Ito medium-term bioassay. (Supported by NIOSH/CDC grant 1 RO1 OH07556)

1320 REACTION NETWORK MODELING OF BENZO(A)PYRENE METABOLIC PATHWAYS: FURTHER DEVELOPMENT.

K. H. Liao[1, 2], A. N. Mayeno[1, 3], K. F. Reardon[1, 2] and R. S. Yang[1, 3]. *[1]Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Colorado State University, Fort Collins, CO, [2]Department of Chemical Engineering, Colorado State University, Fort Collins, CO and [3]Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO.*

We have been developing a modeling tool for predicting the complex biotransformation pathways of xenobiotics by using a chemical engineering approach, Reaction Network Modeling (RNM). RNM generates the reaction pathways automatically based on the chemical structures of the parent compounds and reaction rules. Graph theory is used to encode chemical structures and reaction rules at the molecular level. Benzo[a]pyrene (BaP) was used as the substrate for the first application of RNM to toxicology since it is a human carcinogen as well as a ubiquitous environmental pollutant. Despite thousands of papers in the literature on BaP, quantitative data on human enzyme kinetics useful for RNM are lacking. Thus, to obtain reaction rate constants for RNM, the time course profiles of BaP metabolic pathways were studied using recombinant human cytochrome P450 1A1 and epoxide hydrolase. In addition, considerable effort was devoted to developing HPLC methods for the analysis of 12 major BaP metabolites including dihydrodiols, phenols, tetrols, and quinones with detection limits in the nM range. The high detection sensitivities of BaP-quinones were achieved by an on-line post-column zinc reduction column, which converts the non-fluorescent quinones to their corresponding fluorescent hydroquinones. RNM provided good predictions of the time course profiles of these major BaP metabolites when compared with experimental data. The next phase is to link RNM with PBPK models. Ultimately, we will be able to simulate the entire reaction network of BaP from whole body pharmacokinetics down to the level of molecular interactions among enzymes, BaP, and its metabolites. Since RNM is encoded at the molecular structure level, it is a promising approach to handle the extremely complex biochemical reactions and reaction networks. (Supported by NIEHS Grant R01 ES09655).

1321 QUANTIFYING GENE EXPRESSION NETWORKS USING BAYESIAN METHODS: KNOWN NETWORK STRUCTURE.

H. Toyoshiba, T. Yamanaka, F. M. Parham, J. M. Martinez, H. Sone, N. J. Walker and C. J. Portier. *LCBRA, National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

Gene expression arrays (gene chips) have enabled researchers to roughly quantify the level of mRNA expression for a large number of genes in a single sample. Several methods have been developed for the analysis of gene array data including clustering, outlier detection, correlation studies, etc. Most of these analyses are aimed at a qualitative identification of what is different between two samples and/or is there a relationship between two genes. Several researchers have also suggested the use of gene interaction mapping as a tool for the analysis of gene array data. Dynamic models, usually based on ordinary differential equations, have also been proposed and in some cases used for the analysis of microarray data; in most cases, these analyses have shown limited use of formal statistical methods. In this study, we propose a quantitative, statistically sound methodology for the analysis of gene regulatory networks using gene expression data sets. The model was developed based on Bayesian networks to formalize the direct quantification of gene-expression networks. Using this method, it is not only possible to determine the qualitative relationships between genes but the quantitative relationship and the probabilities associated with these quantitative connections. Using gene expression data from HPL1A lung airway epithelial cells after exposure to TCDD at levels of 0.1, 1.0 and 10.0 nM for 24 hours, a hypothetical gene expression network was assumed and the developed method applied to the data and the hypothetical network. Different from seeing gene by gene correlations, the method allows us to see the relationship between genes in terms of network. A new statistical approach to analyze microarray data is shown in this study.

1322 PATHWAYS TO UNDERSTANDING THE METABOLISM OF CHLORINATED ENVIRONMENTAL CONTAMINANTS THROUGH BIOCHEMICAL REACTION NETWORK MODELING.

A. N. Mayeno, B. Reisfeld and R. Yang. *Quantitative and Computational Toxicology Group, Center for Environmental Toxicology and Technology, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO.*

Chlorinated hydrocarbon solvents, such as trichloroethylene (TCE), perchloroethylene (Perc), methyl chloroform (MC), and chloroform (CF), are common groundwater contaminants, all of which can exert harmful health effects. Toxicity of these chemicals is, in large part, attributed to the formation of reactive species after bioactivation by cytochrome P450 enzymes. A single chlorinated solvent may produce several reactive species through complex metabolic pathways. For a mixture of compounds, the situation is much more complicated. Experimental studies of pure chemicals and the multitude of chemical mixture combinations to understand the formation of these toxic intermediates and the interconnections between these pathways of metabolism can be resource prohibitive; hence, novel, alternative approaches are needed. Here, we report the use of an *in silico* and systems biology approach, "Biochemical Reaction Network Modeling" (BRNM), to describe, interconnect, and understand the metabolism of TCE, Perc, MC, and CF. BRNM is a computer-assisted modeling tool that, when validated, not only predicts possible metabolites but depicts and connects the metabolic pathways through common metabolites, thereby creating a comprehensive and interwoven network of reaction pathways. Using this tool, we were able to predict metabolites of the four pollutants, describe their metabolic pathways, depict metabolites in common and intermediates expected to be unstable or reactive. Each step in the pathway results from "reaction rules" based on empirical metabolism studies and mechanistic organic chemistry. Our work represents the first steps in using BRNM to study, describe, and understand the interwoven and complex metabolic pathways of environmental toxins. (Supported in part by NIEHS Career Development Award 01 K25 ES11146 and Training Grant 1 T32 ES 07321)

1323 APPLICATION OF A STATISTICAL DYNAMIC MODEL INVESTIGATING THE SHORT-TERM CELLULAR KINETICS INDUCED BY RIDDELLIINE, A HEPATIC ENDOTHELIAL CARCINOGEN.

M. V. Smith[1], A. Nyska[2] and C. Portier[2]. *[1]Constella Health Sciences, Constella Group, Durham, NC, NC and [2]NIEHS, Research Triangle Park, NC.*

In recent studies, riddelliine, a pyrrolizidine alkaloid, was found to increase rates of replication and apoptosis and induce hemangiosarcoma in the liver of rats and mice . In our current investigation, applying a kinetic model, we used the data from a short-term study (Nyska et al., 2002) in which 42 male F334/N rats were dosed by gavage with riddelliine (0, 1.0, and 2.5 mg/kg/day) for 6 weeks; 7 animals/group were sacrificed after 8 consecutive daily doses, and the remaining rats were terminated after 30 daily doses, excluding weekends. Cellular kinetics were investigated using immunohistochemical stainings for bromodeoxyuridine (BrdU) and TUNEL. We now report a predictive mathematical model for describing BrdU labeling and apoptotic processes for hepatocytes and hepatic endothelial cells in rats, using the data from this previous experiment. The model predictions were used with multivariable nonlinear regression techniques to estimate replicative and apoptotic rate constants for both cell types and all treatment groups. Information on the total numbers of each cell type in each animal was utilized as a covariate to increase the accuracy of the predictions. Hypothesis tests were used with the predicted rates to separate the competing effects of riddelliine on proliferation and apoptosis of hepatocytes and endothelial cells. That estimated replicative rates were found to be significantly higher for endothelial cells than hepatocytes supports the supposition of induction of hemangiosarcoma by riddelliine in the liver. Additional hypothesis testing suggested a differential effect of BrdU on the kinetic rates of hepatocytes and endothelial cells in the control groups. Although the replicative rates were found to be approximately the same, the apoptotic rates were found to be significantly higher for endothelial cells in the control groups.

1324 A FEEDBACK MODEL FOR TESTICULAR-PITUITARY AXIS HORMONE KINETICS AND THEIR EFFECTS ON THE REGULATION OF THE PROSTATE IN ADULT MALE RATS.

H. A. Barton[2], M. G. Zager[1] and L. K. Potter[1]. *[1]Toxicology, University of North Carolina, Chapel Hill, NC and [2]NHEERL, USEPA, Research Triangle Park, NC.*

The testicular-pituitary axis regulates male reproductive system functions. A model describing the kinetics and dynamics of testosterone (T), dihydrotestosterone (DHT) and luteinizing hormone (LH) was developed based on a model by Barton and Anderson (1997). The model describes the metabolism of T to DHT by 5α-reductase with feedback loops for the positive regulation of T synthesis by LH and negative regulation of LH by T and DHT, and simulates the maintenance of the adult prostate as a function of hormone concentrations. The regulatory processes involved in prostate maintenance include cell proliferation, apoptosis, fluid production and 5α-reductase activity, which is controlled by the occupancy of a single gene by the DHT-androgen receptor (AR) dimerized complex. The other processes are regulated by the occupancy of multiple genes, but each process is represented as a single DNA binding site in the model. The model simulates prostate dynamics for intact, castrated, T injected and T or DHT subcutaneously implanted rats. The activities of environmental antiandrogens, such as the fungicide vinclozolin and the therapeautic drug finasteride, are modeled as competitive ligands for the AR. After calibration, the model is able to simulate the castration-induced regression of the prostate with accuracy compared to experimental data, which show that the prostate regresses to approximately 17 percent of its intact weight at 14 days post-castration and 5 percent at 30 days post-castration. The model demonstrates accuracy compared to the data in predicting serum T and AR levels following castration. This model sets a basis for quantifying the kinetics and effects of exogenous endocrine active compounds on the prostate. Furthermore, it provides a framework to extend modeling efforts to the pubertal developmental period, including growth and changes in hormone synthesis and metabolism. (Funded by EPA/UNC Toxicology Research Program, Training Agreement CT827206. This abstract does not reflect EPA policy.)

1325 PRELIMINARY ANALYSIS OF ALGORITHMS PREDICTING BLOOD:AIR AND TISSUE:BLOOD PARTITION COEFFICIENTS FROM SOLVENT PARTITION COEFFICIENTS.

T. R. Sterner[1], P. J. Robinson[2], D. R. Mattie[3] and G. A. Burton[4]. *[1]OpTech, Dayton, OH, [2]ManTech Environmental Technology, Dayton, OH, [3]Air Force Research Laboratory, Wright-Patterson AFB, OH and [4]Wright State University, Dayton, OH.*

Algorithms predicting tissue and blood partition coefficients (PCs) from solvent properties (octanol:water, saline or water:air, oil:air coefficients) were compared to assess their usefulness for a petroleum mixtures physiologically based pharmacokinetic/pharmacodynamic model. Measured blood:air and tissue:blood PCs were sought from literature resources for 14 JP-8 components. PCs, mainly from *vial* equilibration experiments, were separated by species (rat and human) and averaged by tissue and chemical. Average experimental PCs were then compared with predicted PCs calculated using algorithms from nine published sources. The algorithms chosen use solvent PCs due to the relative accessibility of these parameters. Tissue:blood PCs were calculated from ratios of predicted tissue:air and experimental blood:air values (PC_{EB}). Calculated PCs were evaluated using percent error compared to the experimental value. Of the 231 calculated values, 30% performed within ± 20% of the experimental PC values. Algorithms were divided into three main types. Empirical equations (derived from linear regression of experimental PC data), physiologically based equations (based on water and lipid components of a tissue type), and hybrid equations (physiological parameters and empirical factors combined) each performed equally well. PC_{EB} values were compared with tissue:blood PCs calculated from ratios of predicted tissue:air and predicted blood:air values (PC_{PB}). Overall, 68% of PC_{EB} values had smaller absolute percent errors than PC_{PB} values. Physiological equations should not be used to calculate PC_{PB} values as 100% of these PC_{PB} values had higher absolute percent errors than corresponding PC_{EB} values. If calculated PC values must be used in models, a comparison of experimental and predicted PCs for chemically similar compounds is advisable, so one understands the expected error level in calculated values.

1326 STEADY STATE TOXICOKINETICS OF METHYLMERCURY IN HUMANS.

G. Balagopal and H. Chan. *Nutrition and Dietetics, McGill University, Ste-Anne-de-Bellevue, QC, Canada.*

Chronic low dose exposure to methylmercury (MeHg) above the reference dose recommended by USEPA (0.1 μg/kg/day) is common in populations that consume fish and marine mammals. High recalcitrance property with low body clearance increases the bioavailability of MeHg in different body compartments. In spite of such dogma, steady state toxicokinetic end points have never been characterized for MeHg exposure. The objective of this study is to estimate internal doses at the target organs at steady state of exposure. Multicompartmental toxicokinetic model of Eular and Gear algorithms with mass balanced differential equation were used to simulate MeHg exposure and the model applicability were shown with a scenario, assuming daily intake of 200g of fish resulting in MeHg exposure of 0.1 μg /kg over a year. Steady state burden of both organic and inorganic mercurials was calculated for different tissue groups like brain, liver, kidney, hair and nail. Results showed

that Hg concentrations reached steady state in all the tissue compartments after 150 days of exposure and the minimum clearance time in all the tissue compartments was 120 days. Blood MeHg concentration was 23.7 μg/L and the concentration of MeHg in brain, liver and kidney were 0.169 μg/kg, 3.53 μg/kg and 2.70 μg/kg respectively. The inorganic mercury concentration in the routes of elimination like hair and nail were 1 ng/g and 3.5 ng/g respectively. This approach can be used by policy makers in generating reference values based on MeHg body burden.

1327 DEVELOPMENTAL EXPOSURE TO LEAD PROMOTES NEURODEGENERATION IN THE SENESCENT RAT BRAIN.

B. Brock, N. H. Zawia and M. Basha. *Biomedical Sciences, University of Rhode Island, Kingston, RI.*

The Fetal Basis of Adult Disease (FeBAD) hypothesis states that many adult diseases have a fetal origin. Virtually little data is available that has examined the impact of environmental exposures during early development on delayed dysfunctions or diseases in the adult brain. To bridge this gap we have examined if any associations exist between developmental exposure to lead (Pb) and neurodegenerative diseases in the aging brain. We exposed rats to 0.2% Pb-acetate in the drinking water in the following manner: 1) control unexposed group; 2) early exposure to Pb (from birth to weaning); 3) late exposure to Pb (17-20 months of age); 4) early and late exposure. The brains of all the groups were prepared for immunohistochemistry and analyzed by the Campbell-Switzer silver neurodegeneration stain. The brains of aging animals chronically exposed to Pb exhibited pathological features of neurodegeneration (astrocytic activation), which were more prominent and widespread in aging rats exposed to Pb as neonates, indicating that early exposure to Pb has made the animals more susceptible to chemical stressors later in life.

1328 DEVELOPMENTAL OVEREXPRESSION OF ALPHA(2)-ADRENERGIC RECEPTORS: ROLE IN CELL PROLIFERATION AND CROSS-REGULATION BY BETA-ADRENERGIC RECEPTOR NEUROTOXICANTS.

M. L. Kreider, F. J. Seidler and T. A. Slotkin. *Pharmacology & Cancer Biology, Duke University Med. Ctr, Durham, NC.*

Catecholamines serve as neurotrophins that coordinate cell replication and differentiation in the developing brain. The alpha(2)-adrenergic receptor (A2AR) is overexpressed during periods of high mitotic activity in the fetus and is thought to be involved in promoting cell proliferation. Terbutaline, which is commonly administered to arrest preterm labor, increases the expression of A2ARs in the neonatal rat brain while suppressing DNA synthesis and evoking neural dysmorphology. We examined whether there is a mechanistic link between the effects on A2ARs and neural cell replication by examining regional alterations in [3H]thymidine incorporation into DNA in animals treated with an A2AR agonist (clonidine, 2 mg/kg) or antagonist (yohimbine, 2.5 mg/kg), during three developmental time periods: gestational day (GD) 20, postnatal day (PN) 1 and PN8. Clonidine elicited dramatic decreases in DNA synthesis in the forebrain, brainstem, and cerebellum during both gestational and postnatal time periods. However, blockade of the A2AR with yohimbine also resulted in decreases in DNA synthesis, albeit on a more moderate scale than seen with the agonist; the effects of the antagonist may be related to A2AR autoreceptor control of norepinephrine release and subsequent actions of the neurotransmitter/neurotrophin on cell proliferation. The dual actions of A2AR agonists and antagonists indicate that low, tonic A2AR input is necessary to maintain cell replication in the developing brain but that inappropriate overstimulation inhibits mitotic activity. Involvement of A2ARs in the control of brain cell development implies that perturbations in A2AR expression elicited by terbutaline may contribute to neurobehavioral anomalies. Supported by NIH HD09713.

1329 EFFECT OF ETHANOL ON CARBACHOL-STIMULATED PHOSPHOLIPASE D SIGNALING IN ASTROGLIAL CELLS.

M. Guizzetti[1], B. D. Thompson[1], Y. Kim[1], K. VanDeMark[1] and L. G. Costa[1, 2]. *[1]Environmental and Occupational Health Sciences, University of washington, Seattle, WA and [2]Department of Pharmacology and Human Physiology, University of Bari, Bari, Italy.*

Inhibition of astrocyte proliferation has been hypothesized to be an important event involved in the developmental neurotoxicity of ethanol. We have previously shown that the acetylcholine analogue carbachol induces astroglial cell proliferation and that ethanol strongly inhibits this effect, primarily by targeting activation of PKC z and its down-stream effectors p70S6K and NF-kB. We hypothesized that a primary upstream target for ethanol may be the PKC zeta activator phosphatidic acid (PA), which is formed by hydrolysis of phosphatidylcholine by phospholipase D (PLD). In this study, we investigated whether inhibition by ethanol of the main signal transduction pathway involved in carbachol-induced cell proliferation may be due, at least in part, to inhibition of PA formation. Suramin, an inhibitor of PLD activity, inhibited DNA synthesis induced by carbachol; similarly, 1-butanol, which is a substrate for PLD and inhibits PA formation, inhibited carbachol-induced cell proliferation and the underlying intracellular signaling, while its analog tert-butanol, which is not a substrate for PLD, was ineffective. Furthermore, exogenous PA was able to increase DNA synthesis, and to activate PKC zeta and p70S6K. Ethanol had only a small inhibitory effect on PA-induced astrocyte proliferation. Furthermore, in carbachol-stimulated cells, ethanol increased the formation of phosphatidylethanol and inhibited the formation of PA. Altogether, these results indicate that PLD activation plays an important role in carbachol-induced astroglial cell proliferation, probably by generating the second messenger PA. Moreover, the effect of ethanol on carbachol-induced proliferation appears to be mediated, at least in part, by its ability to interact with PLD leading to the formation of phosphatidylethanol and to inhibition in the formation of PA, thus affecting a major singaling pathway leading to astroglial cell proliferation. (Supported in part by AA-08154 and ES-07033).

1330 THE EFFECTS OF EARLY POSTNATAL CHLORPYRIFOS EXPOSURE ON PERFORMANCE IN THE 12-ARM RADIAL MAZE. FRANK JOHNSON AND RUSSELL L. CARR. CENTER FOR ENVIRONMENTAL HEALTH SCIENCES, COLLEGE OF VETERINARY MEDICINE, MISSISSIPPI STATE UNIVERSITY, MISSISSIPPI STATE, MS 39762, USA.

F. O. Johnson and R. L. Carr. *College Of Veterinary Medicine, Center For Environmental Health Sciences, Mississippi State University, Starkville, MS.*

Developmental exposure to chlorpyrifos (CPF) has been associated with cognitive deficits in children. The current study investigated the effects of early postnatal exposure to CPF on spatial learning and memory using a 12-arm radial maze paradigm. Using an incremental dosing regimen, Sprague-Dawley rats were orally gavaged daily with either corn oil or chlorpyrifos at a low dose of 1 mg/kg from postnatal day 1 (PND1) to 21; a medium dose of 1 mg/kg from PND1-5, 2mg/kg from PND6-13, and 4mg/kg from PND14-21; or a high dose of 1.5mg/kg from PND1-5, 3mg/kg from PND6-13, and 6mg/kg from PND14-21. Rats were then tested beginning on PND36 for 4 weeks (4 sessions per week for 16 total sessions) on a working/reference memory task. Developmental CPF exposure elicited persistent cholinesterase inhibition with 65-70% inhibition on PND20 and 28-32% on PND40. Interestingly, control females committed greater numbers of working and reference memory errors than did control males in the 12-arm radial maze. In females, the number of working errors were increased with the medium dosage of CPF but the number of reference memory errors was not affected. In males, neither working or reference memory were affected. These data suggest that neonatal exposure to CPF alters spatial memory formation in a gender specific manner.

1331 DEVELOPMENTAL EXPOSURE TO CHLORPYRIFOS ELICITS SEX-SELECTIVE ALTERATIONS OF SEROTONERGIC SYNAPTIC FUNCTION IN ADULTHOOD.

J. E. Aldridge, F. J. Seidler and T. A. Slotkin. *Pharmacology & Cancer Biology, Duke University Med. Ctr, Durham, NC.*

During brain development, serotonin (5HT) provides essential neurotrophic signals and we found that gestational or neonatal exposure of rats to the commonly used insecticide chlorpyrifos (CPF) elicits immediate changes in 5HT systems. In the current study, we evaluated the effects in adulthood after CPF exposures from the neural tube stage (gestational days GD9-12) and the late gestational period (GD17-20), through postnatal (PN) neuronal differentiation and synaptogenesis (PN1-4, PN11-14), utilizing treatments below the threshold for systemic toxicity. With exposure on GD9-12, CPF elicited global elevations in 5HT1A and 5HT2 receptors and in the 5HT presynaptic transporter. The GD17-20 treatment elicited larger effects that displayed selectivity for regions with 5HT nerve terminals and that were preferential for males. Although similar receptor upregulation was seen after PN1-4 exposure, the effects were larger in regions with 5HT cell bodies; in addition, the presynaptic transporter was downregulated in the nerve terminal zones of females. The PN11-14 exposure had much smaller effects on receptors but still elicited transporter suppression with the same regional and sex selectivity. Although CPF exposure on GD17-20, PN1-4 or PN11-14 altered the ability of 5HT to modulate adenylyl cyclase, these did not correspond to the effects on 5HT receptors, suggesting an additional set of effects on proteins that transduce the 5HT signal. Our results indicate that CPF elicits long-lasting changes in 5HT receptors, the presynaptic 5HT transporter, and 5HT-mediated signal transduction after exposure in discrete developmental windows that range from the neural tube stage through synaptogenesis. These effects are likely to contribute to neurobehavioral teratology of CPF. Supported by NIH ES10387 and ES10356.

1332 NICOTINE IS A DEVELOPMENTAL NEUROTOXICANT AND NEUROPROTECTANT: STAGE-SELECTIVE INHIBITION OF DNA SYNTHESIS COINCIDENT WITH SHIELDING FROM EFFECTS OF CHLORPYRIFOS.

D. Qiao, F. J. Seidler, J. D. Violin and T. A. Slotkin. *Pharmacology & Cancer Biology, Duke University Med. Ctr, Durham, NC.*

Although nicotine is now well-recognized as a developmental neurotoxicant, it also may have neuroprotectant properties. In the current study, we used PC12 cells to characterize the specific developmental phases in which these effects are expressed. In undifferentiated cells, nicotine had a modest effect on DNA synthesis (10% reduction), that was nevertheless selective, as no significant reductions were seen for RNA or protein synthesis. The effects were blocked by mecamylamine, indicating mediation by nicotinic acetylcholine receptors. Initiation of differentiation with nerve growth factor, which greatly increases the receptor concentration, produced a commensurate increase in the sensitivity of DNA synthesis to nicotine, while RNA and protein synthesis again remained unaffected. The organophosphate insecticide, chlorpyrifos, also interferes with DNA synthesis in undifferentiated PC12 cells, but by mechanisms independent of nicotinic receptors. Accordingly, the effects of a combination of nicotine and chlorpyrifos should be additive. However, simultaneous exposure of undifferentiated cells to both agents produced less-than-additive effects at low concentrations of chlorpyrifos, and at high chlorpyrifos concentrations, nicotine produced outright protection: the combination of nicotine and chlorpyrifos had lesser effects than chlorpyrifos alone. The same neuroprotection was seen when cells were exposed to nicotine for 24 hours, washed free of the drug for 24 hours, and then exposed to chlorpyrifos. We also obtained similar results with human SH-SY5Y neuroblastoma cells. The results indicate that nicotine interferes with neural cell replication, with peak effects in early stages of differentiation. At the same time, nicotine promotes trophic actions that protect against neurotoxicants that work through other mechanisms. Support: USPHS DA14247, ES10387, ES10356, and Philip Morris USA.

1333 PRENATAL NICOTINE EXPOSURE ALTERS THE RESPONSE TO NICOTINE ADMINISTRATION IN ADOLESCENCE.

Y. Abreu-Villaca, F. J. Seidler and T. A. Slotkin. *Pharmacology & Cancer Biology, Duke University Med. Ctr, Durham, NC.*

Maternal smoking during pregnancy leads to increased incidence of smoking in the offspring, with typical onset in adolescence. We used a rat model to investigate whether prenatal nicotine exposure alters the subsequent neurochemical response to nicotine. Pregnant rats were given nicotine (6 mg/kg/day) or saline by minipump infusion throughout pregnancy and the offspring were treated again with nicotine or saline during adolescence (postnatal days PN30-47). We assessed nicotinic cholinergic receptor (nAChR) binding, choline acetyltransferase (ChAT) activity and [3H]hemicholinium-3 (HC-3) binding to the high affinity choline transporter in the midbrain, cerebral cortex and hippocampus. Measurements were made during the period of nicotine administration (PN45) and at various intervals for up to one month after the termination of treatment (PN50, 60, 75). Prenatal nicotine alone did not alter nAChRs in adolescence but adolescent nicotine administration evoked substantial increases during and after treatment. Prenatal nicotine exposure reduced the nAChR response to adolescent nicotine treatment. For ChAT, a constitutive marker for cholinergic nerve terminals, prenatal nicotine exposure elicited increased overall activity, but again the effects of combined prenatal and adolescent exposure were less than additive. HC-3 binding, which is responsive to cholinergic neuronal impulse activity, showed long-lasting decreases with either prenatal or adolescent nicotine exposure. Combined treatment produced either additive (midbrain) or less-than-additive (cerebral cortex, hippocampus) effects but notably, there was synergism toward the effect of adolescent nicotine withdrawal (PN50, cerebral cortex). Our results suggest that prenatal nicotine exposure alters the subsequent effect of nicotine administered in adolescence, both with regard to nAChR upregulation and indices of cholinergic synaptic integrity and neural activity, especially during withdrawal. (Philip Morris External Research Program and CNPq Brazil)

1334 TERBUTALINE IS A DEVELOPMENTAL NEUROTOXICANT: EFFECTS ON NEUROPROTEINS AND MORPHOLOGY IN CEREBELLUM, HIPPOCAMPUS, AND SOMATOSENSORY CORTEX.

M. C. Rhodes[1], F. J. Seidler[1], A. Abdel-Rahman[1], A. Nyska[2], H. L. Rincavage[1] and T. A. Slotkin[1]. [1]*Pharmacology & Cancer Biology, Duke University Med. Ctr, Durham, NC and* [2]*NIEHS, Research Triangle Park, NC.*

Beta2-Adrenoceptor agonists, especially terbutaline, are widely used to arrest preterm labor but they also cross the placenta to stimulate fetal beta-adrenoceptors that control neural cell differentiation. We evaluated the effects of terbutaline administration in neonatal rats, a stage of neurodevelopment corresponding to human fetal development. Terbutaline administered on postnatal days PN2-5 elicited neurochemical changes indicative of neuronal injury and reactive gliosis: immediate increases in glial fibrillary acidic protein and subsequent induction of the 68 kDa neurofilament protein. Quantitative morphological evaluations carried out on PN30 indicated structural abnormalities in the cerebellum, hippocampus, and somatosensory cortex. In the cerebellum, PN2-5 terbutaline treatment reduced the number of Purkinje cells and elicited thinning of the granular and molecular layers. The hippocampal CA3 region also displayed thinning, along with marked gliosis, effects that were restricted to females. In the somatosensory cortex, terbutaline evoked a reduction in the proportion of pyramidal cells and an increase in smaller, nonpyramidal cells; again, females were affected more than males. Although abnormalities were obtained with later terbutaline treatment (PN11-14), in general the effects were smaller than those seen with PN2-5 exposure. Our results indicate that terbutaline is a neurotoxicant that elicits biochemical alterations and structural damage in the immature brain during a critical period. These effects point to a causal relationship between fetal terbutaline exposure and the higher incidence of cognitive and neuropsychiatric disorders reported for the offspring of women receiving terbutaline therapy for preterm labor. Support: USPHS HD09713, ES07031, USEPA STAR Fellowship.

1335 ELEVATED EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN IN THE CEREBELLUM OF THE OFFSPRING AT LATE PUBERTY FOLLOWING MATERNAL EXPOSURE TO NICOTINE DURING GESTATION.

W. A. Khan, A. Abdel-Rahman, A. M. Dechkovskaia, J. M. Sutton, X. Guan and M. B. Abou-Donia. *Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.*

In the present study, histopathological and cholinergic changes were evaluated in the offspring following maternal exposure to nicotine and chlorpyrifos, alone and in combination. Female Sprague-Dawley rats (300-350 gm) with known pregnancy date were treated daily with nicotine (1mg/kg, s.c.) or chlorpyrifos (0.1 mg/kg, dermal) or a combination of nicotine and chlorpyrifos from gestational days (GD) 4-20. Control animals were treated with saline and ethanol. On PND 60, the offspring were evaluated for cholinergic changes and pathological effects. Plasma butyrylcholinesterase (BChE) activity from the female offspring from chlorpyrifos treated mothers showed a significant increase (~183% of control). Male offspring from mothers treated with either chlorpyrifos or nicotine alone showed a significant increase in the AChE activity in the brainstem while female offspring from mothers treated with either nicotine or a combination of nicotine and chlorpyrifos showed a significant increase (~134 and 126 % of control, respectively) in acetylcholinesterase (AChE) activity in the brainstem. No significant changes were observed in the ligand binding densities for a4b2 and a7 nicotinic acetylcholine receptors in the cortex. Histopathological evaluation using cresyl violet staining showed a significant decrease in surviving Purkinje neurons in the cerebellum of the offspring from nicotine treated mothers. An increase in glial fibrillary acidic protein (GFAP) immunostaining was observed in the cerebellum of the offspring from the mothers treated with nicotine. These data suggest that maternal exposure to nicotine causes differential regulation of brainstem AChE activity, a decrease in the surviving neurons and an increased expression of GFAP in cerebellum of the offspring on PND 60. These changes may have long-term adverse health effects later in life. Supported by the USEPA (EPA grant # R829399-01-0).

1336 MATERNAL EXPOSURE TO NICOTINE VIA INFUSION DURING GESTATION PRODUCES NEUROBEHAVIORAL DEFICITS AND ELEVATED EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN IN THE CEREBELLUM IN THE OFFSPRING AT PUBERTY.

M. B. Abou-Donia, A. Abdel-Rahman, A. M. Dechkovskaia, J. M. Sutton and W. A. Khan. *Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.*

Developmental exposure to nicotine due to maternal smoking during pregnancy causes neurological deficits in the offspring. The present studies were carried out in the offspring following maternal exposure to nicotine *via* osmotic pump. Female Sprague-Dawley rats (300-350 gm) with known pregnancy date were treated daily with nicotine (3.3mg/kg, in normal saline *via* s.c. implantation of the pump) from gestational days (GD) 4-20. Control animals were treated with saline *via* s.c. implantation of the pump. On postnatal day (PND) 30 and 60, the offspring were evaluated for changes in the ligand binding for various types of nicotinic acetylcholine receptors, oxidative stress load and pathological effects. On PND 60, the neurobehavioral evaluations for sensorimotor functions, beam-walk score, beam walk time, incline plane and grip time response were carried out. Beam-walk time and forepaw grip time showed significant impairments in both male and female off-

spring. Ligand binding densities for [3H]epibetadine, [3H]cytisine and [3H]abungarotoxin did not show any significant changes in the cortex at PND 30 and 60. A significant decrease in the levels of reduced glutathione (GSH) was observed in the female offspring on PND 30 and 60. Histopathological evaluation using cresyl violet staining showed a significant decrease in surviving Purkinje neurons in the cerebellum, and an increase in glial fibrillary acidic protein (GFAP) immunostaining at PND 30 in both male and female offspring. These data suggest that maternal exposure to nicotine produces significant neurobehavioral deficits, a decrease in the surviving neurons and an increased expression of GFAP in cerebellum of the offspring on PND 30. These changes may have long-term adverse health effects later in life. Supported by a grant from the External Research Program of Philip Morris Inc., USA.

1337 GENE EXPRESSION CHANGES ASSOCIATED WITH A DEVELOPMENTAL PESTICIDE EXPOSURE MODEL OF PARKINSON'S DISEASE.

M. Thiruchelvam[1], A. I. Brooks[2], E. K. Richfield[1] and D. A. Cory-Slechta[1]. *[1]Environmental and Community Medicine, University of Medicine and Dentistry of New Jersey, Piscataway, NJ and [2]Environmental Medicine, University of Rochester, Rochester, NY.*

It still remains to be determined whether developmental insults or cumulative insults across life could underlie the etiology of Parkinson's disease (PD). We have shown that developmental exposure to paraquat (PQ) and maneb (MB) produce permanent and progressive changes in the nigrostriatal dopaminergic (DA) system, with enhanced vulnerability to subsequent adult pesticide re-exposure. Mice were treated daily with saline, 0.3 mg/kg PQ, 1 mg/kg MB or PQ MB from postnatal (PN) days 5-19. A subset were re-challenged as adults (PN AD) with saline, 10 mg/kg PQ, 30 mg/kg MB, or PQ MB and another group treated only as adults (AD). PN AD treatment produced greater neurotoxicity than either PN or AD only regardless of endpoint. Relative to globally normalized signal values, significant changes in expression of >2000 genes were found in striatum of PN and PN AD groups, and >300 in the AD group; 73 genes changed in common across the 3 groups, 1589 in the PN and PN AD groups, and 28 in the AD and PN AD groups. Functional clustering of the outcomes indicated significant changes in dopaminergic (D2, D3 and D4), GABAergic (GABA A and B), and glutamatergic (NMDA, AMPA and kainite) receptor function and expression of glutathione metabolism-related molecules. A significant dose relationship was obtained, with the greatest changes observed with the PN AD treatment paradigm. Similar changes were observed when functional clustering of genes related to ubiquitin and protein degradation as well as apoptotic cell death were analyzed. These findings demonstrate that developmental pesticide exposure can produce permanent and progressive nigrostriatal DA lesions and enhance adult vulnerability, consistent with a developmental etiology for the PD phenotype. Changes in profiles of gene expression can be used to elucidate the mechanism of action of these compounds but possibly also biomarkers associated with the PD phenotype.

1338 ONTOGENY OF PROTEINS FOR USE AS BIOMARKERS OF DEVELOPMENTAL NEUROTOXICITY.

B. L. Robinette and W. R. Mundy. *Neurotoxicology Division, NHEERL, ORD, USEPA, Research Triangle Park, NC.*

The developing nervous system can be uniquely susceptible to adverse effects following exposure to environmental chemicals, and several advisory panels (e.g. ILSI, NRC, NAS) have highlighted the need for rapid and sensitive developmental neurotoxicity testing methods. Measurement of changes in biochemical markers that are linked to the cellular processes underlying nervous system development is one approach to developing such methods. The current research examined the ontogeny of proteins in the CNS that are associated with neuronal differentiation and growth. Frontal cortex and hippocampal tissue were obtained from male Long Evans rats on postnatal days 1, 7, 14, 21, 28, and 83-125 (adults) and the expression of structural and signaling proteins was determined by SDS-PAGE and western blotting optimized with respect to antibody dilution and protein load to obtain semi-quantitative results. The expression of GAP-43 and tau, proteins associated with axonal and dendritic elongation, was high during the early postnatal period and decreased by one half in adulthood. In contrast, expression of the synaptic proteins synapsin and synuclein increased 15-fold from very low levels on postnatal day 1 to maximal levels at adulthood. SMAD, CREB, ERK, and CaMKII are all signaling proteins that are part of pathways activated during neuronal growth and synaptic plasticity. Expression of all four proteins increased during development (SMAD 2-fold; CREB 10-fold; ERK 3-fold, CaMKII 100-fold). However, the levels of the phosphorylated (active) form of these proteins were highest during the early postnatal period then decreased during adulthood. The unique ontogeny of these proteins suggests that they can be used to monitor several aspects of nervous system development. Further research will determine whether changes in the ontogenic profile of these proteins can be used to detect developmental neurotoxicity produced by environmental chemicals. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy).

1339 NEUROPATHOLOGICAL EXAMINATION OF FETAL RAT BRAIN EXPOSED TO THE GENOTOXIC COMPOUND, 5-BROMO-2-DEOXYURIDINE (BRDU).

T. Ogawa[1], K. Muneoka[2], M. Kuwagata[1] and S. Shioda[1]. *[1]Anatomy, Showa University School of Medicine, Tokyo, Japan and [2]Hatano Research Institute, FDSC, Kanagawa, Japan.*

Prenatal exposure to chemicals such as alcohol, nicotine and PCBs is well known to induce developmental abnormalities in the central nervous system (CNS) of children. In the developmental neurotoxicity, the majority of studies focus on the postnatal subjects rather than the fetus. Even in the study of endocrine disrupters, and area of current scientific interest, there has been little focus on the assessment of alteration in the fetal brain shortly after experimental *in utero* toxicant exposure. Among the reasons for this are 1) the difficulty in obtaining histological preparations of satisfactory quality and level of section, 2) the absence of clear endpoints for use in histological evaluation of such material. Despite such difficulties, the evaluation of the fetal brain is an important step in assessing toxic alterations of neurodevelopment. In this study, we show a method of preparing rat fetal brain, using gelatin-embedding, complete vibratome serial sectioning and Nissl or hematoxylin staining. We also demonstrate its suitability for immunohistochemistry using the neuronal marker, MAP2. It has been reported that prenatal treatment with a genotoxic compound, BrdU (50 mg/kg, i.p., gestation days 9-15) induces hyperactivity, impaired learning and memory, and impaired male reproductive behavior in rat offspring. In this study, we examined the fetal rat brain shortly after BrdU exposure (gestation day 16). BrdU induced TUNEL-positive cell death in the neuroepithelial layer and the intermediate zone of the cortex, striatum and hippocampus. Fuzziness or destruction of the cortical plate was also detected. Although almost all offspring born after prenatal exposure to BrdU showed behavioral abnormalities, our pathological studies demonstrated variation in the degree of cell death among the fetuses, even in the same litter. Therefore, even very mild induction of cell death may contribute to the behavioral abnormalities.

1340 EPA SCREEN FOR DEVELOPMENTAL NEUROTOXICITY: RESULTS WITH SIX ORGANOPHOSPHORUS (OP) INSECTICIDES.

L. P. Sheets. *Toxicology, Bayer CropScience, Stilwell, KS.*

Developmental neurotoxicity (DNT) studies (OPPTS 870.6300) were conducted with methamidophos, azinphos-methyl, disulfoton, trichlorfon, coumaphos and fenamiphos in response to a data call-in notice issued by the USEPA. Each OP was administered *via* the diet at three dietary levels, plus control, to mated female Wistar rats (20-23/dietary level) from gestation day (GD) 0 (sperm-positive) through postnatal day (PND) 21. The offspring were evaluated for clinical signs, weight gain, sexual maturation, motor activity (figure-8 maze), auditory startle habituation, associative learning and memory (M-maze and passive avoidance), neuropathology and brain measurements. Whole-brain, erythrocyte and plasma cholinesterase (ChE) activities were measured in the dams on LD 21 and in the pups on PND 4 and PND 21. Overt maternal toxicity at the high dose in some studies (e.g., decreased body weight, clinical signs) was associated with pronounced toxicity in the pups, including mortality, a marked decrease in weight gain and decreased motor activity during exposure, a reduced startle response on PND 22 and some delays in vaginal patency and/or preputial separation, while minimal effects were seen in pups at doses that caused more modest maternal toxicity. Pup exposure during lactation was shown by inhibition of ChE activity. The only effect that persisted to term on PND 70-75 was reduced body weight at high doses. Measures of cognition and habituation (motor activity and auditory startle) were not affected and neuropathology was not evident with any OP at any dose. The lowest NOEL in each study was ChE inhibition in the dam, which is consistent with the results from the two-generation reproduction studies. These results support the continued use of ChE inhibition as a conservative measure to protect for adverse effects in the adult and young.

1341 AUDITORY STARTLE REFLEX HABITUATION IN DEVELOPMENTAL NEUROTOXICITY TESTING: A CROSS-LABORATORY COMPARISON OF CONTROL DATA.

W. Sette[2], K. Crofton[3], S. Makris[1], J. Doherty[1] and K. Raffaele[1]. *[1]OPP, USEPA, Washington, DC, [2]OSA, USEPA, Washington, DC and [3]NHEERL, ORD, USEPA, Research Triangle Park, NC.*

The USEPA Developmental Neurotoxicity (DNT) Study Test Guideline (OPPTS 870.6300) calls for a battery of functional and neuropathological assessments in offspring during and following maternal exposure. Auditory startle reflex habituation is assessed around the time of weaning and around 60 days of age. The Guideline specifies only that mean response amplitude be measured using 5 blocks

of 10 trials per session on each day of testing; the type of device, stimulus intensity, or inter-stimulus interval are not specified. To compare types of devices, testing conditions, age, and laboratory, we evaluated startle reflex data generated in control animals from 26 studies in terms of: 1) variability of mean amplitudes across ages, both within a laboratory and across laboratories; and 2) startle amplitude habituation across ages, within a laboratory and across laboratories. Data from 12 laboratories (1-4 studies/laboratory) were evaluated. Many laboratories submitted reports with incomplete procedural information (e.g., no description of device type). There was large variability in auditory startle habituation data for some studies; in almost all laboratories, variability was greater in adults than in weanlings. Startle amplitude varied among laboratories and with postnatal age. Within laboratories, some demonstrated stable startle amplitude at a given age across studies, while others had variable control data between studies. Degree of habituation also varied across laboratories, with some laboratories finding little habituation in weanlings, while other laboratories demonstrated similar habituation for adults and weanlings. More complete reporting of procedural information would allow for improved understanding of the sources of variability in auditory startle habituation data. Review of control data by testing laboratories as additional studies are generated would also aid in understanding and reducing data variability. *This abstract does not reflect official policy of the USEPA.*

1342 LEARNING AND MEMORY TESTS IN DEVELOPMENTAL NEUROTOXICITY TESTING: A CROSS-LABORATORY COMPARISON OF CONTROL DATA.

K. Raffaele[1], M. Gilbert[2], K. Crofton[2], S. Makris[1] and W. Sette[3]. *[1]OPP, USEPA, Washington, DC, [2]NHEERL, ORD, USEPA, Research Triangle Park, NC and [3]OSA, USEPA, Washington, DC.*

The USEPA Developmental Neurotoxicity (DNT) Study Test Guideline (OPPTS 870.6300) calls for functional tests to assess the impact of chemicals on cognitive function in offspring following maternal exposure. A test of associative learning and memory is to be conducted around the time of weaning and around 60 days of age, and flexibility is allowed in the choice of test. The present study was undertaken to: 1) survey the types of` learning and memory tests typically used by companies submitting DNT data to EPA, 2) evaluate the consistency of protocols for such tests across laboratories, 3) identify the variables routinely reported for any given test protocol, and 4) estimate the variability in control values on a number of test endpoints. Datasets from 15 laboratories were examined, comprising learning and memory tests for 35 test substances and 7 positive control treatments. The survey revealed a total of 8 learning and memory tests were employed. The two most common tests in both age groups were position discrimination in a 3 compartment water maze and passive avoidance conditioning. Preliminary findings reveal a range in the stimulus parameters and protocols for any given test. Similarly the dependent measures recorded and the format of the data report varied broadly, complicating efforts to evaluate control variability and limiting direct comparisons of proficiency among laboratories. A number of cases utilized the same animals for young and adult testing. Data presentation and statistical evaluation were often inadequate; incomplete reporting of individual animal and trial data limited our ability to re-analyze submitted data. More complete reporting of procedural information and inclusion of complete individual animal and trial data may help identify sources of variability and facilitate cross-laboratory comparisons. *This abstract does not reflect official policy of the USEPA.*

1343 DEVELOPMENTAL NEUROTOXICITY OF PYRETHROID INSECTICIDES: CRITICAL REVIEW.

T. J. Shafer[1], D. A. Meyer[2] and K. M. Crofton[1]. *[1]Neurotoxicology Division, NHEERL, ORD, USEPA, Research Triangle Park, NC and [2]Curriculum in Toxicology, University of N. Carolina, Chapel Hill, NC.*

Pyrethroids are widely utilized insecticides whose primary action is the disruption of voltage-sensitive sodium channels (VSSC). Although these compounds have been in use for over 30 years and their acute neurotoxicity has been well characterized, there is considerably less information available regarding the potential developmental neurotoxicity of this class of compounds. The current effort reviewed results of 22 studies on the developmental neurotoxicity of pyrethroids. Nineteen of the studies were from peer-reviewed journals. These studies included developmental neurotoxicity studies of 7 different type I and II pyrethroid compounds, with deltamethrin and allethrin being the most studied compounds. Although numerous endpoints were examined, changes in motor activity as well as muscarinic acetylcholine receptor levels were among the most common endpoints examined and were reported to be altered by developmental exposure to pyrethroids in several studies. Review of all of these studies indicate that many of the developmental neurotoxicity studies contained inadequacies in study design and/or statistical analysis. In addition, several studies utilized formulated products and inadequate controls, which limited interpretation of results from these studies. In conclusion, no definitive statements regarding the potential developmental neurotoxicity of pyrethroid insecticides can be made on the basis of currently available scientific data. *(This is an abstract of a proposed presentation and does not reflect EPA policy)*

1344 NEUROPATHOLOGICAL STUDY OF LONG-TERM CONCURRENT EXPOSURE TO TWO ORGANOPHOSPHATES (OPS) IN RATS.

B. S. Jortner, S. Hancock, J. Hinckley, L. Tobias, L. Flory and M. Ehrich. *Laboratory for Neurotoxicity Studies, Virginia Tech, Blacksburg, VA.*

Exposure to multiple chemicals is a likely event in industrial societies, yet few related neuropathological studies exist. We report results of a 90 day study which sequentially evaluated such effects in rats administered two neurotoxic OPs. Adult male Long-Evans rats were exposed to a 60 mg/kg sc dosage of chlorpyrifos on days 7 and 42. In addition, seven 75, 150, or 300 mg/kg gavage dosages of tri-orthotolyl phosphate (TOTP) were given during both the day 14-28 and 49-63 intervals. Appropriate controls were used. Rats were sacrificed on days 28, 63 and 90 days, for determination of neurotoxic esterase (NTE) levels, or perfusion-fixed for neuropathological study. Activity of NTE was diminished in a dose-related fashion by TOTP on days 28 and 63. At the latter interval, the 300 mg/kg group had regional brain activity below 50% of controls, with recovery seen at day 90. In rats administered this dose of TOTP, the distal (medullary) gracile fasciculus had occasional swollen myelinated axons on day 28. By day 63 in rats given the 300 mg/kg dose, this had evolved into prominent Wallerian-like myelinated fiber degeneration, and some lesions were also noted in more proximal levels of this tract, and in peripheral nerves. Lesser involvement was seen at the 150 mg/kg dose. On day 90, after a 4-week period without additional OP exposure, the fiber degeneration was enhanced in rats given the 300 mg/kg dose (the lower two TOTP doses were not examined at this interval). One reflection of this was the greater extent of the lesions in more proximal levels of the gracile fasciculus and peripheral nerve. These changes are related to TOTP dose. Chlorpyrifos did not elicit this lesion, nor significantly modify the TOTP effect. The nature and location of the fiber degeneration, and its association with brain NTE inhibition indicates this is OP ester-induced delayed neuropathy. This study demonstrates the post-dosing progression of this neuropathic state after cessation of multiple toxicant administration in rats. Supported by US-AMRMC, DAMD 17-99-1-9489.

1345 THE EFFECT OF REPEATED ORAL INGESTION OF CHLORPYRIFOS ON CHOLINESTERASE AND CARBOXYLESTERASE ACTIVITY IN ADULT RATS.

A. M. Betancourt and R. Carr. *Mississippi State University, MS State, MS.*

The organophosphurus (OP) insecticide chlorpyrifos (CPS) is used worldwide in agricultural and commercial settings for insect control. In many developmental studies, appropriate maternal weight gain is used as a marker for no negative effects during OP exposure to pregnant dams. This project was designed to investigate the effects of CPS exposure on cholinesterase (ChE) and carboxylesterase (CbxE) activity in non-preganant female rats to determine the extent to which female rats will be affected by repeated exposure during the gestational and the lactational periods. Every day for 36 days, adult female rats were fed vanilla wafers onto which 5 mg/kg chlorpyrifos, dissolved in safflower oil, had been applied. Treatment had no effect on body weight. Rats were sacrified on days 6, 12, 18, 24, and 36. ChE activity in the cerebral cortex (CC), corpus striatum (CS), cerebellum (CE), hippocampus (HC), and medulla oblongata (MO) and CbxE in the liver and serum were determined. By 6 days of treatment, ChE in the brain regions was inhibited greater than 45% in all regions except the CS which was 26%. As treatment progressed, the amount of inhibition gradually increased to maximum on day 24 with little change through day 36. Inhibition did not exceed 80%. Inhibition of liver CbxE was 79% by day 24 with a slight decrease through day 36. The data agree with previous studies demonstrating brain area specific inhibition of ChE. These data suggest that substantial enzyme inhibition can occur in the absence of negative effects on weight gain. (Supported by NIH 1 P20 RR17661-01).

1346 EFFECTS OF REPEATED EARLY POSTNATAL EXPOSURE TO EITHER CHLORPYRIFOS OR METHYL PARATHION ON SPATIAL LEARNING AND MEMORY AND MOTOR ACTIVITY.

R. L. Carr, A. M. Betancourt, J. E. Chambers, F. O. Johnson and J. A. Kamykowski. *Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.*

There is currently significant concern about the exposure of children to the organophosphorus insecticides (OP). Since the brain is not fully developed during early childhood, introduction of known neurotoxic agents could disrupt this devel-

opment leading to long-term alterations in function and behavior. This project investigated the effects of early postnatal exposure to two agricultural OP insecticides, chlorpyrifos (CPS) and methyl parathion (MPS), on spatial learning and memory in a Morris water maze and on motor activity and habituation using the figure 8 maze. Using an incremental dosing regimen, postnatal rats were gavaged daily with either corn oil or CPS at a low dose of 1 mg/kg from postnatal day 1 (PND1) to 21; a medium dose of 1mg/kg from PND1-5, 2mg/kg from PND6-13, and 4mg/kg from PND14-21; a high dose of 1.5mg/kg from PND1-5, 3mg/kg from PND6-13, and 6mg/kg from PND14-21, or MPS at a low dose of 0.2mg/kg from PND1-21; a medium dose of 0.2mg/kg from PND1-5, 0.4mg/kg from PND6-13, and 0.6 mg/kg from PND14-21; a high dose of 0.3mg/kg from PND1-5, 0.6mg/kg from PND6-13, and 0.9mg/kg from PND14-21. Rats were tested in a figure 8 maze on PND35, in the Morris water maze on PND36-39 followed by a probe trial on PND42, and then retested in a similar manner on PND70, PND71-74, and PND77, respectively. Neither insecticide appears to have an effect on spatial learning or memory. Neither insecticide had effects on overall activity or habituation in males. In females, the low dosage of CPS caused an increase in activity and decreased habituation on PND35 while on PND70, the medium and high dosages of CPS caused a significant increase in activity and decreased habituation. MPS had no effects. These data suggest that developmental exposure to CPS but not MPS can exert gender-related effects on activity and habituation which vary with exposure level and time. (Supported by NIH R01 ES 10386).

1347 DNA MICROARRAY ANALYSIS OF RAT BRAIN TO ASSESS CHANGES IN GENE EXPRESSION AND NEUROTOXICITY OF FOUR CONAZOLES.

L. D. White[1], D. B. Tully[2], W. Bao[2], J. E. Schmid[2], H. Ren[2], A. K. Goetz[4], G. Sun[3], S. Nesnow[3], D. J. Dix[2] and S. Barone[1]. *[1]Neurotoxicology, USEPA, Research Triangle Park, NC, [2]Reproductive Toxicology, USEPA, Research Triangle Park, NC, [3]Environmental Carcinogenesis, USEPA, Research Triangle Park, NC and [4]Department Envir. & Molecular Toxicology., NCSU, Raleigh, NC.*

Conazoles are azole fungicides widely used both pharmaceutically and agriculturally. This study focused on four conazoles that exhibit a range of cancer and non-cancer effects, to ascertain if any neurotoxic effects are present and to identify possible modes of action. Conazoles target cytochrome P450s (CYPs), and the inhibition and induction of various CYP activities may be part of the toxic modes of action in brain. We used gene expression profiling to characterize a broader range of conazole effects and to identify potential modes of action. Adult male Sprague-Dawley rats were dosed by gavage for 14 days with fluconazole, propiconazole, myclobutanil or triadimefon (three doses per chemical). All four conazoles induced cytochrome 2B and/or 3A CYPs in liver microsomes and caused increases in liver weights relative to body weights. Spotted oligonucleotide microarrays utilizing cy3/cy5 labeling interrogated the expression of over 4000 genes in hippocampus from control and treated rats. Log-transformed data were normalized using an intensity dependent local (Lowess) regression model. Genes expressed consistently in at least one treatment group were analyzed individually using a one-way anova. The analysis was repeated with various modifications to the data or analysis: with and without local background subtraction, with and without a range normalization, and with and without a random predictor for date of array experiment added to the anova. Gene expressions consistently different for at least one treatment versus control were selected for further analysis. Comparison of hippocampal expression profiles between control and fluconazole-, myclobutanil-, propiconazole-, and triadimefon-treatment identified 107, 71, 138, and 130 differentially expressed genes, respectively, including genes involved in programmed cell death and neurotrophic factor production. This abstract does not necessarily reflect EPA policy.

1348 BEHAVIORAL AND NEUROCHEMICAL ALTERATIONS ASSOCIATED WITH ACUTE AND CHRONIC ATRAZINE EXPOSURE.

V. M. Rodriguez, M. Thiruchelvam and D. A. Cory-Slechta. *Environmental and Community Medicine, EOHSI, University of Medicine and Dentistry of New Jersey and Rutgers State University, Piscataway, NJ.*

Atrazine (ATR) is a broad-spectrum herbicide commonly used to control weeds and grass in a wide variety of crops. Given its abundant use, ATR has been found as a contaminant in ground and surface water resulting in human exposure. Aside from its effects on the hormonal systems, it may also impact the nervous system. Behavioral and neurochemical alterations associated with chronic ATR exposure were examined in Long Evans rats exposed to 5 or 10 mg/kg ATR daily for 3 or 6 months *via* food. The 6 months exposure to 5 mg/kg ATR increased cortical dopamine (DA) levels but did not alter locomotor activity. Exposure to 10 mg/kg ATR increased locomotor activity, which was more pronounced when challenged with 1 mg/kg amphetamine. This exposure paradigm also decreased both striatal DA and hypothalamic serotonin (5-HT) levels when measured 2 weeks post-termination of exposure. A 3 months exposure to 10 mg/kg ATR also increased locomotor activity level. ATR exposure blunted the increases in locomotor activity produced by MPTP exposure. Acute exposure to either 100 or 200 mg/kg ATR i.p. decreased basal as well as high potassium-evoked striatal DA release measured using microdialysis. Collectively, these studies indicate that ATR can adversely affect monoaminergic systems of the brain, systems important in mediating movement as well as cognition and executive function. Thus, ATR may be an environmental risk factor for disorders related to these systems, underscoring the need for further investigation of its mechanism of action. Supported by ES01247 & ES10791.

1349 INFLUENCE OF 14-DAY EXPOSURE TO ATRAZINE ON STRIATAL NEUROCHEMISTRY IN JUVENILE MALE C57BL/6 MICE.

A. Coban, A. B. Norwood, S. C. Sistrunk and N. M. Filipov. *CEHS, Basic Sciences, Mississippi State University, Mississippi State, MS.*

Previous *in vitro* studies have suggested, and we recently demonstrated *ex vivo*, that the herbicide atrazine (ATR), decreased striatal dopamine (DA) levels. The present study had the objective of assessing the ability of ATR to modulate DA *in vivo*. Juvenile animals appear to be more sensitive to certain neurotoxicants (*i.e.*, lead). Thus, in this study, 1-month-old male C57BL/6 mice were exposed to ATR by daily gavage for 14 d at doses of 5, 25, 125, 250 mg/kg x BW^{-1}. Groups of mice were sacrificed and brains collected at 1, 7 and 49 d post termination of ATR exposure. Sub-groups of mice were administered MPTP (4 X 10 mg/kg, 2 h apart, *i.p.*). DA and its metabolites, 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA), were analyzed in the striatum by HPLC. Expression of several key proteins involved in DA homeostasis (*i.e.*, tyrosine hydroxylase [TH], dopamine transporter, and vesicular monoamine transporter-2), as well as striatal TH immunoreactivity (IR) and the number of TH-positive neurons in the substantia nigra were analyzed by immunoblots and immunocytochemistry/unbiased stereology, respectively. ATR, at doses of 125 and 250 mg/kg, decreased striatal DA, DOPAC and HVA levels at 1, but not at 7 d, after the last ATR dose and this decrease was accompanied by a moderately-decreased TH-IR in the striatum. As expected, MPTP decreased striatal levels of DA and its metabolites. The degree of DOPAC and HVA decrease, but not of DA, was potentiated in animals that were exposed to ATR (125 and 250 mg/kg) prior to the MPTP challenge. Thus, it appears that (*i*) ATR is capable of decreasing striatal DA levels in juvenile mice, (*ii*) this effect is apparently short-lived, and (*iii*) previous ATR exposure enhances the dopaminergic toxicity of MPTP mainly by augmenting its effects on the DA metabolites DOPAC and HVA. Supported by: P20 RR017661 (NIH).

1350 SPECIES, STRAIN, AND SEX INFLUENCE ON THE DOPAMINERGIC TOXICITY OF THE HERBICIDE ATRAZINE *EX VIVO*.

J. A. Whitehead, S. C. Sistrunk and N. M. Filipov. *CEHS, Basic Sciences, Mississippi State University, Mississippi State, MS.*

In light of the suggested link between Parkinson's Disease (PD) and pesticide exposure, using striatal slices derived from male Sprague-Dawley (SD) rats, we demonstrated that exposure to the herbicide atrazine (ATR) selectively decreases tissue levels of the neurotransmitter dopamine (DA) in the absence of general cytotoxicity. However, when it comes to other indexes of toxicity, it appears that there are strains of rats that are more sensitive to ATR than others. For example, in some reproductive toxicity studies, the SD strain was affected by ATR the most. Therefore, an argument could be made that in our previous studies we have used as a source of tissue a strain of rats that is uniquely sensitive to ATR, and thus, not representative for a generalized effect of ATR. To address these issue, series of experiments were performed in which striata derived from male and female SD and Fisher 344 rats, as well as from C57BL/6 and BALB/c mice, were exposed *ex vivo* to ATR at concentrations of up to 500 μM for 4 h and levels of DA and its metabolites 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) in tissue and media were determined. At the highest dose, ATR exposure decreased tissue DA and increased media DA concentrations regardless of sex, strain or species. At lower doses of ATR, there were important sex- and strain-dependent differences in that BALB/c mice and female SD rat striata were the most and least sensitive to ATR, respectively. Taken together, results from this study suggest that ATR, at higher concentrations, acts as a dopaminergic toxicant regardless of sex, strain, and species. At lower ATR concentrations, the source of striatal tissue does matter. However, striata from male SD rats are not the most sensitive to ATR in this experimental paradigm and this fact, when it comes to basal ganglia toxicity, argues against a unique sensitivity of SD rats to ATR. Supported in part by: P20 RR017661 (NIH).

1351 PARAOXONASE ABUNDANCE AND Q192R GENOTYPE ARE IMPORTANT DETERMINANTS OF ORGANOPHOSPHATE TOXICITY DURING DEVELOPMENT.

T. B. Cole[1,2], C. Pettan-Brewer[2], R. Richter[2], D. M. Shih[3], A. Tward[3], A. J. Lusis[3], L. G. Costa[2] and C. E. Furlong[1]. [1]*Genome Sciences and Medicine, Division of Medical Genetics, University of Washington, Seattle, WA,* [2]*Environmental Health, University of Washington, Seattle, WA and* [3]*Microbiology and Molecular Genetics, UCLA, Los Angeles, CA.*

Paraoxonase (PON1) is an HDL-associated enzyme that metabolizes organophosphorous (OP) compounds, drugs, and oxidized lipids. PON1 activity is determined in part by a coding region polymorphism (Q192R) that affects its catalytic efficiency and in part by differences in PON1 levels, which vary widely (>13-fold). Infants are particularly susceptible to OP toxicity due to low abundance of PON1 and variable onset of expression. To assess the importance of PON1 for protecting against the developmental toxicity of chlorpyrifos oxon (CPO), PON1 knockout (PON1-/-) and wild-type (WT) mice were exposed chronically (PN4 to PN21) to low levels of CPO. Endpoints included cholinesterase activity, histopathology, gene expression, and behavior. Even at PN4, when PON1 levels were low in WT mice, PON1-/- mice were more sensitive to inhibition of brain cholinesterase by CPO. At PN22, and persisting as long as 4 months, chronic developmental exposure to 0.18 mg/kg/d or 0.25 mg/kg/d CPO resulted in perinuclear vacuolization of cells in a discrete area of the neocortex and irregular distribution of neurons in the cortical plate, with an increase in the number of affected cells at 0.25 mg/kg/d. To assess the importance of the Q192R polymorphism for OP detoxication, transgenic mice were used that expressed human transgenes encoding hPON1Q192 or hPON1R192 at equal levels in place of mouse PON1. Mice expressing hPON1R192 were significantly more resistant than hPON1Q192 mice to CPO toxicity. The developmental onset of expression was similar for the hPON1Q192 and hPON1R192 transgenes, allowing these mice to be used to assess the importance of the Q192R polymorphism during development. Our studies indicate that children less than 2 yr old, especially those homozygous for PON1Q192, are particularly susceptible to OP toxicity. Supported by T32 AG00057, ES09601/EPA-R826886, ES09883, ES04696, ES07033.

1352 EVALUATION OF EPIDEMIOLOGICAL AND ANIMAL DATA ASSOCIATING PESTICIDES WITH PARKINSON'S DISEASE.

A. A. Li[1], P. Mink[2], L. McIntosh[1], J. Teta[2] and B. Finley[1]. [1]*Toxicology/Human Health Risk Assessment, Exponent, San Francisco, CA and* [2]*Health/Epidemiology, Exponent, Washington D.C., DC.*

The potential role of pesticide exposure in the development of Parkinson's Disease (PD) has been examined in numerous epidemiologic studies and animal models. Although some epidemiologic studies have reported elevated PD risk among those with rural residences and/or a history of farming occupations, no single class of pesticides or any specific pesticide has been linked consistently with PD. The reason for the lack of consistency across studies is due, in part, to methodological differences such as PD case definition, completeness of case ascertainment, selection of appropriate controls in case-control studies and variable quality and specificity of exposure assessments. In addition, animal data, linking pesticide exposure to neurotoxic effects related to those observed in PD patients, has been reported for specific pesticides and for chemicals similar in structure to some pesticides. We have performed a comprehensive evaluation of the epidemiological and animal data. Epidemiological studies of PD that have included information on pesticide exposure (i.e., either pesticide class, specific pesticide, or dose-response for general category "pesticide") were stratified into three different tiers based on strengths and limitations of study design features including case definition, case and control selection, and exposure assessment. The animal studies were evaluated with respect to specificity of PD-like effects and the exposure levels required to produce the observed effects. This qualitative analysis provides an initial systematic weight-of-evidence approach to evaluating the evidence linking pesticide exposure with Parkinson's disease.

1353 NEUROTOXICOLOGICAL AND STATISTICAL ANALYSES OF A MIXTURE OF FIVE ORGANOPHOSPHORUS PESTICIDES USING A RAY DESIGN.

V. C. Moser[1], A. Hamm[2], M. Casey[2], W. H. Carter[2], J. E. Simmons[1] and C. Gennings[2]. [1]*NHEERL, USEPA, Research Triangle Park, NC and* [2]*Biostatistics, VCU, Richmond, VA.*

Pesticide application patterns generally result in exposure to mixtures instead of single chemicals. Of particular importance in the estimation of pesticide mixture risks is the detection and characterization of their interactions. This research tested for interaction(s) in a mixture of five organophosphorus (OP) pesticides (chlorpyrifos, diazinon, dimethoate, acephate, and malathion). The ratio of pesticides in the mixture reflected the relative dietary exposure estimates projected by the USEPA Dietary Exposure Evaluation Model (DEEM). The approach first required characterization of dose-response curves for each OP alone to build an additivity model. Neurochemical (blood and brain cholinesterase [ChE] activity) and behavioral (motor activity [MA], gait score, tail-pinch response score) endpoints were assessed in adult male Long-Evans rats following acute oral exposure. The additivity model was used to predict the effects of the pesticide mixture along a ray of increasing total doses (full ray, 10-450 mg/kg), using the same fixed ratio of components. The mixture data were similarly modeled and statistically compared to the additivity model along the ray. To evaluate the influence of malathion, a second pesticide mixture was tested using the same proportions of OPs but without malathion (reduced ray, 2-79 mg/kg). Analysis of the full ray revealed significant synergism for all endpoints except the tail-pinch response, which was additive. For gait score, the reduced ray was additive, indicating that the non-additivity detected before was due to malathion in the mixture. For blood ChE and MA, the curves for the full and reduced rays were different from each other, but for brain ChE they were not. Therefore, there is evidence that malathion interacts with the other OPs, at least in terms of the effects on blood ChE, MA, and gait; however, the de*via*tion from additivity cannot fully be attributed to the malathion in the mixture. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy

1354 CHOLINESTERASE INHIBITION AND HYPOTHERMIA FOLLOWING EXPOSURE TO BINARY MIXTURES OF ANTICHOLINESTERASE AGENTS: LACK OF EVIDENCE FOR CAUSE-AND-EFFECT.

C. J. Gordon[1], D. Herr[1], C. Gennings[2] and C. M. Mack[1]. [1]*Neurotoxicology, USEPA, Res. Tri. Park, NC and* [2]*Virginia Commonwealth University, Richmond, NC.*

Inhibition of plasma cholinesterase (ChE) activity and hypothermia were used as benchmarks of exposure to anticholinesterase insecticides to test the assumption of additivity of binary mixtures of chlorpyrifos (CHP) and carbaryl (CAR) in the Long-Evans rat. Male rats were dosed orally with mixtures of CHP and CAR in 2:1 and 1:1 ratios while core temperature (Tc) was monitored continuously by radiotelemetry. Plasma ChE activity was measured at 4 hr after dosing in duplicate sets of rats dosed with CHP:CAR mixtures. Doses were selected that coincided with a predicted threshold or maximal decrease in Tc. An additivity model was developed and predicted a threshold total mixture dose for hypothermia of 12 mg/kg for the 2:1 ratio and 14 mg/kg for the 1:1 ratio. The hypothermic response of the 2:1 ratio was antagonistic with significant hypothermia not observed until the dose equaled 35 mg/kg. The hypothermic response of the 1:1 ratio was additive at all doses tested. CHP and CAR reference doses of 30 and 70 mg/kg led to 84 and 37% reductions in plasma ChE activity, respectively. Plasma ChE activity was inhibited by 60 and 50% for total doses of 14 and 35 mg/kg for the 2:1 CHP:CAR ratio; for the 1:1 ratio, ChE was inhibited by 54 and 64% for total doses of 12 and 42 mg/kg. The inhibition in ChE activity was not correlated with the magnitude of the thermoregulatory response. The inhibition in plasma ChE activity was not proportional to mixture dose. Overall, binary mixtures of CHP and CAR were either additive or antagonistic, depending on their ratio in a mixture, in terms of their effects on temperature regulation. These effects on Tc are not reflected in the inhibition in plasma ChE activity. Hence, the physiological response (i.e., decrease in Tc) is not necessarily correlated with the inhibition in plasma ChE activity for binary mixtures of CHP and CAR. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1355 ESTERASE PROFILES FOR A DIALKYLPHOSPHATE SERIES: QSAR APPROACH.

G. Makhaeva[1], V. Malygin[1], A. Aksinenko[1], V. Sokolov[1] and R. J. Richardson[2]. [1]*Institute of Physiologically Active Compounds, RAS, Chernogolovka, Russian Federation and* [2]*Toxicology Program, University of Michigan, Ann Arbor, MI.*

Esterase profiles were generated for a series of O, O-dialkyl-O-(1-carboethoxy-2, 2, 2-trifluoroethyl) phosphates (CFP, R= Me, Et, Pr, i-Pr, n-Bu, i-Bu, n-Pent) by determining the bimolecular rate constants of inhibition (ki) against acetylcholinesterase (AChE), neuropathy target esterase (NTE), butyrylcholinesterase (BChE) and carboxylesterase (CE). Log ki was 1.20 to 4.93 for NTE and 2.62 to 4.32 for AChE. CFP with short R (Me, Et) were more potent inhibitors of AChE than NTE. Anti-NTE activity and NTE selectivity [ki(NTE)/ki(AChE)] increased with rising hydrophobicity. High values for R = Bu (2.19) and Pent (4.07) suggest that these compounds would be neuropathic at sublethal doses. Log ki was 3.55 to 5.37 for BChE and 2.41 to 5.93 for CE. Potency against both esterases increased with rising hydrophobicity. QSAR analysis found a linear dependence of NTE selectivity on R hydrophobicity. To consider the role of nonspecific esterases in CFP

toxicity, relationships between CFP structure and ki(CE)/ki(AChE), ki(BChE)/ki(AChE), ki(CE)/ki(NTE) and ki(BChE)/ki(NTE) were analyzed by polynomial regression. A family of parabolic equations was obtained, which along with the equation for ki(NTE)/ki(AChE), describe the change in esterase profile of CFP with hydrophobicity. The data suggest that interactions with nonspecific esterases would have different potential effects on acute and delayed neurotoxicity. For compounds with short R-groups, binding to CE and BChE could reduce their interaction with NTE. For more hydrophobic compounds, binding to nonspecific esterases has a low effect on NTE inhibition and decreases AChE inhibition. The results agree with data on the low neuropathic potential of OP compounds with short-R (Me, Et) and high neuropathic potential of long-R compounds. Taking the first derivative of the QSAR equations permits determining structures for which binding to BChE and CE is the most effective for toxic effects. (Supported by CRDF projects #RB-2035 and #RB2-2488).

1356 CHARACTERIZATION OF LARGEMOUTH BASS ACETYLCHOLINESTERASE AND ITS INHIBITION BY ANTIESTERASE PESTICIDES.

D. S. Barber and T. Knowles. *Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL.*

The largemouth bass (Micropterus salmoides, LMB) is a ubiquitous freshwater fish that is ecologically and economically important in Florida and much of the United States. Much of its habitat is adjacent to agricultural and residential lands and receives significant inputs organophosphate (OP) and carbamate insecticides through runoff and direct application. It is well documented that OP and carbamate insecticides produce overt toxicity and behavioral impairment in fish by inhibition of acetylcholinesterase (AChE), though susceptibility of fish species to toxicity from these compounds varies widely. These studies demonstrated that LMB brain cholinesterase activity was exclusively AChE, based on lack of inhibition by iso-OMPA. The activity of brain AChE was unchanged between temperatures of 20 and 35°C, but declined by 20% at 15°C. AChE activity was optimal between pH 7.0-7.5 and decreased dramatically below pH 6.0 and above 8.0. Using acetylthiocholine as a susbstrate, brain AChE exhibited Michaelis-Menten behavior with an apparent Km of 250μM and Vmax of 263.4 nmol/min/mg. Plasma cholinesterase activity was 60.5 nmol/min/ml. In contrast with many fish, 65% of this activity was apparently butyrylcholinesterase. Brain AChE was 40-100-fold more sensitive to *in vitro* inhibition by chlorpyrifos-oxon, malaoxon, and carbofuran than diazinon-oxon or propoxur, with IC50s of 11nM, 27nM, and 11nM versus 1.1μM and 1μM, respectively. As in other species, phosphorothioate insecticides were very weak AChE inhibitors compared to their oxygen analogs. While kinetic factors will affect *in vivo* sensitivity, our results suggest that largemouth bass are very susceptible to toxicity from exposure to malathion, chlorpyrifos, and carbofuran.

1357 DELTAMETHRIN INCREASES DOPAMINE TRANSPORTER EXPRESSION AND ENHANCES COCAINE-INDUCED LOCOMOTION.

T. S. Guillot, J. R. Richardson and G. W. Miller. *Center for Neurodegenerative Disease, Rollins School of Public Health, Emory University, Atlanta, GA.*

Deltamethrin (DM), a type II pyrethroid insecticide, was introduced in the early 1980s and has become one of the most widely used insecticides in both agricultural and household settings. Recently, pyrethroid exposure has been shown to preferentially affect nigrostriatal dopaminergic neurons. In particular, a functional upregulation of the dopamine transporter (DAT) has been demonstrated in the striatum of C57BL/6 mice exposed to DM. DAT, in addition to its normal role in dopamine clearance, is a target for psychostimulants such as cocaine. In fact, mice in which DAT was genetically overexpressed by 20-30% exhibited increased locomotor activity in response to cocaine challenge. Therefore, we hypothesized that an upregulation of DAT by DM would enhance cocaine induced hyperlocomotion. Male C57BL/6 retired breeder mice were treated with 6 mg/kg DM i.p. on days 1, 8, and 15. On day 16, half of the treated mice were challenged with cocaine (15 mg/kg) and open field activity was then measured. Animals were sacrificed one day later and the striatum was removed for neurochemical determinations. DM increased habituated locomotor activity (LA) by 400% and enhanced cocaine-induced LA by an additional 58%. DAT expression was increased by about 36% in DM treated animals as measured by both western blotting and [3H] WIN 35, 428 binding. Additionally, DM increased tyrosine hydroxylase (TH) by 52%, serine 40 phosphorylated TH by 13% and vesicular monoamine transporter 2 (VMAT2) by 12%, as measured by western blotting. These data suggest that upregulation of DAT by DM enhances the motor excitatory effects of cocaine and may lead to behavioral sensitization. This could have important implications for interactions between the environment and development of drug abuse. (supported by DOD 00267036).

1358 DIETARY SUGAR-INDUCED MODULATION OF PARATHION TOXICITY IN JUVENILE AND ADULT RATS.

J. Liu, S. Karanth and C. Pope. *Physiol Sciences, Oklahoma State University, Stillwater, OK.*

Sugar feeding (15% glucose in the drinking water) markedly enhanced parathion (PS) toxicity in adult rats, delayed recovery of acetylcholinesterase (AChE) activity, and decreased chow intake. We hypothesized that dietary sugars exacerbate PS toxicity by limiting intake of precursors for AChE synthesis, thereby impairing enzyme and functional recovery. Consumption of high fructose corn syrup (HFCS) has increased dramatically. We evaluated the effects of 15% HFCS in drinking water on PS toxicity in adult and juvenile rats. Rats were given access to feed and either water or 15% HFCS, for 10 days and challenged with PS (6 or 18 mg/kg, sc, in juveniles or adults, respectively) on the 4th day. SLUD signs and involuntary movements, body weight and chow/fluid intake were recorded daily. AChE activity and immunoreactivity (AChE-IR) in frontal cortex and diaphragm were measured at 2, 4 and 7 days after dosing. In another study, adult rats were fed ad lib or pair-fed to evaluate the effects of reduced chow intake alone on PS toxicity. When allowed to choose, there was a marked preference for HFCS over water. HFCS intake was associated with a significant reduction in chow intake in both young (25-35%) and adult (35-40%) rats. Cholinergic toxicity was markedly increased by HFCS in both age groups. HFCS did not, however, affect AChE inhibition in either tissue or either age group. AChE-IR in cortex was generally not affected. Diaphragm AChE-IR was significantly reduced, however, in adults by both PS (14-17%) and HFCS (19-27%), with a greater reduction (32-39%) in sugar-fed/PS-treated rats. In juveniles, diaphragm AChE-IR was reduced only in sugar-fed/PS-treated rats. Food restriction alone had no apparent effect on PS toxicity. The results suggest that consumption of HFCS can markedly increase the acute toxicity of PS in both adult and juvenile rats. Parathion and dietary HFCS reduced AChE-IR in a tissue-dependent manner, possibly by limiting de novo synthesis of new AChE molecules. The interaction between nutrient deficiency and excess sugar intake appears important in the modulation of PS toxicity.

1359 EFFECTS OF *IN VIVO* EXPOSURE TO ENDOSULFAN AND PERMETHRIN ON THE STRIATAL DOPAMINERGIC PATHWAYS.

C. Aguilar[1] and H. P. Misra[1, 2]. *[1]College of Osteopathic Medicine, Blacksburg, VA and [2]Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA.*

Pesticides have been suggested to play an important role in the development of many neurodegerative diseases. Several studies indicate that exposure to certain pesticides may cause degeneration of the nigrostriatal pathway leading to the development of Parkinson's disease. We hypothesized that exposure to mixtures of certain pesticides may exaggerate this effect. We studied the effects of permethrin (a pyretroid type I) and Endosulfan (an organochlorine) on the levels of dopamine and its metabolites in the brain of mice. These pesticides have different structures but both are known to modify the kinetics of voltage-sensitive ion channels and calcium ion flux/homeostasis that could affect the release of several neurotransmitters. C57Bl/6 mice of 7-9 months old were injected, i.p., with each of the pesticides and their mixture every-other day during a two week period. Mice were sacrificed 24 hrs after the last injection. The corpora striatum was extracted and analyzed by HPLC for dopamine and its metabolites. All the treatment groups exhibited lower dopamine levels and higher DOPAC and HVA as compared to controls (corn oil). Because reactive oxygen species have been implicated in the development of Parkinson's disease, and are known to cause degradation of certain neurotransmitters, we monitored the levels of lipid peroxides in brain cortex as an indicator of free radical tissue damage. The peroxide levels were measured by thiobarbituric acid reactive products. Increased levels of lipid peroxides were observed in brain tissues of animals exposed to these pesticides. These results indicate animal exposure to endosulfan and permethrin causes decreased levels of dopamine and increased levels of its metabolites, DOPAC and HVA, in the corpora striatum. Furthermore, a free radical mechanism involving lipid peroxidation may also be involved in pesticide-induced pathogenesis of Parkinson's disease.

1360 THE EFFECTS OF ZINEB AND ENDOSULFAN ON MPTP-INDUCED STRIATUM DOPAMINE DEPLETION IN MICE.

Z. Jia[1] and H. P. Misra[2]. *[1]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA and [2]Edward Via Virginia College of Osteopathic Medicine, Blacksburg, VA.*

The thiocarbamate fungicides are ubiquitous environmental contaminants. These pesticides are known to enhance the depletion of striatal dopamine levels and are implicated behind the pathogenesis of Parkinsons Disease. Zineb (zinc-ethylenebisdithiocarbamate) is used extensively to control unwanted plants. The organochlorine insecticide, endosulfan is a potent inhibitor of acetylcholinesterase (ACHE)

and is known to adversely affect neurotransmitters. Both are used as pesticides on a variety of crops worldwide and pose potential health risks to humans and animals. We hypothesized that these two pesticides either individually or in combination would increase MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine)-induced neurotoxicity. Male C57Bl/6 mice (six weeks old) were exposed to zineb (50 and 100 mg/kg), endosulfan (1.55 and 3.1 mg/kg) and their mixtures every other day over a 2-weeks period. The levels of dopamine and its metabolites in striatum and the activity of ACHE in cerebral cortex were monitored by high-performance liquid chromatography (HPLC) and spectrophotometric analysis, respectively. Results of these studies showed that zineb alone, but not endosulfan, could significantly ($P<0.05$) enhance MPTP-induced striatal dopamine depletion. Preliminary data also showed that mixtures of pesticides produced more exacerbation of MPTP-induced striatal dopamine depletion than when exposed to individual pesticides. However, the activity of ACHE was not significantly affected by these treatments. These data suggest that mixtures of these pesticides enhance the depletion of the nigrostriatal dopamine system.

1361 PREGNANCY ALTERS THE MECHANISM OF ALCOHOL-INDUCED BONE LOSS.

M. J. Ronis[1, 5], M. Hidestrand[1], K. Shankar[1], C. K. Lumpkin[2], T. M. Badger[3, 5], J. Aronson[4], R. Skinner[4], W. Hogue[4], C. Jo[2], P. Simpson[2], M. Zipperman[5], R. Haley[5] and L. Humphrey[5]. *[1]Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR, [2]Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, [3]Physiology, University of Arkansas for Medical Sciences, Little Rock, AR, [4]Orthopedics, University of Arkansas for Medical Sciences, Little Rock, AR and [5]Arkansas Children's Nutrition Center, Arkansas Childrens Hospital, Little Rock, AR.*

We examined skeletal toxicity of ethanol in cycling and pregnant female rats. Rats were infused liquid diets i.g. beginning at gestational d. 6 for 14 d. Diets containing ethanol, 10 g/kg/d or 13 g/kg/d were infused overnight from 1800 till 0800 h. All diets were made isocaloric by substituting ethanol for carbohydrate. Urine ethanol concentrations averaged 93 ± 2 mg/dl and 197 ± 10 mg/dl in the pregnant groups and 137 ± 13 and 271 ± 17 mg/dl in the non-pregnant groups ($p \leq 0.05$). Pregnancy increased tibial trabecular bone mineral density (BMD) in control rats as determined by pQCT scan ($p \leq 0.05$) accompanied by an increase in the plasma concentrations of the bone formation marker osteocalcin and of 1, 25 (OH)2 vitamin D3 ($p \leq 0.05$). Ethanol produced a dose-dependent loss in tibial trabecular and sub-cortical BMD ($p \leq 0.05$) in both pregnant and non-pregnant rats accompanied by reduced serum osteocalcin and 1, 25 (OH)2 vitamin D3 ($p < 0.05$). However, loss of BMD was greater in the non-pregnant females ($p \leq 0.05$). Static histomorphometric analysis of tibial bone in ethanol-treated pregnant rats demonstrated decreases in osteoblast surface (ObS/BS) and osteoid surface (OS/BS) ($p \leq 0.05$). In contrast, ObS/BS was unaffected in the non-pregnant rats and at the high ethanol dose these animals had lower bone volume, decreased trabecular number and increased trabecular separation ($p \leq 0.05$) accompanied by increased mean osteoclast surface (Oc.S/BS) and eroded surface (ES/BS). These data suggest that ethanol inhibits bone formation in pregnant rats while in non-pregnant rats the data are consistent with ethanol-induced bone resorption. Supported in part by AA12928 (M.R.).

1362 DIRECT BONE FORMATION IN NUDE (NU/NU) MICE.

L. Liu[3], J. Aronson[1, 2, 3], E. C. Wahl[3], R. A. Skinner[2], B. G. Fowlkes[3], T. M. Badger[1] and C. K. Lumpkin[1, 3]. *[1]Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, [2]Department of Orthopaedics, University of Arkansas for Medical Sciences, Little Rock, AR and [3]Laboratory for Limb Regeneration Research, Arkansas Children's Hospital Research Institute, Little Rock, AR.*

The combination of a novel limb lengthening protocol (distraction osteogenesis, DO) and T cell deficient (nu/nu) mice has allowed the comparative study of direct bone formation in the absence of functional T cells. To determine how athymic mice deficient in T cells would compare to their euthymic littermate controls in regard to bone formation during DO, twelve male mice (30g) of each genotype (nu/nu; nu/+) underwent placement of an external fixator and osteotomy on the left tibia. Distraction began three days after surgery (3 day latency) at a rate of 0.075 mm twice a day (0.15 mm/day) and continued for 14 days. At sacrifice the left tibias were harvested. Comparison of the distracted tibial radiographs demonstrated excellent regenerate bone formation in both strains ($80.4\% \pm 7.8$ nu/nu vs $87.2\% \pm 4.0$ nu/+). In addition, comparison of the distracted tibial histology demonstrated robust endosteal new bone formation in both groups ($67.4\% \pm 5.6$ nu/nu, $71.2\% \pm 6.7$ nu/+). This suggests that T cell activity is either not necessary for bone formation during DO in adult animals, or that other factors/cells are compensating for the loss. This model should allow for study of the role of T cells in skeletal pathologies impacting direct bone formation. Supported in part by NIH grant AA12223.

1363 ADMINISTRATION OF RMTNF-α INHIBITS ENDOSTEAL BONE FORMATION IN A MOUSE DISTRACTION OSTEOGENESIS MODEL.

J. Aronson[1, 2], L. Liu[2], E. C. Wahl[2], P. S. Daniel[2, 1], R. A. Skinner[1], T. M. Badger[1, 2] and C. K. Lumpkin[1, 2]. *[1]University of Arkansas for Medical Sciences, Little Rock, AR and [2]Arkansas Children's Hospital Research Institute, Little Rock, AR.*

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that modulates osteoblastogenesis. TNF-α has been shown to inhibit osteoblast differentiation and to induce osteoblast apoptosis. In addition, the demonstrated inhibitory effects of chronic ethanol exposure on direct bone formation may be mediated by TNF-α signaling. We hypothesized that administration of recombinant mouse TNF-α (rmTNF-α) would inhibit direct bone formation in a mouse model of limb lengthening (distraction osteogenesis, DO). Nineteen CB57BL/6 12-week-old male mice (average weight 25.5g) were divided into two groups: vehicle group and rmTNF treated group. Following acclimation and under nembutal anesthesia, each mouse underwent placement of an external fixator and osteotomy to the left tibia. Fourteen day, 100 µl Alzet pumps (Model 1002) were placed subcutaneously on the back for systemic delivery of rmTNF-α (10 µg/kg/day) or vehicle. Distraction began three days after surgery (3 day latency) at a rate of 0.075 mm twice a day (0.15 mm/day) and continued for 14 days. All mice were sacrificed on postoperative day 17. The distracted and contralateral tibiae and trunk blood were harvested. The blood was assayed for serum TNF-α measurement by bead array. Systemic rmTNF-α administration increased circulating levels of serum TNF-α over controls (13.05 ± 5.99 pg/ml vs undetected in controls, $p<0.01$). Weight changes were equivalent in both groups. Radiographically, the percent of mineralized new bone per gap was 59.5% in the vehicle group vs 38.8% in the rmTNF-α group, $p<0.001$. Histologically, the percent of endosteal (intercortical/marrow) bone formation per gap was 69.6% in the vehicle group vs 29.3% in the rmTNF-α group, $p<0.001$. Systemic delivery of rmTNF-α leads to prolonged, elevated circulating levels of serum TNF-α and is associated with inhibition of direct endosteal bone formation during DO. Supported by NIH grant AA12223.

1364 EFFECT OF OVARIECTOMY ALONE AND IN COMBINATION WITH CALCIUM/VITAMIN D-DEFICIENT DIET ON THE BONE MINERAL DENSITY IN CYNOMOLGUS MONKEYS.

M. Niehoff[1], S. Mohr[2] and G. Weinbauer[1]. *[1]Covance Laboratories GmbH, Muenster, Germany and [2]F.Hoffmann-La Roche Ltd., Basel, Switzerland.* Sponsor: P. Thomas.

Numerous studies have shown that estradiol, vitamin D and calcium play an important role in bone metabolism in primates. We used peripheral quantitative computed tomography (pQCT) yielding true volumetric density (mg/cm3) and separation of trabecular and cortical bone to investigate the effect of ovariectomy and/or calcium/vitamin D-deficiency in 22 female monkeys (>8 years of age). Assignments were: Group 1: control (standard diet); Group 2: ovariectomy and calcium/vitamin D-deficient diet; Group 3: ovariectomy and standard diet; Group 4: calcium/vitamin D-deficient diet. Bone mineral density (BMD) was measured in distal radius, proximal tibia and femur, lumbar vertebra and femoral neck before and 13, 26, 39, 52, 66 and 79 weeks after treatments. Overall, a decrease of BMD occurred in Groups 2 and 3, being more pronounced in Group 2. Total BMD decrease occurred in Week 13 in tibia, femur and lumbar vertebra in Groups 2 and 3 (approx. 5%) and in femoral neck of Group 2 (approx. 5%). Total BMD of the radius decreased in Week 26 by approx. 5% in Group 2 and by 5-10% in Group 3 whereas trabecular BMD decreased from Week 39 onwards for both groups by approx. 10%. The trabecular BMD decreased in the tibia by Week 13 and in the femur by Week 26 (approx. 6%). Cortical BMD of Groups 2 and 3 decreased in tibia and femur by approx. 3% in Week 52 and in lumbar vertebra by approx. 3% in Week 79 but BMD of radius and femoral neck remained unaltered. BMD in Groups 1 and 4 was unaltered. Unexpectedly, BMD in Group 4 increased slightly. These data indicate that a calcium/vitamin D-deficient diet may not accelerate ovariectomy-induced BMD loss but aggravates BMD loss in the long term. For the purpose of a primate osteoporosis study, a special diet does not provide an experimental advantage over ovariectomy alone.

1365 INHIBITION OF DIRECT BONE FORMATION ASSOCIATED WITH CHRONIC ETHANOL EXPOSURE IN A MOUSE MODEL OF DISTRACTION OSTEOGENESIS.

E. C. Wahl[2], L. Liu[2], D. S. Perrien[2, 1], J. Aronson[1, 2], W. R. Hogue[1], R. A. Skinner[1], M. Hidestrand[2], M. J. Ronis[1, 2], T. M. Badger[1, 2] and C. K. Lumpkin[1, 2]. *[1]University of Arkansas for Medical Sciences, Little Rock, AR and [2]Arkansas Children's Hospital Research Institute, Little Rock, AR.*

Excessive alcohol consumption has been reported to interfere with human bone homeostasis and repair in multiple ways. Previous studies have demonstrated that chronic ethanol (EtOH) exposure in the rat inhibits direct bone formation during

distraction osteogenesis (DO, limb lengthening). The opportunity to extend the rat EtOH studies into mice is now possible due to the development of mouse models of DO. Recent studies support the lack of confounding nutritional effects in the liquid EtOH diet mouse model. This study employed the liquid EtOH diet combined with the murine DO model to test the hypothesis that chronic EtOH exposure would result in deficits in direct bone formation during DO in contrast to the pair-fed controls. Twenty-eight C57BL/6 male 12-month-old mice were acclimated to the Lieber-DeCarli liquid control diet #710027 (Dyets Inc.) over a week's period. The mice were separated into two diet groups (n=14): control and EtOH (diet #710260). The control mice were pair-fed to the EtOH mice. The EtOH group started on 1% EtOH and were slowly increased to 6.45% EtOH over a period of 16 days. After being on diet for 82 days, all mice underwent placement of an external fixator and osteotomy on the left tibia. Following a six-day latency, distraction began at a rate of 0.075mm b.i.d. for 14 days. At sacrifice the left tibiae and contralaterals were harvested. The weight changes were equivalent for both groups. The hypothesis of ethanol toxicity (p= EtOH < control) was supported by radiographic (p=.011) and histologic (p=.002) analyses of the % new bone formation in the DO gaps, by pQCT analysis of the total vBMD of the contralateral proximal tibiae (p<.001) and femoral necks (p=.012), by 3 point bending on the contralateral tibiae (p<.001 energy to break), by pin site bone formation measures (p<.001), and by EtOH associated increased marrow fat percentages (p<.002). Supported by NIH grant AA12223.

1366 EFFECT OF PERCHLORATE ON DISPLACEMENT OF THYROXINE FROM SERUM BINDING PROTEINS AND BINDING OF PERCHLORATE TO SERUM PROTEINS.

J. L. Campbell[1], L. Narayanan[3], D. C. Ferguson[2], M. Mumtaz[4], H. El-Masri[4] and J. Fisher[1]. *[1]Interdisciplinary Toxicology Program, University of Georgia, Athens, GA, [2]Physiology and Pharmacology, University of Georgia, Athens, GA, [3]GeoCenters, Inc., Wright-Patterson AFB, Dayton, OH and [4]Division of Toxicology, ATSDR, Atlanta, GA.*

Perchlorate, a highly water soluble and widely dispersed water contaminant, exerts its primary mode of action by inhibiting the active uptake of iodide into the thyroid gland. In animals perchlorate has been shown to cause thyroid cancer, hypothyroidism, and possible neurodevelopmental defects. We examined another feasible mode of action for perchlorate based on reports that perchlorate could displace thyroid hormones from proteins. Under normal conditions most of the circulating thyroid hormones are protein bound in serum, thus conserving the thyroid hormones for use by the body. Serum binding of perchlorate was determined by dialyzing perchlorate, dissolved in physiological saline at concentrations of 0, 10, 50, 100, 200, 300, 400, 500, 1, 000 µg/l, against undiluted rat serum until the system was at equilibrium. Both the dialysate and deproteinated serum were analyzed for perchlorate by HPLC. Displacement of thyroxine from serum proteins was investigated by spiking rat or human serum with ^{125}I-thyroxine followed by perchlorate at concentrations of 0, 1, 10, 50, 100, 200, 300 mg/l and then determining the amount of free thyroxine by dialyzing the serum against phosphate buffer and then determining the amount of ^{125}I-thyroxine in the dialysate and serum with a gamma counter. In the rat, approximately 100% of the mass of perchlorate added was bound to serum constituents for concentrations up to 100 µg/l, followed by a rapid decline in the bound fraction from 100 µg/l to 500 µg/l where only 0.355% was bound. Over the range of concentrations tested, perchlorate did not significantly alter the percent of thyroxine bound to serum proteins for either rat (p=0.1302) or human (p=0.9610). Displacement of thyroxine from serum proteins is not an imporatant mechanism of action for perchlorate induced thyroid disturbances. Funded by ATSDR (Grant No: U61/ATU472105-01).

1367 ENHANCED RAT HERSHBERGER ASSAY APPEARS RELIABLE FOR DETECTION OF NOT ONLY (ANTI-) ANDROGENIC CHEMICALS BUT ALSO THYROID HORMONE MODULATORS.

T. Yamada, T. Kunimatsu, K. Miyata, S. Yabushita, T. Sukata, S. Kawamura, T. Seki, Y. Okuno and N. Mikami. *Environmental Health Science Laboratory, Sumitomo Chemical Company, Ltd.,, Osaka, Osaka, Japan.*

Development of an internationally recognized standard for the Hershberger assay as a screening tool to detect potential (anti-)androgenic chemicals is in progress. In the present preliminary study, we evaluated the reliability and specificity of the enhanced Hershberger assay to detect thyroid hormone modulating activity, while concentrating attention on possible confounding influence on evaluation of (anti-)androgenic activity. Castrated or testosterone propionate (TP, 0.2 or 0.25 mg/kg/day)-injected castrated male Crj:CD(SD)IGS rats (7 weeks old) were dosed for 10 days by oral gavage with vehicle (corn oil) or the following chemicals (mg/kg/day): propylthiouracil (PTU, 2.5), a potent inhibitor of thyroid hormone synthesis, phenobarbital and 2, 2-bis(4-chlorophenyl)-1, 1-dichloroethylene (PB, 125; and *p, p'*-DDE, 100), two hepatic enzyme inducers that enhance the clearance of thyroid hormones. PTU markedly increased thyroid weights, and decreased serum T3 and T4, and increased serum TSH, also causing marked microscopic alteration of the thyroid gland. PB and *p, p'*-DDE treatment was also associated with similar findings but less pronounced. The alterations appeared to be equally induced irrespective of the presence/absence of TP. Furthermore, data for *p, p'*-DDE demonstrated anti-androgenic effects as documented in our earlier publication, whereas PTU and PB had no effects on the weights of androgen-related accessory glands/tissues (the ventral prostate, dorso-lateral prostate, seminal vesicles with coagulating glands, glans penis, Cowper's glands, and *levator ani plus bulbocavernosus* muscles). Although further investigations, including dose-response studies, should be performed, the present study provides evidence that the enhanced Hershberger assay, with evaluation of thyroid histopathology and weights, and hormone levels, is reliable for screening for not only (anti-)androgenic chemicals but also thyroid hormone modulators.

1368 HIGH-THROUGHPUT REPORTER GENE TRANSCRIPTION ASSAY FOR THE DETERMINATION OF THYROID HORMONE DISRUPTING EFFECTS OF FOOD ADDITIVES OR CONTAMINANTS.

J. Cho, S. Jeong and J. Park. *Toxicology Division, National Vet. Res. Quarant. Service, Anyang City, South Korea.*

Thyroid hormonal system is very important for the regulation of metabolism, growth and reproductive organs development. Thyroid hormones (T3 and T4) bind to thyroid receptor or retinoic acid receptor (RXR) and then the complexes are located to thyroid hormone responsive elements (TREs) that induce thyroid hormonal activity. In this study, recombinant human uterus carcinoma cells (HELA) express TREs and luciferase reporter genes were used to determination of thyroid hormone disrupting effects of chemicals. The limit of response of this recombinant cell to T3 and T4 were 6.5 fg and 8.9 fg, respectively. 14 compounds of food additives or contaminants were investigated on the thyroid hormone disrupting effects using the recombinant HELA cells. Ethoxyquin, sorbic acid, genisteine and tetrabromodiphenyl ether (TBDE) induced transcription activity with dose-responsive pattern. In addition, butylated hydroxytoluene (BHT), butylated hydroxylanisole (BHA), ethoxyquin, zearalane, genisteine, and TBDE showed agonistic reaction with T3 while cadmium showed antagonistic reaction in the study of agonistic and antagonistic transcription assay. These results demonstrate that reporter gene transcription assay using recombinant HELA cells can be recognized as high-throughput bioassay system for the detection of thyroid hormone disruptors.

1369 CHANGES IN SERUM TSH LEVEL AND T4/T3 RATIO IN DEVELOPMENTAL TOXICITY STUDIES OF PERCHLORATE IN RATS FED HIGH-IODINE DIETS REFLECT ADAPTIVE INCREASES IN IODIDE UPTAKE NOT RELEVANT TO HUMANS.

G. Goodman. *Intertox, Inc., Seattle, WA.*

The T4/T3 ratio is the most stable measure of thyroid hormones (TH) in the fetal and postnatal rat; T4 and T3 are changing rapidly but their ratio is highly regulated. A research study in pregnant rats fed an iodine-restricted diet with perchlorate added at > 3.6 mg/kg-day found a lower T4/T3 ratio and an order-of-magnitude higher TSH level in both maternal and fetal serum compared to controls (Schroeder-van der Elst et al., Endocrinology 142: 3736-3741, 2001). These results conform to the pattern observed in both rats and humans when iodide uptake is completely blocked or dietary iodine is severely deficient. A quite different pattern emerged in rats fed a high-iodine diet (ordinary lab chow) in subchronic and developmental toxicity studies of perchlorate performed for regulatory purposes (USEPA 2002): an *unchanged or higher* T4/T3 ratio (calculated in the present analysis) and an *unchanged or only slightly higher* TSH. The effects were extremely inconsistent between studies and across groups, suggesting an external variable such as iodine. Importantly, the effects were found at doses for which (in other studies in rats given a high-iodine diet) upregulation of the sodium-iodide symporter leads rapidly to full compensation for inhibition of thyroidal iodide uptake (Yu et al. 2000). Nevertheless, the observed changes in hormone levels were interpreted by USEPA as evidence of an adverse effect at the lowest dose tested (0.01 mg/kg-day). To the contrary, the changes were similar in pattern and magnitude to those found in iodine-sufficient rats and humans given supplemental dietary iodine in excess; they are best explained as being secondary to the compensatory increase in thyroidal iodide uptake in rats that occurs when the uptake is pharmacologically inhibited. Humans exposed to perchlorate at doses as high as 0.5 mg/kg-day do not upregulate iodide uptake. The adaptive hormone changes observed at lower doses in the regulatory studies in rats are irrelevant to humans and should not be interpreted as adverse for risk assessment purposes.

1370 MARGINAL IODINE DEFICIENCY EXACERBATES PERCHLORATE THYROID TOXICITY.

P. C. Das[1], J. M. Hedge[2], D. C. Wolf[2] and K. M. Crofton[2]. *[1]Curriculum In Toxicology, UNC, Chapel Hill, NC and [2]NHEERL, ORD, USEPA, Research Triangle Park, NC.*

The environmental contaminant perchlorate disrupts thyroid homeostasis *via* inhibition of iodine uptake into the thyroid. This work tested whether iodine deficiency exacerbates the effects of perchlorate. Female 27 day-old LE rats were fed a custom iodine deficient diet with 0, 50, or 75% iodine deficiency for 28 days. After 14 days on the diet 0, 31, or 250 mg/L perchlorate were provided in drinking water for 14 days. Serum total T4, T3, and TSH were assayed by RIA, and standard histopathology was performed on thyroid. Neither the 50% deficient diet nor 31 mg/L perchlorate produced effects when given alone, but together reduced T4 ~35%, and elevated TSH ~5- fold. A 75% deficient diet plus 250 mg/L perchlorate also caused additive effects on T4 and T3. TSH was induced ~14-fold. Follicular cell hypertrophy was present in all treated rats, but was more severe with both treatments than either alone. Colloid depletion was present only after perchlorate treatment but not iodine deficiency. The marginal iodine deficient rat could represent a model for a sensitive sub-population for studying disruptions in thyroid homeostasis. *This is an abstract of a proposed presentation and does not necessarily reflect University.S.EPA policy.*

1371 AN ADDITIVE EFFECT OF A MIXTURE OF AMMONIUM PERCHLORATE AND SODIUM CHLORATE ON THE PITUTARY-THYROID AXIS IN MALE F344 RATS.

M. A. Khan[1, 2], S. E. Fenton[3], A. E. Swank[1], G. W. Knapp[1], S. D. Hester[1] and D. C. Wolf[1]. *[1]Environmental Carcinogenesis Division, USEPA, Research Triangle Park, NC, [2]NRC, Research Triangle Park, NC and [3]Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.*

Humans are exposed to mixtures of chemicals through drinking water. Ammonium perchlorate (AP) and Sodium chlorate (SC) are present in public drinking water supplies in many parts of the US. AP is used as an oxidizer in solid rocket propellants, munitions, fireworks, and air bag inflators. SC is a drinking water disinfection by-product of chlorine dioxide and is also used as an oxidizing agent in industrial processes. Exposure to either of these chemicals causes perturbations in pituitary-thyroid homeostasis. AP and SC competitively inhibit iodide uptake preventing the synthesis of thyroglobulin, thus reducing circulating T4. Persistent long-term exposure to either of these chemicals can result in persistent stimulation of the pituitary-thyroid axis resulting in thyroid tumor development in rats. The present study was designed to describe the potential of an additive effect in rats following short-term exposure to both chemicals. Adult male F344 rats were exposed to AP (0.1, 1.0, 10 mg/L) or SC (10, 100, 1000 mg/L) or their mixtures through the drinking water for 7 days. Serum and tissues were collected for biochemical, histological, and molecular endpoints. Serum T4 was decreased ($p < 0.05$) in rats exposed to the mixtures, but not to the individual chemicals. Serum T3 levels were unaffected by each treatment and TSH was only elevated in the high dose chlorate group. Histological examination of the thyroid gland showed, colloid depletion and significant ($p < 0.05$) hypertrophy of follicular epithelial cells in all the single chemical and mixture treated animals while hyperplasia was observed only in AP 10 + SC 10, AP 0.1 + SC 1000, and AP 10 + SC 1000 mg/L mixture groups. The data suggest that the combined exposure to chlorate and perchlorate results in an additive effect on the rat thyroid. This abstract does not reflect the policies and opinions of the USEPA.

1372 CUMULATIVE EFFECTS OF ENDOCRINE DISRUPTERS (EDCS): SYNERGY OR ADDITIVITY.

L. E. Gray[1], J. Ostby[1], J. Furr[1], C. Lambright[1], A. Hotchkiss[2] and V. S. Wilson[1]. *[1]ORD, NHEERL, RTD, EB, USEPA, Research Triangle Park, NC and [2]Psychology, Ohio State University, Columbus, OH.*

Several EDCs have been shown to disrupt sex differentiation by interfering with androgen signaling. Vinclozolin (V) and procymidone (P) are androgen receptor (AR) antagonists while some phthalate esters (PE) inhibit fetal testis hormone production. Linuron (L) and prochloraz act as AR antagonists and by inhibiting steroid hormone production. Of the above EDCs, only PEs reduce insl3 gene expression required for gubernacular development. Although risk assessments are typically conducted on a chemical-by-chemical basis, the 1996 FQPA mandated that the USEPA consider cumulative risk from chemicals that act *via* a common mechanism. Our studies begin to provide a framework for assessing the cumulative effects of "antiandrogenic" EDCs. SD rats were dosed orally on days 14-18 of gestation with EDCs singly or in pairs at dosage levels equivalent to about 50% the ED50 for hypospadias including 1) two AR antagonists (V plus P, each at 50 mg/kg/d), 2) two PEs with a common metabolite (DBP and BBP, each at 500 mg/kg/d), 3) two PEs with different metabolites (DEHP and DBP (500)), 4) a PE plus an AR antagonist (DBP (500) plus P(50)), and 5) L (75 mg/kg/d) plus BBP (500). We expected that V, P, L, DBP, DEHP and BBP individually would not induce hypospadias using this dosing regimen, but mixing any two together would induce hypospadias in about 50% of the males and induce cumulative effects on other androgen-dependent organs. In the current study, all combinations produced cumulative effects on every androgen-dependent tissue. However, only the PE combinations caused agenesis of the insl3-dependent gubernacular ligaments. We found that EDCs that alter differentiation of the same reproductive tissues during sexual differentiation produce cumulative, apparently dose-additive effects when combined. However, the relative potency factors vary from tissue to tissue based upon the mechanism and mode of toxicity of each chemical. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1373 MALFORMATIONS IN GUBERNACULAR LIGAMENT DEVELOPMENT INDUCED BY DEHP, DBP, AND BBP ARE ASSOCIATED WITH DECREASES IN INSL3 GENE EXPRESSION IN THE FETAL RAT TESTIS.

V. S. Wilson, C. Lambright, J. Furr, C. Wood, G. Held and L. E. Gray. *Reproductive Toxicology Division, USEPA, ORD, NHEERL, Research Triangle Park, NC.*

Targeted inactivation of the insulin-like hormone 3 (insl3) gene in male mice results in altered gubernacular development, disrupted testis decent, and cryptorchidism. Cryptorchidism is a fairly common human malformation, being displayed in about 3 males per 100 at birth, but only a small percentage has been linked directly to genetic defects. Recently, concern has arisen over the apparent increase in male reproductive health problems and the potential role of endocrine disrupting chemicals in the etiology of these conditions. The phthalate esters (PE) are high production volume, ubiquitous environmental chemicals, some of which alter sexual differentiation, inducing gubernacular agenesis and other male rat reproductive tract malformations. We hypothesized that phthalate-induced gubernacular lesions likely result from an inhibition of Leydig cell insl3 gene expression. Three phthalates, di-n-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP) and benzyl butyl phthalate (BBP) were administered orally to the dam (750 mg/kg/day) on gestation day (GD) 14 through 18 and the fetal testes examined on GD18 for effects on steroid hormone production and insl3 gene expression. Compared to chemicals like vinclozolin, linuron, and prochloraz that act as AR antagonists and/or inhibit fetal Leydig cell testosterone production, only the three phthalates significantly reduced both *ex vivo* testosterone production and insl3 gene expression when quantified by real-time rtPCR. Dose response studies with DEHP (0, 100, 300, 600 or 900 mg/kg/day) also showed a dose dependent decrease in both testicular testosterone production and insl3 message levels in the GD18 rat fetus. These results provide the first demonstration of dose dependent PE-induced alteration of insl3 mRNA in the fetal male rat testis. Disclaimer: Abstract of a proposed presentation and does not necessarily reflect EPA policy.

1374 DI-BUTYL PHTHALATE ACTIVATES THE NUCLEAR RECEPTORS CAR AND PXR AND ENHANCES THE EXPRESSION OF CYP 2B1 AND 3A1 IN MATERNAL AND FETAL LIVER IN THE RAT.

L. You[1], M. Wyde[1], S. Kirwan[1], A. Laughter[1], E. Bartolucci-Page[1], K. Gaido[1] and B. Yan[2]. *[1]CIIT Centers for Health Research, Research Triangle Park, NC and [2]Department of Biological Sciences, University of Rhode Island, Kingston, RI.*

The plasticizer di-butyl phthalate (DBP) is a reproductive toxicant in rodents at high doses. Exposure to DBP *in utero* causes alterations in sexual and reproductive development in male rats as a result of inhibition of testicular steroidogenesis during sexual differentiation. DBP is also known to alter expression of hepatic enzymes. This study was to delineate the effects of DBP exposure during gestation on the expression of enzymes in the maternal and fatal liver that are involved in the metabolism of steroid hormones. Pregnant Sprague-Dawley rats were dosed with DBP through gavage in the dose range of 10 to 500 mg/kg/day from gestation day 12 to 19. Maternal and fetal liver samples were collected approximately 2 hrs after the last dosing. Protein and mRNA levels of CYP 2B (testosterone 16β-hydroxylase), CYP 3A (testosterone 6β-hydroxylase), and CYP 4A were increased in both the maternal and fetal liver tissue in the 500 mg dose group, but not in the groups of 50 mg or lower. The high-dose DBP treatment also caused increase in the mRNA of hepatic estrogen sulfotransferase and UDP-glucuronosyltransferase 2B1 in the dams, but not in the fetuses. *In vitro* transcriptional activation assay showed that DBP, but not its metabolite mono-butyl phthalate, was able to activate the nuclear receptor pregnane X receptor (PXR). DBP by itself did not increase the activity of the constitutive androstane receptor (CAR), but it did activate androstanol-repressed CAR. These data indicate that maternal and fetal liver enzymes are susceptible to DBP induction during pregnancy. Such effects on enzyme expression are likely mediated through DBP interactions with the nuclear receptors that tran-

scriptionally regulate these liver enzymes. While these enzymes are known to participate in controlling steroid bioavailability, the potential role of their altered expression in DBP-caused reproductive toxicity remains to be understood.

1375 EFFECTS OF GESTATIONAL PROCHLORAZ ADMINISTRATION ON MALE REPRODUCTIVE DEVELOPMENT IN RATS. *IN VIVO* ASSESSMENTS OF A FUNGICIDE WITH MULTIPLE *IN VITRO* EFFECTS.

N. Noriega, J. Ostby, C. Lambright, V. S. Wilson and E. Gray. *USEPA, Research Triangle Park, NC.*

Prochloraz (PZ) is an imidazole fungicide that shows multiple endocrine activities, including androgen receptor (AR) antagonism and inhibition of testosterone and estradiol synthesis *via* reductions in P450 activity. In the current study, we confirmed in-vitro reports that PZ is an AR antagonist, and described PZ effects on male sex differentiation. PZ concentrations above 1μM caused a dose-dependent inhibition of DHT (0.1nM)-induced AR-mediated activity in cells (MDA-kb2) containing endogenous AR and stably expressing the MMTV-luc reporter. PZ inhibited androgen (1nM R1881) binding to the rat AR (IC_{50} approx 60μM). *In vivo*, pregnant Sprague-Dawley rats were gavaged daily from GD 14-18 at PZ doses of 31.25, 62.5, 125 and 250 mg/kg bodyweight/day. Pup delivery was delayed in a dose-dependent manner and resulted in stillbirths at the two highest doses. In male pups, AGD was reduced at birth but was highly correlated with reduced bodyweight. Female-like areolas were observed in PZ-treated 13 day old males at frequencies ranging from 31% in the low-dose group, to 71% at the high dose. Males in the two highest dose groups showed reduced androgen-dependent tissue weights (testis, epididymis, seminal vesicle, ventral prostate and levator ani + bulbocavernosus) and 40% of high-dose males had vaginal pouches. All high-dose males and some in the 125 mg/kg treatment group showed permanent phallus abnormalities including hypospadias. In animals without malformations, PZ did not affect age at preputial separation. PZ appears to affect sexual differentiation *via* two independent mechanisms; steroidogenesis inhibition (similarly to other antifungal imidazoles) and AR antagonism. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1376 STEROID AND THYROID HORMONAL RECEPTOR GENE TRANSCRIPTION ASSAY AND ONE-GENERATION REPRODUCTION STUDY OF CHLORPYRIPHOS-METHYL.

S. Jeong, B. Kim, S. Kim, J. Cho and J. Park. *Toxicology Division, National Vet. Res. Quarant. Service, Anyang City, South Korea.*

Chlorphyriphos-methyl (CPM) has been widely used as an organophosphate insecticide especially useful in controlling foliage crop pests and household insects. It is classified as suspected carcinogen and endocrine disrupter. We investigated endocrine disrupting effects of CPM in steroid and thyroid hormone receptor gene transcription assay system and in rat one-generation reproduction study. CPM induced weak transcription activity by binding with estrogen, androgen or progesterone receptor as induction ratio (IR) compared to β-estradiol, testosterone, and progesterone was 1.48% at 10-8 M, 1.50% at 10-8 M and 1.39% at 10-6 M, respectively. While it did not induce any apparent transcription activity or agonistic reaction with T3 in thyroid receptor transcription assay. In one-generation reproduction study, CPM (1, 10, 100 mg/kg BW) induced increase of relative organ weight of liver, kidney, adrenal gland and brain in F0 generation. Also, estradiol and testosterone content in serum of female rats were increased while estradiol in serum of male rats was decreased. Lowering of mating rate, sperm head width and T4 in serum were observed in F0 rats. In F1 rats, increase of organ weights of liver, kidney, spleen, adrenal gland and brain and decrease of that of prostate gland were observed. Sex ratio of male rats was higher but number of implantation, living births, anogenital distances, sperm number and estradio1 and testosterone content in serum were lower than control. Conclusively, CPM is a disruptor of steroid hormonal system *in vitro* and *in vivo* study and NOAEL and ADI of CPM according to the results from one-generation reproduction study were estimated as 1 mg/kg B.W. and 0.01 mg/kg B.W./day, respectively.

1377 EFFECTS OF PRENATAL EXPOSURE TO PCB METABOLITES 4-OH-CB 107 AND 4-OH-CB 187 ON ENDOCRNE STATUS AND REPRODUCTIVE CYCLE OF THE FEMALE RAT.

C. Buitenhuis[1], P. Cenijn[1], A. Bergman[2], A. Gutleb[1], J. Legler[1] and A. Brouwer[1]. *[1]Institute for Environmental Studies (IVM), Vrije Universiteit, Amsterdam, Netherlands and [2]Department of Environmental Chemistry, Stockholm University, Stockholm, Sweden.*

Recently, hydroxylated metabolites of organohalogenated compounds have been identified in human and cord blood. The major aim of this study was to determine the long-term consequences of low-level prenatal exposure to hydroxylated polychlorinated biphenyls (PCBs) on brain development, reproduction and endocrine status of the rat. Pregnant rats were orally dosed with hydroxylated PCBs during gestation day 10-16. The major human plasma PCB metabolites 4-OH-CB 107 (0.1, 0.5 and 5 mg/kg/day) and 4-OH-CB 187 (0.5 and 5 mg/kg/day) were chosen as test compounds. Starting on postnatal day (PND) 1, offspring were scored for developmental landmarks including onset of hair grow, pinna detachment, bilateral eye opening, vaginal opening and preputial separation. Endocrine endpoints were measured, such as thyroid hormone- and estrogen levels. Female rats were monitored daily for estrous cycle length during 3 different periods in the experiment. The first period was directly after vaginal opening (PND 30-68), the second between PND 150-170 and the last between PND 210-230. No effects of the PCB metabolites were observed on developmental landmarks. A marked reduction in plasma total thyroxin was found at PND 4 in male and female offspring following prenatal exposure to 0.5 mg/kg/day of 4-OH-CB 107 and 0.5 mg/kg/day 4-OH-CB 187. Analysis of the estrous cycle showed a prolongation the estrous cycle length in the high dose groups of 4-OH-CB 107 only in the last period of estrous cycle monitoring. This suggests an aging effect of prenatal exposure to hydroxylated PCB metabolites. Coinciding with this increase in estrous cycle length, a 250% increase in plasma estradiol concentrations in the same female rats was found, implying involvement of sex steroid hormones in prolongation of the estrous cycle. This work has been supported by the EU, contract no. QLK4 2000-00261.

1378 THE CHARACTERIZATION AND HORMONAL REGULATION OF KIDNEY ANDROGEN-REGULATED PROTEIN (KAP)-LUCIFERASE TRANSGENIC MICE.

S. Malstrom[1], A. F. Purchio[2] and D. B. West[1]. *[1]LPTA, Xenogen Corporation, Alameda, CA and [2]Xenogen Corporation, Alameda, CA.*

Transgenic mice expressing luciferase (luc) under the control of the kidney androgen regulated protein (Kap) promoter were characterized. Kap-luc expression was observed at high levels in kidneys, epididymies, testes and seminal vesicles in male mice; and in kidneys, ovaries and uterus in female mice. Kap-luc expression was modulated by androgen and anti-androgen treatment in male and female mice. Male mice were treated daily with the anti-androgenic compound, cyproterone acetate, flutamide or vehicle for 16 days. Endpoints evaluated included *in vivo* biophotonic imaging, body weight, organ weights (liver, kidney, testes, epididymies, preputial gland and seminal vesicles) and protein luciferase assays. Biophotonic images were acquired from each animal throughout the experiment using a sensitive imaging system. These imaging results were correlated with traditional endpoints of body and organ weights. Following treatment with anti-androgens, luciferase signal significantly decreased in the intact male mouse *in vivo* and by measuring luciferase activity in homogenized organ extracts. The decrease in epididymal and seminal vesicle weight confirmed the action of the anti-androgens. *In vivo* imaging documented significant changes in luciferase expression within the first few days of the experiment, indicative of the anti-androgenic activity of the drugs. Testosterone treatment significantly increased the Kap-luc bioluminescent signal in female mice. Biophotonic imaging may provide a useful approach for tracking noninvasively the effects of endocrine disruptors in specific tissues.

1379 AIRWAY HYPERRESPONSIVENESS AND PULMONARY INFLAMMATION IN PULMONARY HYPERTENSIVE RATS EXPOSED TO CONCENTRATED AMBIENT PARTICLES.

T. Cheng[1], Y. Lei[1], M. Chen[1], C. Chan[1] and P. Wang[2]. *[1]Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, Taipei, Taiwan and [2]Institute of Environmental Engineering, National Central University, Chung Li, Taiwan.* Sponsor: T. Ueng.

Epidemiology studies have shown associations between particulate air pollution and exacerbation of respiratory disease, but the exact biological mechanism remains unclear. In this study, we exposed pulmonary hypertensive rats to concentrated ambient particles (CAPs) using particle concentrator to examine airway hyperresponsiveness and inflammation. This study was conducted at an EPA supersite located at a traffic busy urban area nearing Taipei. Exposure group (n=5) was exposed to CAPs for 6 hrs each day for 3 consecutive days (mean mass concentration = 371.7 μg/m3), and control group (n=6) was exposed to HEPA filtered air. Airway responsiveness was measured as enhanced Penh using whole-body barometric plethysmography (BUXCO, NY) before and after PM exposure. We also controlled temperature, humidity and the restrictive stress resulted from nose-only exposure chambers when pulmonary function test was performed. Bronchoalveolar lavage fluid (BALF), lung tissue and peripheral plasma were collected 48hr after the experiment. Our results showed airway responsiveness difference of Penh with methacholine challenge measured before and after experiment were higher for CAPs than that for filtered air group. Additionally, BALF neutrophils and total protein were increased in CAPs group. Elevated plasma 8-OHdG and depletion of total GSH in

the lung tissue were also observed in CAPs group, although it did not reach a statistic significance. Our findings indicate that potential confounders need to be controlled when we use whole-body barometric plethysmography. We conclude that CAPs could induce airway hyperresponsiveness and increase lung inflammation and injury in pulmonary hypertensive rats after exposed to concentrated ambient PM.

1380 RESPONSES TO SUBCHRONIC INHALATION OF DIESEL EXHAUST (DE) AND HARDWOOD SMOKE (HWS) MEASURED IN RAT BRONCHOALVEOLAR LAVAGE FLUID.

J. Seagrave[1], S. K. Seilkop[2] and J. L. Mauderly[1]. *[1]Lovelace Respiratory Research Institute, Albuquerque, NM and [2]SKS Consulting, Siler City, NC.*

Air pollution exposure is associated with adverse health effects, but the components and mechanisms that cause these effects are unclear. As part of a larger program to develop a database to correlate health effects with chemical constituents of various types of air pollution, we exposed male (M) and female (F) F344 rats to DE or HWS. Exposure conditions were 0 (filtered air), 30, 100, 300, or 1000 μg/m^3 of TPM, 6 h/d, 7 d/w. After 6 mo, the rats were killed and lungs were removed. The right lung was lavaged with PBS. Cell-free lavage fluid was assayed for lactate dehydrogenase (LDH), alkaline phosphatase (APase), βglucuronidase (βgluc), MIP-2, IL-1β, TNFα, and total glutathione (GSH). Statistical analyses included pair-wise comparisons with control and exposure-related trends. Most effects were mild, but the largest effects often occurred at sub-maximal exposures. LDH increased in HWS-exposed M and DE-exposed M and F. βGluc decreased in HWS-exposed M and F and DE-exposed M. MIP-2 in HWS-exposed M and F and DE-exposed F showed exposure-related decreases. In contrast, APase showed a statistically insignificant increase in DE-exposed F, but decreased in HWS-exposed M and F. TNFα levels decreased in DE-exposed M and F, but HWS-exposed M showed some evidence of an increase. In F, HWS significantly decreased GSH but DE weakly increased it, while in M, DE caused little change, but 100 μg/m^3 HWS increased GSH with a trend to decrease from the peak response at higher doses. These results show that these two combustion emissions may differentially affect various types of responses in the lung, and that effects may be non-monotonic functions of exposure levels. This work is supported by the National Environmental Respiratory Center, which is funded by numerous industry, state, and federal sponsors, including the USEPA, US Department of Energy (Office of Freedom CAR and Vehicle Technologies), and US Department of Transportation. This work does not represent the views of any sponsor.

1381 TOXICOLOGICAL ASSESSMENT OF DIESEL-WATER EMULSION [PURINOX™ (SUMMER FUEL BLEND)] EXHAUST EMISSIONS.

R. Kraska[1], E. Barr[2], I. Daly[3], R. Gudi[4], J. D. McDonald[2], M. Mercieca[5], D. Naas[8], J. O'Callagan[6], N. Ronsko[1], S. Seilkop[7], V. Wagner[4] and M. D. Reed[2]. *[1]Lubrizol Corporation, Wickliffe, OH, [2]Lovelace Respiratory Research Institute, Albuquerque, NM, [3]Regulatory and Technical Associates, Lebanon, NJ, [4]BioReliance, Rockville, MD, [5]Pathology Associates, Frederick, MD, [6]CDC/ NIOSH, Morgantown, WV, [7]SKS Consulting, Silver City, NC and [8]AccuTox Consulting, Midland, MI.*

A type of fuel shown to decrease combustion emissions vs. traditional diesel is the diesel-water emulsion. Utilizing a collaborative approach, a toxicological assessment of the exhaust of PuriNOx™ fuel emulsion was recently conducted. Whole exhaust from one of two, 2001 model Cummins 5.9L ISB engines was diluted to exposure levels of 100, 200, and 400 μg particulate matter (PM) /m^3. The engines were operated on a repeating EPA heavy-duty certification cycle. F344 rats were housed in Hazleton H2000 exposure chambers and exposed to exhaust 6 h/day, 5 days/wk for the first 11 weeks and 7 days a week thereafter. Exposure days ranged from 58-70 days. Exposure- start was staggered to account for multiple health endpoints. Toxicological assays were conducted during and following exposures and subsequent to a recovery period. General toxicity (body weight, organ weight, clinical pathology, and histopathology), neurotoxicity (glial fibrillary acidic protein assay, GFAP), genotoxicity (Ames, micronucleus, sister chromatid exchange), and reproduction and development were assessed. Effects observed were mild. Exhaust was not associated with neurotoxicity, reproductive/developmental toxicity, or *in vivo* genotoxicity. Small decreases in serum cholesterol and small increases in platelet values were treatment related. PM accumulation within alveolar macrophages was noted in all exposure groups. Other sporadic statistically significant responses were observed but not clearly treatment related. Exhaust subfractions induced mutagenic responses in S. typh. Based on the cholesterol and platelet results, the 100 μg/m^3 exposure level was the NOAEL. In general, these observations were consistent with rodent and bacteria exposure to petroleum diesel exhaust.

1382 USING TOXICOLOGY TO PREDICT THE HEALTH EFFECTS OF DIESEL PARTICLE MATTER (DPM) IN THE University.S.

L. C. Green, M. Ames and E. Crouch. *Cambridge Environmental, Cambridge, MA.*

Background and methods: Diesel particle matter (DPM) is a complex, well-studied mixture. We used exposure-response information from rodent bioassays of DPM to estimate the health benefits of a proposal to reduce non-road DPM emissions. In rats, the most sensitive response to chronic DPM exposures is lung inflammation: the log-probit exposure-response has an EC50 of 3.1 mg DPM/m3 "human equivalent concentration" and a standard de*via*tion of a factor of 3.2. We applied this relationship to US populations with extreme occupational or average ambient exposures, and compared our results to the non-toxicologic analysis performed by USEPA. Results: About 8, 000 underground, non-coal miners in the US are exposed to 8-hr DPM levels of up to 2 mg/m3. Applying the inflammation exposure-response curve to these miners yields an expected occurrence of 13 lung effects. Enforcement of EPA's proposed non-road diesel engine rule would reduce airborne DPM concentrations in mines by some 56% and the expected prevalence of lung effects to 1 in 8, 000. In contrast, ambient, non-occupational concentrations of DPM in the US average < 0.001 mg/m3. Such small ambient exposures yield an estimated 0.0004 (i.e., 0) lung effects in the US population as a whole, so that the non-road diesel rule is expected to have no effect on morbidity or mortality in the general population. Disregarding the toxicology on DPM, USEPA applied "health damage functions" from selected observational epidemiologic studies of total PM2.5 to assess the health benefits of the proposed rule. EPA thus estimated that reducing ambient DPM levels by 0.00046 mg/m3 would avert 9, 600 premature deaths each year in the US, and would substantially reduce morbidity. Conclusions: The toxicology on DPM predicts some morbidity and no mortality due to current DPM exposures to underground miners, and no morbidity or mortality for the general US population. In contrast, extrapolating selected observational epidemiologic data on PM2.5 predicts substantial morbidity and mortality from current ambient DPM exposures. Both approaches cannot be correct.

1383 INHALATION OF DIESEL EXHAUST AFFECTS CALCITONIN GENE-RELATED PEPTIDE (CGRP) DENSITY IN F344 RATS.

S. S. Wong[1], I. M. Keith[2], N. N. Sun[1], C. Kweon[3], J. J. Schauer[4], D. E. Foster[3], R. Lantz[1] and M. L. Witten[1]. *[1]Center for Toxicology, The University of Arizone, Tucson, AZ, [2]School of Veterinary Medicine, The University of Wisconsin, Nadison, WI, [3]Engine Research Center, The University of Wisconsin, Madison, WI and [4]Wisconsin College of Engineering & State Laboratory of Hygiene, The University of Wisconsin, Madison, WI.*

CGRP is a neuropeptide of sensory C-fibers with proposed pro-inflammatory properties. To characterize the mechanism underlying diesel exhaust (DE)-induced neurogenic inflammation, female Fischer 344 rats (~175g, 4-weeks old) were randomly assigned to 6 groups in a 2 X 3 factorial design [Capsaicin vs. non-capsaicin (vehicle) pretreatment to stimulate C-fiber and room air vs. low and high levels of DE]. The rats were then exposed nose-only to DE at 0 (filtered room air), 35.3, and 669.3 μg/m3 particulates directly from a Cummins N14 research engine run at 75% throttle for 4 hours/day, 5 days/week for 3 weeks. Immunohistochemical data indicated that airway and alveolar parenchyma of rats exposed to the high level of DE appeared to have more CGRP fibers than their controls. More alveolar CGRP-containing neuroendocrine cells were also observed, which were accompanied by plasma extravasation increase and inflammatory response. Histological evidence showed that activation of mast cells and alveolar macrophages was associated with CGRP. However, capsaicin pretreatment did not significantly attenuate DE-induced changes in the CGRP profile in the lungs. This finding suggests that DE-induced inflammatory responses may be mainly attributed to CGRP-containing neuroendocrine cells. Moreover, the efforts were made to determine the physical aspects and the chemical composition of DE particles that induce the deleterious effects. The results showed that the neurogenic response highlighted above may possibly be related to the particle number, volume, and mass concentration as well as its carbon and sulfate contents, rather than particle sizes or the trace metals (Na, Mg, Ca, Fe, Cr, Mn, and Ph) measured in this study. (Supported by Health Effects Institute).

1384 SURFACE AREA AS DETERMINANT OF ULTRAFINE PARTICLE-INDUCED OXIDATIVE STRESS.

Y. Lei and T. Cheng. *Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University, Taipei, Taiwan.* Sponsor: T. Ueng.

Epidemiological studies have shown that exposure to particulate matters (PM) is associated with increasing cardiopulmonary morbidity and mortality. Recent studies further suggest that ultrafine fraction of PM may play an important role in car-

diopulmonary toxicity. It has been proposed that surface area of ultrafine PM is more closely associated with PM-related toxicity. In this study, we investigated the effect of ultrafine polystyrene particles on inflammation and oxidative stress markers in pulmonary hypertensive rats to determine whether surface area of ultrafine polystyrene particles play an important role in ROS generation in diseased animals. Each exposed group (n=5) were treated with ultrafine polystyrene in the combination of three sizes (64, 202 and 535 nm) and two concentrations (100 and 50 μg/ml) using intratracheal instillation. Control group (n=4) received PBS. Bronchoalveolar lavage fluid (BALF), lung tissue and peripheral plasma were collected 24hr after experiment. The results showed that instillation with 100 and 50μg/ml of ultrafine polystyrene particle can induce increased total cells, neutrophil proportion, total protein and LDH compared to fine polystyrene particle at same mass concentration in pulmonary hypertensive rats. We also observed that ultrafine polystyrene induced significant higher proinflammatory cytokine IL-6 and TNF-α as compared to fine particles. Furthermore, the result revealed that the depletion of total GSH in the lung tissue and formation of plasma 8-OHdG were associated with total surface area of particles instilled. In conclusion, we found inert ultrafine polystyrene may cause lung inflammatory effects on pulmonary hypertensive rats, and the formation of ROS was associated with instilled total surface area of ultrafine PM. The exact mechanism warrants further study.

1385 TOXICOLOGICAL ASSESSMENT OF DIESEL-METHANOL-WATER EMULSION [PURINOX™ (ALL WEATHER) GENERATION 2 FUEL] EXHAUST EMISSIONS.

M. D. Reed[1], I. Daly[3], R. Gudi[4], J. D. McDonald[1], M. Mercieca[5], J. O'Callagan[6], N. Ronsko[2], S. Seilkop[7], V. Wagner[4] and R. Kraska[2]. *[1]Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM, [2]Lubrizol Corporation, Wickliffe, OH, [3]Regulatory and Technical Associates, Allendale, NJ, [4]BioReliance, Rockville, MD, [5]Pathology Associates, Frederick, MD, [6]CDC/ NIOSH, Morgantown, WV and [7]SKS Consulting, Silver City, NC.*

A type of fuel shown to decrease combustion emissions vs. traditional diesel is the diesel emulsion. Utilizing a colaborative approach, a toxicological assessment of the exhaust of PuriNOx™ fuel emulsion was recently conducted. Whole exhaust from one of two, 2002 model Cummins 5.9L ISB engines was diluted to exposure levels of 125, 250, and 500 μg particulate matter (PM)/m^3. The engines were operated on a repeating EPA heavy-duty certification cycle. F344 rats were housed in Hazleton H2000 exposure chambers and exposed to exhaust 6 h/day, 5 days/wk for the first 11 weeks and 7 days a week thereafter. Exposure days ranged from 58-70 days. Exposure- start was staggered to account for multiple health endpoints. Toxicological assays were conducted during and following exposures and subsequent to a recovery period. General toxicity (body weight, organ weight, clinical pathology, and histopathology), neurotoxicity (glial fibrillary acidic protein assay, GFAP), genotoxicity (Ames, micronucleus, sister chromatid exchange), and reproduction and development were assessed. Effects observed were mild. Exhaust was not associated with neurotoxicity, reproductive/developmental toxicity, or *in vivo* genotoxicity. Small decreases in serum cholesterol were noted in males and females of the high level exposure. PM accumulation within alveolar macrophages was noted in all exposure groups. Other sporadic statistically significant responses were observed but not clearly treatment related. Exhaust subfractions induced mutagenic responses in S. typh. Based on the cholesterol results, it can be concluded that the 250 μg/m^3 exposure level was the NOAEL. In general, these observations were consistent with rodent and bacteria exposure to petroleum diesel exhaust.

1386 INDUCTION OF IL-6 IN LUNG CELLS BY PM2.5 PARTICLES FROM DESERT SOILS AND COAL FLY ASH.

J. M. Veranth, M. M. Veranth and G. S. Yost. *Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.*

Fine particle air pollution is a complex mixture containing materials from geological, combustion, and atmospheric sources. The mechanisms linking various particle types to specific health effects remain elusive, but many studies have associated ambient particles with pro-inflammatory cytokine signaling pathways. In this study, PM2.5 particles were aerodynamically separated from three western United States desert soils and from a sample of fly ash collected at a power plant burning bituminous coal. Cultured human lung epithelial cells (type BEAS-2B) were treated with doses from 10 - 160 microgram/cm2 of the particles. Viable cell count and the interleukin-6 (IL-6) concentration in the media were determined 24 h after treatment. The IL-6 response correlated with negative charge on the particles (zeta potential) but did not correlate with particle surface area or with transition metal content. Capsazepine, an antagonist of the TRPV-1 receptor, decreased the IL-6 response. One desert dust induced an exceptionally high IL-6 response compared to treatment with the other soils, kaolin clay, or soluble metal salts. The LAL chromagenic assay showed that this dust contained the highest level of endotoxin (20-50 EU/mg particles which equals <10 EU/mL as applied to the cells). The measured endotoxin concentration in the dust was considerably lower that the amount of soluble lipopolysaccaride required to elicit a comparable IL-6 response (> 2000 EU/mL). This suggests that either the particles interfere with the LAL endotoxin assay or that other soil dust properties are responsible for the cytokine response. This work was sponsored by NIH research career award 1 K25 ES011281-01A1 by Southwest Center for Environmental Research and Policy project EH-03-03.

1387 EFFECTS OF PARTICULATE MATTER ON GLUTAMATE CYSTEINE LIGASE IN RAW CELLS.

S. M. Leaman[1], P. Vliet[1], D. L. Luchtel[1], M. E. Rosenfeld[2] and T. J. Kavanagh[1]. *[1]Department of Occupational and Environmental Health Sciences, University of Washington, Seattle, WA and [2]Department of Pathobiology and Nutritional Sciences Program, University of Washington, Seattle, WA.*

Epidemiological studies have consistently shown associations between ambient particulate matter (PM) and respiratory and cardiovascular mortality and morbidity. The mechanisms responsible for this association remain unclear. One hypothesis with supportive toxicologic evidence is that PM acts to cause oxidative stress in the lung leading to upregulation and systemic release of pro-inflammatory mediators from immune defense cells such as macrophages. These pro-inflammatory mediators such as cytokines and reactive oxygen species can negatively impact cardiovascular function. Many studies have revealed the importance of antioxidants in protection of the lung from oxidative stress. The thiol antioxidant glutathione (GSH) is a major antioxidant in the lung and inhibition of its synthesis has been shown to exacerbate lung injury by a large number of toxicants. Glutamate cysteine ligase (GCL), a heterodimer composed of a catalytic (GCLc) and a modifying (GCLm) subunits, catalyzes the rate-limiting step in glutathione (GSH) biosynthesis. Exposing the murine macrophage cell line, RAW 264.7 to 100 μg/ml PM1648 for 3-24 hours caused an upregulation of the GCLm protein while exposing cells to 300 μg/ml PM1648 upregulated GCLc protein over the same time course. These data suggest that GCL plays a role in protecting cells in the lung, such as alveolar macrophages from injury caused by PM exposure.

1388 EFFECTS OF COMBUSTION-DERIVED PARTICULATE MATTERS CONTAINING ARSENIC IN NF-κB LUCIFERASE TRANSGENIC MICE.

J. Park[1], B. Park[2], G. Yang[2], M. Young[3], N. Colburn[3] and M. Cho[1]. *[1]Laboratory of Toxicology, College of Veterinary Medicine, Seoul National University, Seoul, South Korea, [2]Laboratory of Combustion and Air Pollution Control, College of Environmental Engineering, Chunbuk National University, Chunju, South Korea and [3]Basic Research Laboratory, National Cancer Institute, Frederick, MD.*

Inhalation of combustion-derived particulate matters (PMs) can have a variety of negative impacts on human health. Some heavy metals are also known to play a substantial role in adverse health effects. The potential toxic effects of inhaled combustion-derived PMs containing arsenic, one of the most frequently present heavy metals in the air, were examined usinf NF-KB luciferase reporter mice. The male mice (5-6 weeks old) were exposed to different concentrations of PMs (124±24.5, 220±34.5, 426.4±40.3 μ/m3) for 6h per day, 5 days per week for 4 weeks in 1 m3 whole-body chambers. Significant increase of the luciferase activity in heart, liver, and lung was observed. Among them, the pulmonary luciferase activity was changed dramatically. EMSA indicated that the exposed PMs inhibited NF-kκB DNA binding activity in the lung. Western blot analysis in the lung indicated that p65 and p50 protein levels were increased while IκB was decreased in a concentration-dependent manner. The PMs also induced increase of Akt, Bcl-2, c-Myc and Erk proteins. Our results suggest that combustion-derived PMs containing arsenic can be a significant cause of adverse human health. Supported by BK21 grant

1389 CYTOTOXICITY AND CELL SIGNALING IN MH-S CELLS: RELATIVE POTENCY OF DIESEL AND COAL COMBUSTION PARTICLES.

P. Singh[1], Y. Kostetski[2], M. Daniels[1], T. Stevens[3] and I. Gilmour[1]. *[1]ORD/NHEERL, USEPA, Research Triangle Park, NC, [2]NUS, Singapore, Singapore and [3]UNC, Chapel Hill, NC.*

Pulmonary exposures to combustion source particulate matter (PM) are known to induce inflammatory lung diseases, however it is not well understood how these particles exert their effects and how different sources and types of PM rank in potency. We have previously demonstrated in mice that diesel exhaust particles (DEP) high in organic carbons produced macrophage influx, while DEP, high in elemental carbon, induced neutrophilic inflammation in the lung. In the present study we tested the *in vitro* effects of these DEP (SRM-DEP, A1-DEP) along with a third

DEP sample (A2-DEP) and two coal particulate samples (M-Coal, K-Coal) on ATP levels and necrosis as well as JNK and p38 MAP kinase activation in a mouse alveolar macrophage cell line. The particulate samples were analyzed by electron paramagnetic resonance (EPR) to determine whether surface free radical concentrations were associated with cytotoxicity or bioactive potency. All particle treatments produced a dose-dependent decrease in ATP levels at 1, 4, and 24 h post exposure, however the relative potencies of the different samples were distinct: SRM-DEP = A2-DEP > A1-DEP > M-Coal = K-Coal. All particle-exposed cells showed significant increases in necrosis above control levels at 24 h. A2-DEP stimulated the earliest increase in phosphorylated p38 MAP kinase, while A1-DEP induced a later but far more dramatic rise. A1-DEP was also the most potent stimulator of phosphorylated JNK in these cells. EPR analysis showed that SRM-DEP had the highest concentration of surface free radicals followed by A2-DEP, which had about half and A1-DEP, which had about 1/10 the activity. These results indicate that surface free radical concentration is related to a reduction in ATP levels. However, the relative potency of activation was not associated with levels of surface free radicals, suggesting that mechanisms of PM-induced cellular activation and injury are distinct. (This abstract does not reflect EPA policy.)

1390 INFLAMMATORY AND GENOTOXIC RESPONSES AND PULMONARY FUNCTION CHANGES DURING 60 DAYS OF WELDING FUME EXPOSURE PERIOD.

J. Sung[1], I. Yu[1], S. Maeng[1], S. KIm[1], B. Choi[1], K. Song[2], J. Han[1], Y. Chung[1] and J. Hyun[1]. *[1]Center for Occupational Toxicology, Occupatinal Safety & Health Research Institute, KOSHA, Daejeon, South Korea and [2]College of Veterinary Medicine, Seoul National University, Seoul, South Korea.*

Respiratory effects in full time welders include bronchitis, airway irritation, lung function changes, and lung fibrosis. In previous studies, we developed a three-phase lung fibrosis model to study the pathological process of lung fibrosis. In this study, we have attempted to investigate the inflammatory responses and pulmonary changes during this 60 day welding fume inhalation exposure period to elucidate the process of fibrosis. The rats were exposed to manual metal arc-stainless steel welding fumes (MMA-SS) with concentrations of 64.8 ± 0.9 (low dose) and 107.8 ± 2.6 mg/m^3 (high dose) total suspended particulates for 2 hrs per day in an inhalation chamber for 60 days. Animals were sacrificed after the initial 2 hr exposure and after 15, 30 and 60 days. Pulmonary function was measured every week. The rats exposed to the welding fumes showed a statistically significant (P<0.05-0.01) body weight decrease as compared to the control during the 60 days exposure period. Elevated levels of cellular differential count, albumin, LDH, B-NAG, TNF-α and IL-1β were measured in the acellular bronchoalveolar lavage fluid of the rats exposed to the MMA-SS fume for 60 days. In addition, the DNA damage from 60 days welding fume exposure was also confirmed by Comet assay and inmmunohistochemistry for 8-OH dG. Among the parameters for pulmonary function test, only tidal volume showed a statistically significant and dose dependent decrease during 60 days of MMA-SS welding fume exposure. The elevation of inflammatory and genotoxic indicators along with lung function change confirms the lung injury and inflammation caused by the MMA-SS welding fume exposure.

1391 INDUCTION OF TGFβ PRODUCTION IN HAMSTER LUNGS FOLLOWING PERTURBATION WITH PULMONARY FIBROTIC AGENTS AMIODARONE AND PARAQUAT.

H. M. Conway, University. Doshi and J. M. Cerreta. *Pharmaceutical Sciences, St. John's University, New York, NY.*

Pulmonary fibrosis, regardless of its etiology, is characterized by increased accumulation of cells and excessive synthesis and deposition of extracellular matrix components. Several different agents can set in motion a series of pathogenic events that result in significant changes in lung connective tissue content and distribution. However, the precise events from initial insult of the lung to fibrosis remains uncertain. The aim of the current study was to investigate perturbation of the lung in Syrian Golden hamsters by known pulmonary toxicants amiodarone and methyl viologen (paraquat). Such agents were introduced into the respiratory system of experimental animals by intratracheal insufflation. At various time points (6 to 12 weeks following treatment) the animals were euthanized, lungs removed, fixed in formalin and processed for histologic examination. Lungs from animals euthanized at earlier time points (24 to 72 hours) were frozen in liquid nitrogen or lavaged with sterile saline. Tissue sections from different time periods were examined by light microscopy to study the process of developing fibrosis. Frozen sections were stained using immunohistochemical methods to determine the location of and cell types producing transforming growth factor beta (TGFβ) in toxicant treated lungs. It was found that TGFβ levels peaked at 24 hrs. after amiodarone treatment with declining levels at 48 and 72 hrs. With paraquat treatment peak levels of TGFβ appeared at 48 hrs with subsequent decline at 72 hrs. TGFβ was seen in the lung epithelial cells and pulmonary macrophages. Bronchioalveolar lavage also demonstrated a rise and decline in TGFβ levels parallel to those seen by immunohistochemical staining. These data suggest that hamster lung cells are responding to different pulmonary toxicants that produce fibrosis through similar pathways.

1392 SEASONAL METAL CONTENT MEASURED IN BALTIMORE $PM_{2.5}$ SEAS SAMPLES CORRELATES WITH CYTOKINE AND CHEMOKINE RELEASE IN AN *IN VITRO* ASSAY SYSTEM.

R. J. Mitkus[1], J. L. Powell[1], J. P. Pancras[2], J. M. Ondov[2] and K. S. Squibb[1]. *[1]Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD and [2]Chemistry and Biochemistry, University of Maryland, College Park, MD.*

The association between human cardiopulmonary disease and exposure to fine particulate matter ($PM_{2.5}$) has been well established. Metals are hypothesized to be responsible for this relationship. To test this hypothesis, we exposed human alveolar type II cells (A549) and monocytic-like cells (RAW 264.7) to fine particulate matter ($PM_{2.5}$) collected at the Baltimore Supersite during summer and winter intensive sampling periods in 2002. Production of the chemokine, MCP-1, was measured from alveolar epithelial cells, while the release of the pro-inflammatory cytokine, TNF-α, was quantitated from monocytes in two similar *in vitro* assay systems. Mean levels of TNF-α for July 17-19 were 1800.8 (+/- 1092.1) pg/ml, while mean levels for November 19-25 were 1994.7 +/- 1455.0 pg/ml. MCP-1 release for July had a mean value of 101.1 +/- 223.4 pg/ml, whereas it was -836.2 +/- 246.5 pg/ml for November. A comparison of the concentrations of eleven metals measured by GFAAS revealed that Al, Fe, and Zn were the most abundant in both the July (Fe>Al>Zn) and the November (Fe>Zn>Al) samples. Statistical analyses demonstrated significant Pearson correlations (P<0.05) between ambient Fe and Cu concentrations and subsequent TNF-α and MCP-1 release for both sampling periods. Ambient Al concentrations from both July and November correlated with TNF-α release. In contrast, Zn correlated well with TNF-α (r = 0.507) released in response to the November samples and MCP-1 (r = -0.576) released in response to the July samples. Our results demonstrate that summer and winter samples differed in the associations between metal content and induced cytokine or chemokine release and that MCP-1 inhibition correlates well with Zn concentration in these samples. They also support other reports that certain metals found in $PM_{2.5}$ affect the expression and release of specific intercellular signaling molecules in *in vitro* systems. *EPA grant R828063*

1393 METHOD OF DUST EXTRACT PREPARATION AFFECTS CYTOKINE PRODUCTION BY RESPIRATORY EPITHELIAL CELLS AFTER DUST EXPOSURE.

R. D. Massengale and J. J. Balsam. *Biology, Baylor University, Waco, TX.* Sponsor: M. Kanz.

A number of studies have been conducted in recent years to determine the effect of agricultural dust on the respiratory immune response. Many of these studies involved *in vitro* exposure of cell cultures to various concentrations of dust and subsequent measurement of cytokine production. However, the method of preparing the dust for cell exposure varied widely between studies. This variation in protocol limits data comparison between studies. Therefore, the overall objective of the current study was to develop a systematic method of agricultural dust exposure assays by comparing three different methods of dust extract preparation for their effect on cytokine production by respiratory epithelial cells. Samples of various agricultural dusts were collected and dust extracts of each type were prepared (unfiltered dust extract, filtered dust extract, heat-killed dust extract). A549 respiratory epithelial cells were then exposed to each of the dust extract preparations and assessed by ELISA for induction of interleukin-8. The heat-killed dust extracts induced the highest levels of interleukin 8 from A549 cells (range 50-250 ng IL-8 per million A549 cells). The levels of IL-8 induced by filtered dust exposures were significantly less (0-20 ng IL-8 per million A549 cells) than both unfiltered and heat-killed dust exposures at some dust concentrations. Furthermore, the results of the study showed that dust concentrations of 1000-10, 000 g/ml from some dust types were toxic to the A549 cells regardless of dust preparation method. Based on these results, the heat-killed method of dust extract preparation appears most appropriate for agricultural dust exposure assays. This study suggests that 1) direct dust-to-cell interaction is critical for maximum cytokine induction in dust-exposure assays, 2) heat-killed dust extract preparations induce the highest cytokine levels, and 3) developing a standard method of dust extract preparation is critical to effective comparison between experiments and between studies.

1394 ROLE OF TNFα AND CAVEOLIN-1 IN OZONE-INDUCED INFLAMMATORY MEDIATOR RELEASE AND TOXICITY.

L. Fakhrzadeh, J. D. Laskin and D. L. Laskin. *Environmental and Occupational Health Sciences Institute, Rutgers University/UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

Exposure to toxic levels of ozone (O_3) cause alveolar epithelial damage. We have previously demonstrated that alveolar macrophages (AM) and inflammatory mediators including nitric oxide generated *via* nitric oxide synthase, PGE2 and TNF-α contribute to the pathogenesis of tissue injury. The generation of these mediators is regulated, in part, by the transcription factor, NF-κB. Our findings that NF-κB p50 knockout mice are unable to generate inflammatory mediators and are protected from O_3 induced lung injury, demonstrate a critical role for this protein in the pathogenic process. In the present studies we analyzed mechanisms regulating NF-κB activation in the lung after O_3 inhalation. Treatment of wild type (WT) mice with ozone (0.8 ppm, 3 h) resulted in a rapid increase in NF-κB binding activity in AM which peaked after 6-12 h. This response was attenuated in mice with a targeted disruption of TNF-α. TNF-α signaling involves p42/44 (ERK 1/2) MAP kinase, and phosphatidylinositol 3'-kinase/protein kinase B (PI3K/PKB) which are important in NF-κB activation and the generation of inflammatory mediators. O_3 inhalation resulted in increased ERK1/2 expression and activation in WT mice which was evident immediately after exposure. Activated ERK 1/2 is known to downregulate caveolin-1 (Cav-1), a negative regulator of PI3K. O_3 inhalation markedly reduced constitutive Cav-1 expression in AM. This was directly correlated with O_3-induced activation of PI3K and PKB. In contrast, in TNF-α-/- mice, O_3 had no effect on Cav-1 or PI3K expression. These data, together with our findings that TNF-α suppresses Cav-1 expression *in vitro*, demonstrate that TNF-α and downstream signaling molecules are important in activation of NF-κB and the regulation of inflammatory genes involved in O_3 toxicity. Supported by NIH grants ES04738, GM34310 and ES05022.

1395 DIFFERENTIAL EXPRESSION OF TREFOIL FACTORS 1 AND 3 FOLLOWING AIRWAY EPITHELIAL CELL INJURY.

G. L. Baker, L. S. Van Winkle, M. V. Fanucchi, D. C. Kim and C. G. Plopper. *Veterinary Medicine: Anatomy Physiology and Cell Biology, UC Davis, Davis, CA.*

Trefoil factors are rapidly induced following mucosal injury in the gastrointestinal tract and function to protect the intestinal mucosa, enhance epithelial cell migration and are essential for epithelial repair in the GI tract. Recently TFF1 and TFF3 have been identified in the human respiratory tract, suggesting that trefoil factors may play a similar role in airway mucosal protection and injury repair processes. The present study was designed to determine whether trefoil peptides are modulated following injury in a well-defined model of airway epithelial cell injury using the Clara cell specific bioactivated pulmonary toxicant naphthalene (NA). Male Swiss Webster mice received an intraperitoneal injection of 200-mg/kg NA dissolved in corn oil carrier. Airways RNA was isolated 0, 1, 3, 6, 12 and 24 hours following naphthalene treatment. Real-time RT-PCR was performed on the isolated RNA to measure airway TFF1 and TFF3 expression. Six hours following naphthalene treatment TFF1 expression was increased 4 fold in the airways and at 24 hours it was increased 7 fold. TFF3 expression was increased 2 fold 1-3 hours following naphthalene treatment and was non-detectable from 6-24 hours following NA treatment. These results demonstrate that TFF1 and TFF3 are differentially modulated following acute airway injury. The increase in TFF3 that occurs immediately following NA administration may represent in an immediate mucosal protective mechanism, while the later TFF1 increase and the loss of TFF3 expression, which first occurs at a time when glutathione depletion occurs, may represent involvement in both airway mucosal protection and repair of injury. Supported in part by NIEHS ES06700, ES04311, ES05707 and the California Tobacco Related Disease Research Program of the University of California Grants TRDRP 121T-0191 and 11RT-0258.

1396 PRE-TREATMENT WITH DIESEL EXHAUST EXTRACT ALTERS INFLUENZA VIRUS REPLICATION IN LUNG EPITHELIAL CELLS.

I. Jaspers[1, 2], J. Ciencewicki[3], M. Beck[2], W. Zhang[1] and M. Brighton[1]. *[1]Center for Env. Med., Asthma, & Lung Biology, University of North Carolina, Chapel Hill, NC, [2]Pediatrics, University of North Carolina, Chapel Hill, NC and [3]Curriculum of Toxicology, University of North Carolina, Chapel Hill, NC.*

Diesel Exhaust (DE) has been demonstrated to generate inflammatory responses in the lung and modify immune responses to inhaled allergens. However, little is known about the effects of DE on common respiratory viral infections. Previous studies have demonstrated that exposure to DE enhances influenza virus replication in mice. We examined whether exposure to DE extracts (DEE) modifies influenza infections of human lung epithelial cells. Differentiated human bronchial epithelial (HBE) cells or A549 cells were exposed to DEE prior to infection with influenza A Bangkok/1/79. At 24 hours - 5 days post-infection total RNA was analyzed for the expression viral genes, as well host cell inflammatory and antiviral genes. Analysis of hemagglutinin (HA) mRNA, a marker for viral propagation, indicates that pre-treatment with DEE enhances viral replication in both differentiated HBE and A549 cells as early as 24 hours post-infection in a DEE dose-dependent manner. This was not caused by a reduced antiviral defense response of the host cells, since influenza-induced mRNA levels for interferon β and MxA, a major interferon β-inducible antiviral defense gene were also enhanced by pre-treatment with DEE. In addition influenza-induced nuclear translocation of phospho-STAT1 and ISGF3γ, transcription factors mediating interferon β-induced gene expression, were also up-regulated by prior exposure to DEE, indicating that interferon β-induced signal transduction pathways are also enhanced by pre-treatment with DEE. Taken together these data indicate that exposure to DE can enhance influenza virus replication in human lung epithelial cells possibly without altering the ability of the host cell to defend itself against the invading pathogen.

1397 SOLUBLE METALS ASSOCIATED WITH ROFA SUPPRESS LUNG IMMUNE DEFENSE AND ALTER CYTOKINE PROFILES AFTER INFECTION IN RATS.

J. R. Roberts[1, 2], M. D. Taylor[1], V. Castranova[1, 2] and J. M. Antonini[1, 2]. *[1]NIOSH, Morgantown, WV and [2]WVU, Morgantown, WV.*

Residual oil fly ash (ROFA), a by-product of fossil fuel combustion and a component of air pollution, has been associated with increased morbidity in susceptible populations. We have shown that soluble metals in ROFA cause lung inflammation and increase susceptibility to infection in rats. The objective was to examine the mechanisms by which soluble metals in ROFA may enhance susceptibility to infection. ROFA was suspended in saline, separated into soluble and insoluble fractions, and the soluble portion (ROFA-SOL) was retained. At day 0, rats were intratracheally instilled (IT) with ROFA (2.0mg/rat) or equivalent quantities of ROFA-SOL, or saline. At day 3, rats were separated into two groups and received an IT dose of either $5x10^4$ or $5x10^5$ *Listeria monocytogenes*. Rats were euthanized on days 3 prior to infection, and on days 6, 8, and 10, bronchoalveolar lavage (BAL) was performed on the right lungs, and bacterial clearance was assessed using the left lung. BAL fluid was centrifuged and the supernatant was retained for measurement of cytokine levels. Exposure to ROFA-SOL significantly decreased pulmonary clearance of bacteria at both doses and decreased animal survival after treatment with the high bacterial dose. Prior to bacteria inoculation on day 3, IL-2 was decreased and IL-6 was increased in rats treated with ROFA-SOL compared to saline. After exposure to both doses of bacteria, IL-2 was decreased whereas IL-6 and IL-10 were elevated in ROFA-SOL-treated rats compared to saline. An elevation in IL-6, a pro-inflammatory cytokine associated with the acute phase response, may partially account for the ROFA-SOL-induced inflammation. An increase in IL-10, a cytokine involved in macrophage inhibition, and a reduction in IL-2, a cytokine promoting T-cell growth and proliferation, may result in a suppression of the innate and adaptive immune response to infection, respectively. ROFA and its associated soluble metals alter cytokine production which may affect the ability of the animals to respond to the infection.

1398 SHORT-TERM EXPOSURE TO INHALED DIESEL EXHAUST PARTICLES ENHANCES ASTHMA-LIKE SYMPTOMS AND INCREASES CYP1A1 mRNA LEVELS.

M. J. Whitekus[1], N. Brechun[2], S. K. Nelson[2], O. Hankinson[1] and D. Diaz-Sanchez[3]. *[1]Department of Pathology and Laboratory Medicine and Jonsson Comprehensive Cancer Center, UCLA, Los Angeles, CA, [2]Webb-Waring Antioxidant Research Institute, Denver, CO and [3]Division of Clinical Immunology and Allergy at UCLA School of Medicine, UCLA, Los Angeles, CA.*

Epidemiological studies suggest a correlation between exposure to ambient particulate matter and adverse health effects in humans, however, there is still a fundamental lack of understanding of the mechanisms involved. We have established a C57BL/6 mouse model to study the adjuvant effects of short-term inhaled diesel exhaust particle (DEP) exposure on asthma. In this model, animals treated with DEP + ovalbumin (OVA) display cellular infiltration in the lung (eosinophila) and demonstrate increased levels of IgG1 in sera compared to ovalbumin treated mice. Our current hypothesis is that DEP mediates some of its adjuvant effects through oxidative stress. However, when we measure the carbonyl content of total mouse lung in our model we found that there was no difference between DEP+OVA and OVA treated mice, suggesting that oxidative stress is not responsible for DEPs modulatory effects on cellular infiltration. Preliminary data using qPCR also suggests that CYP1A1 mRNA levels in total mouse lung increased by 18 fold in DEP+OVA treated mice. Thus, in our mouse model the adjuvant effect of DEP on cellular infiltration might function through another pathway besides oxidative stress, possibly through stimulation of the Ahr. Supported by US Public Health Service Grant AI50495.

1399 DISPARATE ALLERGIC AIRWAY RESPONSES TO DIESEL EXHAUST INHALATION DURING ALLERGEN SENSITIZATION VERSUS ALLERGEN CHALLENGE.

J. Wagner[1], E. Barrett[2], J. McDonald[2] and J. Harkema[1]. *[1]Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI and [2]Lovelace Respiratory Research Institute, Albuquerque, NM.*

Diesel engine exhaust (DEE) has been studied as a potential contributor to allergic responses in laboratory animals and humans. We compared the response of rodents exposed to DEE in two allergic models; one focused on allergen sensitization and one on allergen challenge. Brown Norway rats were sensitized to ovalbumin (OVA) by 3 daily intranasal (IN) instillations of 0 or 0.5% OVA, and then 2 weeks later were challenged with *IN OVA* for 3 days. Rats were exposed to DEE diluted to 30 or 300 micro-g/m3 particulate matter or clean air during either sensitization or challenge to OVA. Rats were sacrificed 24h after the last OVA challenge, bronchoalveolar lavage fluid (BALF) was collected and analyzed for cellularity and secreted mucins, and lungs were processed for morphology and histochemical analysis of intraepithelial mucosubstances (IM). OVA sensitization and challenge caused increased total inflammatory cells, neutrophils, eosinophils, and mucin glycoprotein 5AC in BALF, and IM in proximal (2-fold increase) and distal (10-fold) pulmonary axial airways. The lowest DEE exposure during OVA sensitization enhanced OVA-induced BALF cellularity by 1.6-fold, mostly due to neutrophils. By contrast, exposure to this same exposure level during OVA challenge resulted in attenuation of OVA-induced BALF total cells, neutrophils and eosinophils by 50%. Exposure to the high DEE exposure level had no effect on OVA-induced changes in BALF cellularity or mucin secretion. OVA-induced increases in IM were unaltered by DEE exposure during allergen sensitization, but both DEE exposure levels during allergen challenge inhibited OVA-induced increases in IM in distal airways by 50%. These data demonstrate that inhalation exposure to DEE can both inhibit and enhance allergic airway inflammation and mucus production. Modulation of these responses is dependent on the time of DEE exposure during the development of allergic disease. (Supported by the Health Effects Institute 03-8)

1400 CHANGES IN THE COMPOSITION OF DIESEL EXHAUST RESULTS IN CHANGES IN THE MAGNITUDE OF SEVERAL ACUTE INHALATION RESPONSES.

J. McDonald, K. S. Harrod, J. Seagrave, S. Seilkop and J. Mauderly. *Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM.*

Diesel engine exhaust changes in composition with modification of engine operation/configuration, including the addition of emission reduction technologies such as low sulfur fuel and catalyzed ceramic traps. While previous studies have shown that diesel exhaust can cause inflammation in rodents and humans and decreased resistance to respiratory infection in mice (Harrod et al., Am. J. Resp. Cell Mol. Biol., V. 28, pp. 451-463), there is little information on the impact of changes in exhaust composition on these effects. To address this, identical inflammation, oxidative stress, and respiratory infection assessments were conducted after exposure of mice to diesel exhaust generated from a single cylinder diesel engine generator under three steady-state operating conditions: 1) High-load operation, number 2 cert. fuel, diluted to 200 micro-g/m3 particle mass (PM), 2) Low-load operation, number 2 cert. fuel, diluted to 200 micro-g/m3 PM, and 3) High-load operation with ultra-low sulfur fuel and catalyzed ceramic trap, with same dilution rate as condition 1. Changing the operation (load) of the engine resulted in a 4-fold change in particle organic content and a difference in particle size. The high load engine condition resulted in increased lung response (inflammatory/oxidative stress signals, altered viral clearance) for all endpoints, while low engine load resulted in mild increases in an oxidative stress indicator. No lung responses were observed with the emission reduction technologies in place. The ultimate goal of this work is to develop a framework that can be used to identify the physical/chemical components, or interactions among components, that are the most important in driving the acute toxicity of engine emissions. Work supported by the FreedomCar and Vehicle Technology Program of the US Department of Energy.

1401 *IN VITRO* AND *IN VIVO* EFFECTS OF WORLD TRADE CENTER DUSTS: STUDIES IN HUMAN AND MOUSE CELL LINES AND IN INFLUENZA-COMPROMISED YOUNG AND OLD RATS.

A. Elder[1], J. Finkelstein[2], R. Gelein[1], N. Corson[1], P. Mercer[1], C. Reed[2], D. Oakes[3], S. Eberly[3], D. Topham[4] and G. Oberdorster[1]. *[1]Environ Med., University of Rochester, Rochester, NY, [2]Peds, University of Rochester, Rochester, NY, [3]Biostat, University of Rochester, Rochester, NY and [4]Immunol, University of Rochester, Rochester, NY.*

It is estimated that the initial airborne dust concentration after the collapse of the World Trade Center (WTC) towers was in the tens of g/m^3. Emergency respondents were exposed to these high concentrations in the first several hours after the disaster and their lingering cough may be due to the inhaled bolus of dust. Aerosol samples were taken at indoor and outdoor sites and then fractionated by size. *In vitro* studies in human and mouse IL-8 reporter cell lines were done first with all 20 samples to identify the most active ones. This was followed by single *in vivo* dust instillations of 500 μg (to mimic responders' exposure) in young and old rats that were compromised with influenza virus either one or 29 days after dust exposure. The three most active dusts from *in vitro* studies were compared to fine-mode TiO_2, a frequently-used "inert" control particle. Parameters of lung inflammation and oxidative stress were measured 3 and 31 days after dust exposure. Influenza virus caused significant lung inflammation by itself, as expected. The very mild WTC dust-induced inflammatory cell influx was characterized by maximal lavage neutrophil responses of 2.8% and 4.4% in young and old rats, respectively. The WTC dusts had significant main effects on total cell number, percentage of macrophages, and *via*bility of lavage cells and the percentage of blood neutrophils (all small decreases). In general, there were no significant interactions between the dusts and virus; the exception to this was for total cell number, which increased slightly when the two factors were combined. There were no large increases in response from 3 to 31 days post-exposure. Lastly, there were no consistent differences between the WTC dusts and TiO_2. These results suggest that an instilled high-dose bolus of WTC dust produces minimal inflammation that is much like the response to a nuisance dust and that subsequent viral infection does not unmask any toxicity associated with dust exposure.

1402 OXIDATIVE STRESS OF POLAR AND NONPOLAR AIR PARTICULATE MATTER COMPONENTS.

A. Kubatova[1], L. C. Dronen[1], S. B. Hawthorne[1] and M. J. Picklo[2]. *[1]EERC, University of North Dakota, Grand Forks, ND and [2]School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND.*

Extensive epidemiological and inhalation studies link atmospheric particulate matter (PM) to cardiopulmonary diseases. PM exacerbates oxidative stress and inflammation in the respiratory system. Most of the toxicological research is focused on inorganic species (metals, sulfates) and nonpolar organics (PAHs), however, few studies have addressed polar organic PM. This is due to the analytical difficulty in purifying and identifying specific fractions, and the necessity to employ more than one solvent to extract a wide polarity range of organics. In this work, two samples representing different polarity matrices were studied: nonpolar diesel exhaust and polar wood smoke PM. We employed hot pressurized water (25°-300°C) at pressures sufficient to maintain the liquid state to fractionate these samples into polar and nonpolar components. At low temperatures water extracts polar organics while at high temperatures water extracts nonpolar organics. The importance of different polarity fractions was evaluated in relation to oxidative stress, based on the glutathione (GSH) depletion, and cytotoxicity to murine alveolar macrophages RAW 264.7. In addition, a bacterial genotoxicity assay SOS Chromotest was performed on each PM fraction. Both polar and nonpolar species contributed significantly to the toxicity of both PM samples. For wood smoke particulate, mid-polarity and nonpolar fractions exhibited more pronounced GSH depletion. In the mid-polarity fractions, methoxyphenols (syringol dimers), oxyPAHs, and PAHs were selectively extracted. For diesel exhaust particulate, the highest GSH depletion was observed in the nonpolar fraction, but also (unexpectedly) in the polar fraction, possibly corresponding to dicarboxylic acids. The genotoxic response was observed in mid-polarity fractions corresponding to the presence of nitropyrene. In summary, the results indicate toxicological importance of polar air PM fractions. Moreover, this work demonstrates the potential of hot pressurized water to selectively extract polar and nonpolar species for toxicological testing.

1403 THE ALLERGY ADJUVANT EFFECT OF PARTICLES: CHARACTERISATION OF THE PRIMARY CELLULAR RESPONSE IN THE LOCAL LYMPH NODE.

University. C. Nygaard, A. Aase and M. Lovik. *Environmental Immunology, Norwegian Institute of Public Health, Oslo, Norway.* Sponsor: E. Dybing.

Traffic pollution may contribute to the increased incidence of asthma and allergy in the western countries. Diesel exhaust particles (DEP) is the main particle type in traffic pollution, consisiting of a complex mixture of small particles with several hundred different compounds adsorbed. Polystyrene particles as a surrogate for the DEP core have been shown to have an adjuvant effect on the allergic response in mice. In the present study, the primary immune response in the draining lymph node was examined after footpad injection of polystyren particles (PSP) and allergen (ovalbumin, OVA). PSP and OVA were injected into the hind footpad of Balb/cA mice. The popliteal lymph node (PLN) was excised on different days after injection, and cell numbers, cell types, several cell surface molecules and cytokine production were assessed. Particle-containing cells were observed in the lymph node already on day 1 after injection of PSP or OVA+PSP, and the number increased continuously until day 21. In the OVA+PSP group, the total and B-cell

numbers increased 3-4 times compared to the control groups, peaking on day 5 after injection. The increase in B cell numbers was twice the increase in T cell numbers. On day 5, most surface markers measured (MHCclassII, CD86, CD23, CD69) increased significantly in the OVA+PSP group compared to the control groups. PLN-cells from OVA+PSP injected animals gave an increased production of IL-4, but not IFN-γ, when stimulated *in vitro* by ConA. We conclude that the adjuvant effect of PSP on the IgE antibody response to OVA was associated with a cellular response in the draining lymph node, peaking on day 5 after injection. The cellular response in the OVA+PSP group on day 5 included increased cell numbers, altered surface marker expression and a selective increase in Th2 cytokine production. PSP alone, however, did not influence any of the immunologic markers studied in the PLN.

1404 IMMUNOLOGIC SENSITIZATION OF GUINEA PIGS VIA INHALATION.

W. Lee, C. Banks and A. Viau. *CTBR, Senneville, QC, Canada.*

In October 2002 the FDA finalized guidelines for the Immunotoxicology Evaluation of Investigational New Drugs the requirements are not all inclusive as the European guidelines (promulgated by EMEA in July 2000), but rather specified assays according to the dose route or indication. For drugs to be administered by the inhalation route, respiratory sensitization must be evaluated. CTBR has been conducting immunologic sensitization studies according to GLP in guinea pigs *via* the inhalation route for over 10 years. The design utilized consists of sensitizing Hartley albino guinea pigs to the test material *via* the inhalation route. Additional control groups such as a positive control sensitized with a proven sensitizing agent (ovalbumin) and saline control groups are included. There are 2 phases to the study, the sensitization phase consisting of nose-only inhalation exposure to either saline, ovalbumin or the test material for a period of 30 minutes over 5 days, followed by a latent untreated period of 17 days. The second phase consists of the challenge inhalation exposure when animals are exposed for 15 minutes to the test material, saline or ovalbumin. Two days prior to the challenge exposure, animals that have been exposed during the sensitization phase to ovalbumin will undergo a skin reaction test to confirm that they have been sensitized. Half the population of each group will be protected from acute anaphylaxis by intraperitoneal injection of pyrilamine maleate 30 minutes prior to challenge exposure. Several endpoints may be evaluated following or during the challenge exposure and include respiratory minute volume (derived from the tidal volume and respiratory rate of the animal is monitored prior to the challenge and during the challenge exposure), antibody titers, histopathological examination and bronchiolo-alveolar lavage (BAL), performed 24 hours post challenge. From the lavage fluid obtained, total cell number, cell differentials for total protein, albumin and globulins are determined. Over the years, this GLP model has been shown to be successful as an indication of respiratory sensitization.

1405 DIFFERENTIAL GENE EXPRESSION PROFILES IN RAT TRACHEAL EPITHELIAL (RTE) CELLS IN RESPONSE TO COMBUSTION-SOURCE PARTICULATE MATTER (PM) AND VANADIUM (V) A PRIMARY METAL CONSTITUENT.

S. Nadadur, J. A. Dye and D. L. Costa. *Pulmonary Toxicology Branch, USEPA, Research Triangle Park, NC.*

Epidemiological studies have associated increased mortality and morbidity in people with cardiopulmonary diseases with increased levels of ambient particulate matter (PM). The molecular basis for the cardiopulmonary health effects of PM and its constituents are still largely unknown. As one of our efforts to identify key target cell populations within the cardiopulmonary system, in the present study, we examined the acute cell responses in a primary airway epithelial cultures using microarray analysis. The RTE cells were exposed to an aqueous extract of model combustion PM, residual oil fly ash (ROFA, 50 μg/ml) and one of its major metal constituents, vanadium (V, at 1 and 7.7 μM), or saline for 25 min. Atlas Rat 4K plastic microarray (Clontech, Palo Alto, CA) was hybridized with ^{33}P-labeled cDNA probe generated from poly A$^+$ RNA isolated from the saline, ROFA or V exposed RTE cells. The spot intensities on the microarray were analyzed using AtlasImage 2.7 software (Clontech, Palo Alto, CA). Gene expression data was analyzed using GeneSpring software (Silicon Genetics, Redwood City, CA). A minimum three-fold exposure specific differential expression was observed in 870 genes. Of these, 479 genes were found up regulated. ROFA induced up regulation was seen in 139 genes, while 82 genes were suppressed. Induction of 293 genes by 1μM of V and suppression of an equivalent number of genes by 7.7 μM of V suggested concentration dependent transcriptional activation. Differential induction of chemokines, colony stimulating factor-2 by ROFA and V, and induction of colony stimulating factor-3 and CXC chemokine LIX by V only suggest possible roles for these genes in the initiation and progression of epithelial cell injury. (This abstract does not reflect USEPA policy).

1406 ANALYSIS OF GENE EXPRESSION IN RAT ALVEOLAR EPITHELIAL CELLS IN RESPONSE TO ORGANIC EXTRACT OF DIESEL EXHAUST PARTICLES.

E. KOIKE[1], S. Hirano[2] and T. Kobayashi[2, 1]. [1]*PM2.5 and DEP Research Project, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan and* [2]*Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan.*

Previous reports have been shown that diesel exhaust particles (DEP) induce pulmonary diseases including asthma and chronic bronchitis. The comprehensive evaluation has been required to know the toxic effects of air pollutants including DEP on the lung diseases. We focused the lung cells such as alveolar macrophages and alveolar epithelial cells, and searched the toxicological index. In this study, we investigated the gene expression in rat alveolar epithelial cells in response to organic extract of DEP using cDNA microarray. The DEP extract was prepared using dichloromethane. Rat alveolar type II cells (SV40T2) were subcultured extended to confluent and exposed to 30 μg/ml DEP extract for 6 h. Total RNA was extracted from the cells using TRIZOL. The gene expression was analyzed by using Motorola CodeLink Bioarray (KURABO, Japan) that includes 10000 rat cDNAs. Major indicator observed significant changes in the cDNA microarray analysis were further confirmed these protein activities. The expressions of many transcriptions on the cDNA microarray were changed by exposure to DEP extract. These were categorized according to the function. Significant increases were observed in the genes associated with drug metabolisms (aldose reductase-like protein), antioxidative enzymes (heme oxygenase1, glutathione S-transferase, heat shock protein 90), regulation of extracellular matrix (plasminogen activator inhibitor 2, tissue inhibitor of metalloproteinase 3) and so on. Furthermore, the expression of heme oxygenase 1 protein was also induced by exposure to DEP extract and the induction was decreased by treatment of N-acetyl cysteine as an anti oxidative agent. These results shows that the early response against oxidative stress caused by DEP may induce the pulmonary defense. Present study suggests that cDNA microarray analysis provides useful information to investigate the biological response to toxicants and to search the toxicological index.

1407 HEAVY METALS AND ELEMENTAL AND ORGANIC CARBON IN ATMOSPHERIC FINE PARTICLES (PM2.5) FROM PUERTO RICO.

B. D. Jimenez[1, 2], D. Acevedo[2] and C. Rodriguez-Sierra[3, 2]. [1]*Biochemistry, University of Puerto Rico, San Juan, PR, Puerto Rico,* [2]*Center for Environmental and Toxicological Research, University of Puerto Rico, San Juan, PR, Puerto Rico and* [3]*Public Health, University of Puerto Rico, San Juan.*

Fine atmospheric particulate PM2.5 were sampled in an urban industrialized area (Guaynabo) and in a reference unpolluted site (Fajardo) from Puerto Rico. Samples (72 hrs) were obtained during November/2000 to September/2001 using a RAAS2.5-400 Andersen Instrument. Composite samples were prepared representing a month of digested material using the appropriate field blanks. As, Cd, Cu, Fe, Ni, Pb and Zn were analyzed by Atomic Absorption and elemental and organic carbon (EC/OC) were also determined. Factor and cluster analyses were performed in order to grouped variables indicating common origin sources. All metal species analyzed, except Fe, were significantly higher in Guaynabo when compared to Fajardo. Average levels of PM2.5 in Guaynabo were 11.6 ug/m3 versus 8.5 ug/m3 in Fajardo. The average levels of EC and OC in Guaynabo were 1.5 ug/m3 and 2.2 ug/m3, respectively. Fajardo, EC levels were <0.14 ug/m3 and OC levels were <1 ug/m3. However, Ni and V concentrations from Guaynabo, were the highest from all sites studied and were the highest concentrations found when compared to other studies from different regions of the World (Ni= 17 ng/m3 and V=40 ng/m3). The levels of Ni and V found in the Guaynabo are of a serious health concern. These metals have been identified as causative agents of cancer and acute lung injury. Multivariate analyses showed a strong relationship between Ni and V, PM2.5 and Fe, As, Cu and Pb in samples from the urban site (Guaynabo). Similarly, PM2.5 and Fe, were highly correlated in Fajardo but other relationships were found between Cd and V, as well as a negative relationship between Ni, Pb and Cu. Studies performed in PR have shown that the population living in Guaynabo had more types and cases of cancer than any other regions in the island and suffered respiratory health problems such as asthma, particularly children and elder people.

1408 QSAR STUDY FOR THE ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR BY POLYCHLORINATED NAPHTHALENES.

J. Olivero-Verbel[1] and K. Kannan[2]. [1]*University of Cartagena. Environmental and Computational Chemistry Group, Cartagena, Colombia and* [2]*Wadsworth Center and Department of Environmental Toxicology and Health, State University of New York, Albany, NY.*

Polychlorinated naphthalenes (PCNs) are persistent environmental toxicans derived from the chlorination of naphthalene and also formed during the combustion of municipal waste. Due to their planarity and molecular similarity to dioxins, these

molecules can induce several toxic effects by activating the aryl hydrocarbon receptor (AhR). Using the potencies relative to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) for 20 individual PCN congeners according to the H4IIE-luc *in vitro* bioassay, and quantum chemically-derived molecular descriptors generated by the functional B3LYP model from the density function theory (DFT), a discriminant analysis was performed for a series of active and non-active PCN congeners to generate a suitable QSAR model for understanding the molecular characteristics responsible for the activation of AhR. The model suggests that PCNs with less than four chlorine atoms are not activators of the AhR. In addition, congeners IUPAC number 47, 50, 51, 52, 60, 63, 64 and 69, were predicted as compounds that can activate the AhR. The sum of atomic charges of the fused carbons within the PCN ring (SQC9-10) and the number of chlorine atoms, were identified by the QSAR model as the best predictors of AhR-dependent biochemical changes induced by these compounds.

1409 APPLICATION OF CALUX BIOASSAY FOR DETERMINING DIOXIN EXPOSURE LEVEL IN HUMAN BLOOD AND ENVIRONMENTS.

K. Joung and Y. Y. Sheen. *Pharmacy, Ewha Womans University, Seoul, seoul, South Korea.* Sponsor: Y. Cha.

For the determination of dioxin and dioxin like compounds, high resolution gas chromatography/mass spectrometer (HR-GC/MS) has been used as a standard method. Chemical analysis using HR-GC/MS has limitations to its application for large scale screenings. However, *in vitro* bioassay may overcome limitations of chemical analytic methods. Numberous bioassay systems have been developed that are mainly based on the AhR-dependent mechanism. Chemically Activated LUciferase gene eXpression assay (CALUX assay) is based on the CYP1A1 gene transcription. which is widely used as a functional parameter for AhR activations. In this study, we tried to establish CALUX bioassay and compare the results with that of chemical analysis. We have carried out CALUX assay for over 200 human blood samples and environmental samples such as Korean water, sediment, and soil. When the results measured by both HR-GC/MS and CALUX assay were compared, the correlation coefficient was as high as 0.96 in case of blood samples and also good in environmental samples except few samples. Dioxin exposure of Korean women without endometriosis turned out to be 14.6 pg/L TEQ and endometriosis patients turned out to be 31.9 pg/L TEQ. Most Korean river water showed minimal levels of dioxin like activity. Korean river sediment showed that 154 pg/g TEQ for Kumho river, 325 pg/g TEQ for Kum river, 216 pg/g TEQ for Mankyung river, 251 pg/g TEQ for Miho stream. These results suggest that the CALUX assay is applicable as an economical and quick method. However, the TEQ values of CALUX assay tended to be higher than that of HR-GC/MS.

1410 RECOVERY DETERMINATIONS FOR DIOXIN ANALYSIS WITH THE CALUX® BIOASSAY.

G. C. Clark[1], A. C. Chu[1], J. D. Gordon[1], D. J. Brown[1], M. Nakamura[2], M. D. Chu[3], H. Murata[2] and M. S. Denison[4]. *[1]Xenobiotic Detection Systems, Inc., Durham, NC, [2]Hiyoshi Corporation, Omihachiman, Shiga, Japan, [3]Alta Analytical Perspectives, Wilmington, NC and [4]Department of Environmental Toxicology, University of California, Davis, Davis, CA.*

Recovery determinations are important for quantitative methods such as high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) and bio-analytical methods such as CALUX® for dioxins. Recovery determinations in HRGC/HRMS are performed by spiking isotopically labeled congeners into the sample prior to extraction, with recovery based on the amount of labeled compound recovered. Bioassays do not differentiate between isotopically labeled and unlabeled analytes. Recovery determinations in bioassays can be accomplished with a surrogate sample spiked with a radiolabeled congener of dioxin. We demonstrate here that 1, 2, 3, 4-TCDD, a biologically inactive congener of the dioxin family of chemicals, can be used as an internal spike to determine recoveries of dioxin-like chemicals. Samples were spiked with ^{14}C labeled 2, 3, 7, 8-TCDD or 1, 2, 3, 4-TCDD and submitted to extraction and clean up using Xenobiotic Detection Systems, Inc. patent pending XCARB sample clean up method (acid silica column in series with an XCARB column). The XCARB column is differentially eluted to yield a PCB and PCDD/F fraction. The 1, 2, 3, 4-TCDD spiked samples were resuspended in toluene containing four PCB injection standards, and recoveries determined by gas chromatograph with electron capture detection or scintillation counter. Average recoveries determined by 1, 2, 3, 4-TCDD with paired samples spiked with ^{14}C- 2, 3, 7, 8-TCDD indicated that the recoveries determined by the two methods were very similar, 88.5% (± 1.2%) and 87.2% (± 2.4%), respectively. Recovery determinations were also verified by HRGC/HRMS. This procedure allows for quantitative determination of dioxin-like chemicals in various sample matrices. Supported by SBIR Grant from NIEHS ES 08372-03.

1411 ACTIVATION OF ARYL HYDROCARBON RECEPTOR BY TCDD INDUCES GENE SILENCING BY PROMOTER METHYLATION: A NOVEL MECHANISM FOR TCDD MEDIATED TUMOR PROMOTION.

S. S. Ray and H. I. Swanson. *Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, KY.*

The aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor that belongs to the bHLH-PAS superfamily and has been shown to mediate most of the effects of TCDD or dioxin. Our previous study has shown that in the primary human keratinocytes, TCDD inhibits the expression of the tumor suppressor proteins p53 and $p16^{INK4a}$, while decreasing senescence and increasing proliferation. This study focuses on determining whether $p16^{INK4a}$ and p53 are direct targets of the TCDD/AHR signaling pathway. Using both the pharmacological antagonist MNF (3'-methoxy-4-nitroflavone) and siRNA designed specifically for AHR we have found that the inhibition of p53 and $p16^{INK4a}$ occurs in an AHR-dependent manner. Analysis of newly synthesized unspliced RNA reveals that the TCDD mediated inhibition is transcriptional. Downregulation of p53 and $p16^{INK4a}$ has been reported as a crucial first step in the onset of many cancer types and often occurs *via* promoter methylation. Using 5-aza-2'-deoxycytidine (5-aza-dC), a DNA hypomethylating agent that can transiently reverse the transcriptional silencing, we have observed a complete reversal in TCDD's ability to inhibit the expression of p53, whereas only partial reversal occurs with respect to $p16^{INK4a}$. The evidence for promoter methylation was further confirmed using methylation specific PCR. In addition, we report that in the primary keratinocytes, the antagonist MNF inhibits nuclear uptake and 26S mediated degradation of AHR similar to that shown in other cell types. Further, the presence of a 26S proteosome inhibitor reverses the inhibitory effects of TCDD on p53 and $p16^{INK4a}$ expression. These novel findings imply that p53 and $p16^{INK4a}$ are targets of the TCDD/AHR signaling pathway and that this regulation involves increased methylation in the promoter regions of these genes. It also suggests that the AHR may be an important player in determining epithelial cell fate (i.e., senescence versus immortality) *via* its ability to regulate $p16^{INK4a}$ and p53.

1412 SUPPRESSOR OF CYTOKINE SIGNALING-2: A NOVEL TCDD INDUCIBLE GENE IN CH12.LX MURINE B-CELLS.

E. Tam, D. R. Boverhof, R. B. Crawford, N. E. Kaminski and T. R. Zacharewski. *Department of Biochemistry and Molecular Biology, Department of Pharmacology and Toxicology, and Institute for Environmental Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI.*

The B-cell, a major component of humoral immunity, is a sensitive target for the immunotoxic effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) possibly by rendering cells less responsive to antigenic or mitogenic stimulation. Potential mechanisms of TCDD action on B-cells were examined in murine B-cell lymphoma cells (CH12.LX) treated with 3 nM TCDD or DMSO vehicle for 0, 2, 4, 6, 8, 12 and 24 hrs using sequence verified cDNA microarrays representing 3068 genes/ESTs. One transcript that was significantly induced by TCDD was Socs2, a suppressor of cytokine signaling. Changes in Socs2 mRNA levels paralleled that of Cyp1a1 with maximal induction of 3-fold observed at 4 hrs as determined by quantitative real-time PCR. TCDD-mediated induction of Socs2 mRNA was dose-dependent and exhibited the characteristic structure activity relationships observed for the aryl hydrocarbon receptor (AhR) ligands PCB-126, ICZ and BNF. Experiments with cycloheximide and AhR deficient B-cells indicated that Socs2 mRNA induction is a primary effect that is AhR dependent. Western analysis confirmed that Socs2 and Cyp1a1 protein levels were also induced in CH12.LX cells. Promoter analysis revealed the presence of 4 dioxin response elements within 1000 bp upstream of the Socs2 transcriptional start site and Socs2 promoter regulated reporter gene activity is inducible by TCDD. These results indicate that Socs2 is a primary TCDD inducible gene that may represent a novel mechanism by which TCDD elicits its immunosuppressive effects. Supported by NIEHS grants ES11271 and ES02520.

1413 EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN EXPOSURE ON HYPOXIA DRIVEN GENES IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS.

K. N. De Abrew[1], K. K. Graven[1, 2] and B. Allen-Hoffman[1, 3]. *[1]Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI, [2]Medicine, University of Wisconsin, Madison, WI and [3]Pathology and Labarotary Medicine, University of Wisconsin, Madison, WI.*

Although the cellular responses to 2, 3, 7, 8-tetrachlorodebenzo-p-dioxin (TCDD) and the environmental stress of hypoxia are well studied, investigations of potential cross-talk are limited. Endothelial responses to these combined stresses are un-

known. Aryl hydrocarbon receptor nuclear translocator (ARNT) is a common dimerization partner for both aryl hydrocarbon receptor (AhR) and hypoxia inducible factor-1α (HIF-1α). Possible cross-talk between these two pathways was studied using gene array technology. Human lung microvascular endothelial cells were exposed to either 10nM TCDD, hypoxia (0%O_2) or both TCDD and hypoxia for 24 hrs and resulting RNA was analyzed using Affymetrix gene arrays. Statistical analysis of triplicate studies was performed using Affymetrix microarray suite and Affymetrix data mining tool. Twenty one genes were upregulated during exposure to TCDD and 3 of these genes contain known active XREs. Five hundred and eighteen genes were upregulated during exposure to hypoxia alone which included 18 known HRE-driven genes. A total of 365 genes were upregulated during exposure to both TCDD and hypoxia. Fourteen of the 18 HRE driven genes upregulated during exposure to hypoxia alone were also upregulated when exposed to both TCDD and hypoxia. Four HRE-driven genes were upregulated when exposed to hypoxia only but were not upregulated when exposed to both hypoxia and TCDD together. These 4 genes are insulin growth factor binding protein-3, erythroblastosis virus oncogene homolog-1, vascular endothelial growth factor receptor-1 and endothelial cell specific molecule. This data suggests that cross-talk exists between the hypoxia and TCDD mediated pathways but only for a specific subset of genes. This cross-talk may be cell type specific, may involve multiple transcription factors and may or may not occur at the level of transcription *via* the transcription factor ARNT.

1414 GENE EXPRESSION RESPONSES TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN MULTIPOTENTIAL C3H10T1/2 FIBROBLASTS EXHIBIT CLUSTER ACCORDING TO THE FUNCTIONAL STATE OF THE CELLS.

P. Hanlon[2] and C. Jefcoate[1, 2]. *[1]Pharmacology, University of Wisconsin, Madison, WI and [2]Molecular & Environmental Toxicology Center, University of Wisconsin, Madison, WI.*

Although the environmental contaminant 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) produces most of its toxicity through activation of the aryl hydrocarbon receptor (AhR), other signaling pathways are now recognized. Genomic profiling of multipotential C3H10T1/2 fibroblasts shows that TCDD produces very different effects on quiescent cells versus cells exposed to a mitogenic epidermal growth factor (EGF) or an adipogenic differentiation stimulus of insulin, dexamethasone, isobutylmethylxanthine, and the thiazolidinedione BRL46593, hereafter referred to as IDMB. In quiescent confluent cells, a 72 hour TCDD exposure changed the expression of over 1000 genes without visibly affecting the cells. These changes included several large functional gene clusters: increased cell cycle regulation and cell signaling mRNA and decreased glycolytic, mitochondrial, and ribosomal mRNA. TCDD completely reversed many gene responses induced by EGF (10 percent) and the IDMB (30 percent), while these stimuli after 24 hours suppressed the quiescent responses. By contrast, 33 genes responded to TCDD regardless of these other stimuli, including 4 genes with recognized AhR response elements (CYP1B1, CYP1A1, NQO1 and ALDH3A1) but also genes that could mediate broad secondary effects (glypican 1 and cysteine dioxygenase). 76 of 311 IDMB responses which were reversed by TCDD were similarly reversed by EGF (R^2=0.87) suggesting shared regulatory factors. 42 IDMB-responsive genes, including the adipogenesis regulator PPARγ, were cooperatively reversed by a combined EGF/TCDD addition in parallel with the need for cooperation in the suppression of differentiation. Most of these genes showed small but equal reversals by TCDD and EGF alone (R^2=0.73) which suggests mechanistic overlap in nuclear regulation. TCDD treatment did not alter EGF stimulated MEK/ERK/Rsk signaling.

1415 INHIBITION OF INTERFERON-γ INDUCED APOPTOSIS BY TCDD IN HUMAN PERIPHERAL LUNG EPITHELIAL CELLS.

M. Richards[1, 2], J. M. Martinez[2], D. M. Mays[2] and N. J. Walker[2]. *[1]Toxicology, University of North Carolina, Chapel Hill, NC and [2]Laboratory of Computational Biology and Risk Analysis, NIEHS, NIH, Research Triangle Park, NC.*

The major route of human exposure to 2, 3, 7, 8 tetrachlorodibenzo-p-dioxin (TCDD) and other dioxin-like compounds is through the ingestion of food containing animal fat. Minute mounts of TCDD can be found in meat, fish and dairy products since it bioaccumulates in the food chain. High levels of TCDD exposure in humans have been associated with lung cancer and chronic obstructive pulmonary disease. We have previously performed toxicogenomic microarray analyses that showed repression, following TCDD exposure, of at least four Interferon (IFN) inducible genes of interest, IFIT1, IFIT2, ISG15, and MX1. We hypothesize that TCDD interacts with the IFN signaling pathway and results in repression of IFN induced phenotypes in human peripheral lung epithelial (HPL1A) cells. The purpose of this study was to look at the anti-apoptotic effects of TCDD on human lung cells treated with IFN-gamma. Initially, the effect of TCDD on cell proliferation was investigated. HPL1A cell proliferation, as measured by the MTS cell *via*bility assay, returned to near basal levels with simultaneous exposure to TCDD [10 nM] and IFN gamma, at the following concentrations of IFN gamma: 1000, 5000, and 10, 000 Units/ml. Flow cytometry was used to quantify the level of apoptosis observed in the HPL1A cells after treatment with TCDD [10 nM], IFN gamma, and a combination thereof. Increased apoptosis levels were observed in HPL1A cells treated with IFN gamma, as compared to cells exposed only to vehicle. With concurrent TCDD exposure, the level of apoptosis we observed was attenuated. These data show that the IFN-gamma induced phenotypes in HPL1A cells, apoptosis and reduced cell proliferation, are inhibited following TCDD exposure. While the correlation between the repression of IFN regulated genes and the attenuation of phenotypic responses to IFN in HPL1A cells is not yet known, this study underscores the interference of TCDD on biological signaling pathways regulated by interferon.

1416 TCDD ATTENUATES VITAMIN A INDUCED GROWTH AND DIFFERENTIATION IN HUMAN LUNG EPITHELIAL CELLS.

D. M. Mays[1], J. M. Martinez[1], M. P. Richards[2, 1] and N. J. Walker[1]. *[1]Laboratory of Computational Biology and Risk Analysis, NIEHS, Research Triangle Park, NC and [2]Toxicology, University of North Carolina, Chapel Hill, NC.*

Human exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is linked to chronic obstructive pulmonary disease and lung cancer. Studies indicate that depletion of Vitamin A is also linked to cancer. Our laboratory used *in vitro* cell culture of non-malignant human alveolar type II epithelial cell line (HPL1A) to determine if TCDD alters or disrupts cell growth and differentiation caused by Vitamin A. To understand the method of action, cells were treated with TCDD 10 nM, Vitamin A (all trans retinoic acid) [10, 20, and 40 nM], or a combination of 10 nM TCDD and Vitamin A. The MTS assay was used to determine the amount of *via*ble cells after 48 hours of treatment. These experiments show that Vitamin A [10, 20, and 40 nM] significantly inhibits cell proliferation in HPL1A cells when compared to basal levels. Vitamin A decreases the percentage of *via*ble cells in a dose-dependent manner to 45% at 10nM, 34% at 20nM, and 26% at 40 nM. However, TCDD added in combination with Vitamin A attenuates Vitamin A inhibition of cell proliferation. The *via*ble cells increase to 50% at 10 nM, 59% at 20 nM, and 69% at 40 nM Vitamin A. Western blot analysis indicates that acute exposure to TCDD induces, while Vitamin A decreases, the differentiation marker human keratin 1/cytokeratin (HK1). Overall results suggest that TCDD attenuates the effect of Vitamin A on cell proliferation and also causes an altered state of differentiation as compared to Vitamin A. How TCDD disturbs Vitamin A homeostasis is unclear, but studies such as this and their relationship to gene expression will help to clarify the biochemical mechanism of action.

1417 HISTONE MODIFICATION IN ARYL HYDROCARBON RECEPTOR MEDIATED GENE TRANSCRIPTION.

A. Fretland[1, 2] and O. Hankinson[1, 2]. *[1]Department of Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, CA and [2]Jonsson Comprehensive Cancer Center, Los Angeles, CA.*

The ligand activated aryl hydrocarbon receptor (AHR) mediates a variety of transcriptional responses to environmental insults. Ligands include a diverse array of common environmental compounds, including 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). Upon ligand binding, AHR requires dimerization with the aryl hydrocarbon nuclear translocator (ARNT) protein to exert its toxic effects. The AHR/ARNT heterodimer is able to activate gene transcription of target genes, including cytochrome P4501A1 (*CYP1A1*). Activation of transcription by the AHR/ARNT complex requires the recruitment of numerous coactivators to the enhancer region of target genes. Certain coactivators probably cause local modifications of histone structure resulting in a more permissive state for gene transcription. Our laboratory has previously shown the recruitment of coactivators with histone modifying activity to the 5'-region of *mCYP1A1* in response to TCDD treatment. To more fully understand the transcriptional regulation of *CYP1A1* by TCDD, we have characterized the histone modifications induced by TCDD using the chromatin immunoprecipitation (ChIP) assay. The mouse hepatoma cell line, Hepa-1, was treated with 10 nM TCDD and cells were harvested at selected time points. ChIP assays using antibodies to specific modified histones and PCR primers specific for the 5'-region of *mCYP1A1* were used to identify histone modifications induced by TCDD. Our results show TCDD-dependent acetylation of lysine 14 and

lysine 23 of histone H3 and methylation of arginine 17 of histone H3 in the 5'-region of *CYP1A1*. We also observed a TCDD-dependent acetylation of lysines in histone H4 in the 5'-region of *CYP1A1*. These results suggest that histone modification in the 5'-region of *mCYP1A1* is an important step in the regulation of transcription in response to TCDD. Further studies will investigate which specific coactivators are responsible for each histone modification. Adrian Fretland is a postdoctoral trainee supported by USHHS Institutional NRSA #T32 CA09056.

1418 EVIDENCE OF AN INDUCTION THRESHOLD IN LIVER CELL LINES TREATED WITH 3, 3′, 4, 4′, 5-PENTACHLOROBIPHENYL.

C. Broccardo[1], R. E. Billings[1], L. S. Chubb[1], M. E. Andersen[2] and W. H. Hanneman[1]. [1]*Department of Environmental & Radiological Health Sciences, Colorado State University, Fort Collins, CO and* [2]*CIIT, Research Triangle Park, NC.*

Some chemicals are known to induce a threshold response versus a linear response at low doses. Further, the shape of the dose-response curve may vary depending on whether one examines response at a population or a single cell level. Often a population of cells will exhibit a graded response whereas on a single cell basis the response appears threshold or switch-like. By studying the single cell level of gene induction one may be able to determine the mechanistic basis for a switch-like response to a chemical. The goal of this research was to determine the mechanistic basis for the switch-like behavior of Cytochrome P450 1A1 (CYP1A1) to 3, 3′, 4, 4′, 5-pentachlorobiphenyl (PCB 126). CYP1A1 is the prototypical biomarker of activation of the aryl hydrocarbon receptor pathway. Two hepatoma cell lines, H4IIE and Hepa 1c1c7 cells were used in these studies. Both cell lines were analyzed *via* dose-response and time-course studies using quantitative real-time PCR. A sigmoidal dose-response curve for CYP1A1 induction was found in the two cell lines, describing the shape of the curve at the population level. Induction shifted to maximal between 2.5 x 10^{-11} to 2.5 x 10^{-9} M PCB 126 in both cell lines. Increased expression of CYP1A1 with time was observed, with the H4IIE cells showing maximal induction of 2000-fold by 16 h, and Hepa 1c1c7 cells showing 100-fold induction at 48 h. In order to study CYP1A1 induction on a single cell level, flow cytometry was employed. Both cell lines displayed a well-defined shift of induction from the ″off″ to the ″on″ state with increasing doses of PCB 126, with induced cells displaying similar levels of CYP1A1. The shape of the dose-response curve at the single cell level was sigmoidal. Moreover, immunocytochemistry studies also support this conclusion. Thus, in conclusion, these data support the hypothesis that PCB 126 induces CYP1A1 in an all-or-none fashion, indicating a threshold at the single cell level.

1419 INHIBITION OF AROMATASE ACTIVITY BY METHYL SULFONYL PCB METABOLITES IN H295R CELLS AND IN PRIMARY CULTURE OF HUMAN MAMMARY FIBROBLASTS.

M. Heneweer[1], M. van den Berg[1], P. C. de Jong[2], A. Bergman[3] and J. T. Sanderson[1]. [1]*Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands,* [2]*Department of Internal Medicine, St. Antonius Hospital, Nieuwegein, Netherlands and* [3]*Department of Environmental Chemistry, Stockholm University, Stockholm, Sweden.*

Aromatase activity in human mammary tissue is mainly located in fibroblasts and a positive feedback loop is thought to be responsible for accelerating estrogen-responsive breast tumor growth through stimulation of estrogen synthesis. Tumor cells secrete factors such as PGE2 and IL-6 that are able to upregulate aromatase gene expression in fibroblasts surrounding tumors. Various *in vitro* studies have shown that pharmacological agents and xenobiotics are able to interfere with the aromatase enzyme. In this study we compared the aromatase expressing human cell line H295R with primary human mammary fibroblasts as *in vitro* screening tools for aromatase inhibition by methyl sulfonyl PCB metabolites. Both cell types were exposed for 24h to 100 nM dexamethasone (DEX) to induce aromatase activity. Levels of aromatase induction in fibroblasts differed greatly among patients, varying from 20- to 650-fold; aromatase activity was induced about 4-fold in H295R cells. In both cell types the EC_{50}-value of DEX was around 20 nM. Since DEX upregulated aromatase activity in a highly effective manner in both cell types, we hypothesized that methyl sulfonyl PCB metabolites, which have anti-glucocorticoid properties, would decrease DEX induced aromatase activity. A 24h-exposure to 100 nM DEX, together with 0.1-10 μM 3-$MeSO_2$-CB-91, 3-$MeSO_2$-CB-101, 3-$MeSO_2$-CB-132, 4-$MeSO_2$-CB-132, 4-$MeSO_2$-CB-149, and 3-$MeSO_2$-CB-174 resulted in dose-dependent reduction of aromatase activity in both cell types with IC_{50}-values around 1 μM. The anti-glucocorticoid RU486 inhibited aromatase activity (IC_{50}-value of 5 nM), indicating that inhibition was glucocorticoid receptor mediated. Relative potencies of methyl sulfonyl PCB metabolites were between 2 and 3 orders of magnitude less compared to RU486. Catalytic inhibition and cytotoxicity were not observed at tested concentrations.

1420 2, 2', 4, 4'-TETRACHLOROBIPHENYL STIMULATES RELEASE OF ARACHIDONIC ACID FROM NEUTROPHILLIC HL-60 CELLS.

S. Bezdecny[1, 2, 3], R. A. Roth[1, 2, 3] and P. E. Ganey[1, 2, 3]. [1]*Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI,* [2]*Institute for Environmental Toxicology, Michigan State University, East Lansing, MI and* [3]*National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI.*

Polychlorinated biphenyls (PCBs) affect a number of cellular systems including neutrophils. Effects in rat neutrophils are mediated by ortho- substituted, non-coplanar PCBs like 2, 2', 4, 4'-tetrachlorobiphenyl (2244-TCB). The objective of these experiments was to explore further the mechanisms by which 2244-TCB affects neutrophil function using a human-derived cell line. The promyelocytic leukemia (HL-60) cell line was differentiated with dimethylsulfoxide to a neutrophil-like phenotype. The effect of 2244-TCB and a coplanar congener of the same molecular weight, 3344-TCB, on release of arachidonic acid were examined. In addition, alterations in expression of mRNA for cyclooxygenase-2 (COX-2) were evaluated using real-time PCR. Treatment of differentiated HL-60 cells with 30 μM 2244-TCB caused a time-dependent increase in COX-2 mRNA expression that was maximal after 30 minutes of exposure and returned to baseline after 90 minutes of exposure. Treatment with 30 μM 3344-TCB did not increase COX-2 mRNA levels. 2244-TCB increased release of [^{3}H]-arachidonic acid ([^{3}H]-AA) from labeled neutrophillic HL-60 cells in a concentration-dependent manner. The inhibitor of calcium-independent phospholipase A_2 (iPLA2), bromoenol lactone, significantly decreased the release of [^{3}H]-AA from cells treated with 30 μM 2244-TCB. PCB-stimulated release of [^{3}H]-AA was unaffected by pretreatment with either an inhibitor of secreted PLA_2 or an inhibitor of the MAP kinase p38. Treatment with 30 μM 3344-TCB did not increase release of [^{3}H]-AA. These results demonstrate that the noncoplanar PCB congener 2244-TCB upregulates COX-2 and increases $iPLA_2$ activity in neutrophillic HL-60 cells, whereas the coplanar PCB congener 3344-TCB lacks these effects. (Supported by ESO4911.)

1421 A NON-COPLANAR POLYCHLORINATED BIPHENYL INDUCES OXIDATIVE STRESS AND CELL DEATH IN A MID-BRAIN DOPAMINERGIC CELL LINE.

R. F. Seegal[1], A. G. Kanthasamy[2] and S. Kaul[2]. [1]*Wadsworth Center, New York State Department of Health, Albany, NY and* [2]*Department Biomedical Sciences, Iowa State University, Ames, IA.*

Polychlorinated biphenyls (PCBs) are associated with developmental deficits in children and behavioral and neurochemical changes in laboratory animals. Following *in vivo* and *in vitro* exposure PCBs reduce dopamine (DA) concentrations and elevate intra-neuronal or media concentrations of DA. These changes may be mediated by inhibition of monoamine transporters, including the DA transporter and the vesicular monoamine transporter. To better understand the consequences of PCB exposure on neuronal function, we exposed the rat mesencephalic dopaminergic neuronal cell line (N27) to a non-coplanar PCB congener (2, 3, 6, 2', 5'-PtCB, 10, 30 and 100 μM) and measured changes in oxidative stress (using dihydroethidine and flow cytometry) and cell death (using the Sytox Green Cytotoxicity assay). PtCB resulted in significant time and dose dependent increases in ROS formation with elevations seen at 30 and 100 μM and as early as five min after exposure. Pretreatment with a cell permeable superoxide dismutase mimetic MnTBAP (5 μM for 15 min) significantly attenuated PtCB-induced ROS production, indicating that PtCB predominantly generates superoxide species. Cell death was measured in serum free media following 1, 2 or 4 hr exposure to PtCB with significant elevations seen at 30 and 100 μ M after 2 and 4 hr. These results demonstrate that ROS formation occurs prior to cell death and may thus contribute to loss of cell *via*bility. Most importantly, PtCB induced ROS formation in N27 cells was greater than that reported following exposure to the same concentrations of MPP+, the active metabolite of MPTP. Taken together, these results provide continuing support for the hypothesis that PCBs may contribute to mid-brain DA neuronal cell loss and thus ultimately play a role in the etiology of parkinsonism. Supported by NIH grant ES11263 and EPA grant 829390 to RFS and NIH grants ES10586 and NS45133 to AGK.

1422 PHARMACOKINETICS AND IMPROVED ORAL BIOAVAILABILITY OF TWO NANOSTRUCTURED DRUG CRYSTALS: COMPARISON OF PARTICLE ENGINEERING TECHNOLOGIES.

S. A. Saghir, G. B. Kupperblatt, D. A. Markham, T. L. Rogers, C. J. Tucker, J. E. Hitt and E. J. Elder. *The Dow Chemical Company, Midland, MI.*

As many as 60% of drug candidates exhibit poor water solubility in physiochemical testing and poor absorption from the GI tract. Poor oral absorption limits the usefulness of a drug. In order to overcome this limitation, one of the emerging tech-

niques is particle engineering of the drug crystals to increase the surface area-to-volume ratio and adding excipients to prevent reaggregation. Nanosizing drug crystals traditionally involves milling, however other emerging techniques are more effective. The Dow Chemical Company has developed a number of technologies to produce novel nanostructured drug particles that enhance oral bioavailability of poorly water soluble drugs. Early determination of ways to enhance oral bioavailability of a drug candidate is important in order to generate accurate kinetic and toxicological data. Two poorly absorbed drugs (danazol and ketoconazole) were processed by various technologies and excipients and evaluated in beagle dogs. Male dogs were dosed orally with danazol (200 mg/dog) or ketoconazole (100 mg/dog) in capsule or tablet form and blood collected from the jugular vein at 0, 0.5, 1, 2, 4, 8, 12, and 24 h post-dosing. Concentration of parent drugs were determined in plasma by LCMS. WinNonlin was used for kinetic analysis. The technologies evaluated for danazol showed 15-64 times higher AUC than the micronized drug (as received). Two technologies, emulsion and controlled precipitation, were effective in enhancing the oral bioavailability of danazol by 64 and 51 times, respectively. The Tmax of the nanostructured and micronized drug remained same, however plasma t1/2 of the nanostructured danazol formulations was 2-3 times longer than micronized drug, similarly Cmax of the nanostructured drug was 18-45 fold higher. The nanostructured ketoconazole showed 2-8 times higher AUC and Cmax, no change in Tmax and plasma t1/2 was observed. The oral bioavailability of ketonazole prepared by emulsion and controlled precipitation technologies was significantly higher than controls.

1423 γ-SECRETASE (γ-SEC) INHIBITORS THAT MODULATE NOTCH PROCESSING CAUSE INTESTINAL GOBLET CELL METAPLASIA (GM) AND SPLENIC MARGINAL ZONE LYMPHOID DEPLETION. PART I, PATHOLOGY.

P. Ciaccio[1], J. McKay[2], C. Louden[1], C. Dagenais[1], R. Gadient[1], Q. Jiang[1], L. Foster-Brown[1], F. Pognan[1], T. Piser[1], J. Stahl[1] and B. Greenberg[1]. *[1]AstraZeneca Pharmacology R&D, Wilmington, DE and [2]AstraZeneca Pharmacology R&D, Alderley Park, United Kingdom.*

Amyloid deposits constitute a primary neuropathological hallmark within vulnerable regions of the Alzheimer patient brain. These deposits consist of Aβ amyloid peptide, derived from amyloid precursor protein (APP) by a series of proteolytic events, the last of which is mediated by an enzyme complex called γ-sec. In addition to APP, γ-sec cleaves a variety of type-1 transmembrane proteins, including the Notch receptor. γ-Sec inhibitors reduce the cleavage of such substrates. This relaxed substrate specificity could potentially lead to untoward mechanism-based side-effects for e.g., signals transduced from the intracellular domain of Notch play an essential role in cell fate decisions. Thus, it was anticipated that γ-sec inhibitor treatment will alter differentiation of cell populations in tissues whose architecture is governed by Notch signaling. To explore this hypothesis, we selected compounds from 3 chemical series that lower Aβ amyloid *in vivo* and which variously inhibit Notch *in vitro* (Notch IC50): an arylsulfonamide (AS) (250 nM), a dibenzazepine (DBZ)(2 nM), and a benzodiazepine (BZ)(1 nM). Rats were dosed ip for up to 5 days at 0, 10, 30 and 100 μmol/kg bid. The AS produced no detectable *in vivo* toxicity despite relatively high exposure. The 2 potent inhibitors caused dose-dependent intestinal GM with villous stunting and splenic marginal zone lymphoid depletion (SMLD). There was a temporal progression of intestinal histopathology with mid-dose BZ. Small intestinal crypt and large intestinal glandular epithelial apoptosis was observed on days 1-4, followed by GM on days 2-5 and crypt epithelial and glandular epithelial regenerative hyperplasia on days 4-5. SMLD occurred from day 2. While these lesions are consistent with Notch pathway alteration, the absence of toxicity in animals dosed with AS suggests that it is possible to inactivate the processing of γ-sec substrates selectively *in vivo*.

1424 OTOTOXICITY STUDY WITH CIPRODEX STERILE OTIC SUSPENSION® IN THE GUINEA PIG.

L. E. Lemke[1], D. H. McGee[1], D. M. Prieskorn[2], R. A. Altschuler[2], R. B. Hackett[1] and J. M. Miller[2]. *[1]Toxicology, Alcon Research, Ltd.,, Fort Worth, TX and [2]Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI.*

This study investigated the ototoxic potential of a combination of Ciprofloxacin 0.3% and Dexamethasone 0.1% (CiproDex®) when administered directly to the round window niche of the guinea pig middle ear. Animals from four test groups (n=10) and two control groups (n=5) were all confirmed to have hearing within normal limits before selection. Positive and negative control groups were treated with 10% neomycin or normal saline, respectively. Test groups were treated with CiproDex Vehicle, CiproDex, Ciprofloxacin 1.0% plus Dexamethasone 0.3%, or Ciprofloxacin 0.3%. Each subject was implanted with an injection port mounted on the skull and attached to a cannula which passed through the temporal bone into the bulla and terminated at the round window niche. Test or control article (10 μL) was injected through the cannula BID for 4 weeks. Administration of test and control articles to the round window niche provided a rigorous test of the compatibility of these test and control articles for otic administration by ensuring access to this portal into the inner ear. Auditory brainstem responses were collected at 2, 8 and 16 kHz after 2 and 4 weeks of dosing. At the termination of the study middle ear tissues were examined grossly and histopathology was performed on inner ear tissues. No statistically significant hearing losses were observed in any of the test groups at either timepoint. Histopathologic examination of the Organ of Corti for the CiproDex Otic, other test groups and saline control each revealed hair cell counts within normal limits. A significant elevation in hearing threshold was observed in animals receiving Neomycin. Profound hair cell loss, corresponding to loss of auditory function was observed in the neomycin positive control group only. The results of this study support the conclusion that CiproDex Sterile Otic suspension is safe for administration to the open middle ear cavity.

1425 THG213.29: SAFETY OF A NOVEL PEPTIDE FOR TREATMENT OF ACUTE RENAL FAILURE.

G. Washer[1], E. Ferdinandi[1], K. High[1], K. Peri[1], J. Praslicka[2], J. Laliberte[2], C. Pare[3], C. Thompson[3] and S. Cote[4]. *[1]Theratechnologies Inc., Montreal, QC, Canada, [2]ITR, Montreal, QC, Canada, [3]CTBR, Montreal, QC, Canada and [4]Anapharm, Quebec City, QC, Canada.*

THG213.29 and its shortmers: THG213.291 (N-1) and THG213.292 (N-2), are potent EP4-receptor antagonists. EP4 mRNA is detected in renal glomeruli & collecting ducts. Potency of THG213.29 was established by *in vitro* screens and *in vivo* efficacy has been demonstrated in rat models of ischemic renal failure and acute tubular necrosis. Acute IV toxicity & toxicokinetics were studied in rats & dogs. Escalating doses, up to 200 mg/kg in rats and 50 mg/kg in dogs, indicated no effect levels of 30 (rat) and 10 mg/kg (dog). In rats, body weight, clinical pathology and renal pathology effects were found at 100 mg/kg and mortality occurred at ∝150 mg/kg. In dogs, transient dose-related reductions in blood pressure and alterations in urine volume & electrolytes occured at 20 & 50 mg/kg. Toxicokinetics, determined by LC-MS/MS plasma analysis, indicated dose-related increased exposure (based on Cmax & AUC) to THG213.29 and shortmers, in both species. Low THG213.29 levels (with high levels of shortmers) 10 min post dose, indicated rapid cleavage of parent. Renal & cardiovascular IV safety pharmacology studies were conducted in rats & dogs, respectively. Doses of 5 & 10 mg/kg to dogs had no effect on hemodynamics or electrocardiograms (ECG). At 20 & 50 mg/kg there were transient, dose-related reductions in blood pressure and increases in heart rate, but no ECG changes. In rats, effects at 1 & 10 mg/kg were limited to slight increases in creatinine clearance. At 100 mg/kg there were also transient alterations in Ca, urea, glucose & protein excretion. Mouse lymphoma & Ames tests were negative for genotoxicity. These studies established a safety profile for THG213.29 and its metabolites. Effects were either transient & related to the pharmacological action, or were at a high multiple of the clinical dose, providing an acceptable safety margin for initiation of human trials.

1426 SAFETY EVALUATION OF TELBERMIN IN RABBITS USING A FULL THICKNESS EXCISIONAL DERMAL WOUND MODEL.

T. R. Gelzleichter[1], A. L. Fuller[3], N. Pelletier[2], S. M. Eppler[2], D. Fei[2] and S. Brignoli[2]. *[1]Safety Assessment, Genentech, South San Francisco, CA, [2]Develepment Sciences, Genentech, South San Francisco, CA and [3]Covance, Madison, WI.*

Telbermin (recombinant human vascular endothelial growth factor-165) is currently in clinical trials as an investigational drug for the treatment of diabetic foot ulcers. The following study was designed to evaluate the safety of telbermin using a full thickness excisional dermal wound model in rabbits. Dermal wounds (2.5 x 2.5 cm^2)were surgically created on the dorsal skin of New Zealand white rabbits. Rabbit wounds were treated by topical administration of telbermin in 3 percent methyl cellulose at a concentration of 0, 0.08, 0.8, or 1.7 mg/mL in a 0.6 mL delivery volume. Treatments were repeated every 2 or 3 days for a total of 12 administrations over 4 weeks. Following each administration, wounds were covered with semi-occlusive dressing and protected with a wound jacket. All doses were well tolerated and no adverse effects were observed that were directly attributable to telbermin. Dose site infections did occur more often in telbermin-treated rabbits, but the incidence was low and therefore difficult to attribute directly to telbermin administration. All infections were readily treatable with antibiotics. Systemic absorption of telbermin was relatively slow with peak serum concentrations detected three hours after telbermin administration. Only a small fraction of the total dose (0.1 percent) was adsorbed systemically. The maximum mean telbermin plasma concentration did not exceed 400 pg/mL for any of the dose groups at any of the measured time

points, however, peak values were roughly six-fold higher than the mean endogenous plasma level of 66±16 pg/mL. Assuming a similar rate of systemic absorption occurs in humans, telbermin treatment is not anticipated to significantly affect plasma VEGF concentrations in clinical use. By week 4, 83 percent of rabbits developed antidrug antibodies; therefore, longer-term safety studies in this model may not be feasible. Overall, telbermin was safe and well tolerated at all doses tested throughout the study.

1427 TOXICOLOGICAL PROFILE OF AVI-4020, AN ANTISENSE MORPHOLINO OLIGOMER FOR TREATMENT OF WEST NILE VIRAL INFECTIONS.

V. Arora, M. L. Cate, M. T. Reddy, D. H. Mason and P. L. Iversen. *AVI BioPharma, Inc., Corvallis, OR.*

Antisense oligomers can selectively inhibit target gene expression and have great therapeutic potential. AVI-4020 is an antisense phosphorodiamidate Morpholino oligomer (PMO) targeted to the translation initiation region of the open reading frame of West Nile virus. The toxicological profile of AVI-4020 has been examined in rodents and non-human primates. ● A 28-day once-daily IP administration study in Sprague-Dawley rats identified no significant toxicological concerns. A slight elevation of liver:body weight ratio was observed in male rats (two-tailed p value = 0.0179). ● A 7-day twice-daily bolus IV administration study in cynomolgus monkeys identified no significant toxicological concerns. Liver:body weight ratio changes were not observed in either sex. ● Biodistribution studies following bolus IV administration in rats indicated that organs with the largest distribution were kidney, followed by liver. Smaller quantities were recovered from lungs, spleen and heart. AVI-4020 residence time in these organs was rather prolonged and not determined by plasma pharmacokinetics. ● An examination of blood coagulation indices revealed no changes in prothrombin time or activated partial thromboplastin time following twice-daily administration for five days in Sprague-Dawley rats. ● Degradative metabolism was not observed. Only full-length oligomers were recovered from various tissues and excreta in rat studies. ● Urine and feces were the major routes of elimination in rat studies. ● Full-length oligomer was recovered in cerebrospinal fluid following bolus IV administration in rats at time points well beyond its presence in plasma. This observation is remarkable for a macromolecule (FW=7624) yet desirable for treatment of West Nile disease, which often has central nervous involvement. The toxicity profile of the anionic PMO AVI-4020 is remarkably different when compared to multiple reports for the negatively-charged phosphorothioate class of antisense agents. This drug is currently being evaluated in a Phase I clinical trial.

1428 GENOTOXICITY STUDIES WITH PURE *TRANS*-CAPSAICIN.

S. Chanda[1], G. Erexson[2], C. Riach[3], D. Innes[3], F. Stevenson[3], H. Murli[2] and K. Bley[1]. *[1]Toxicology, NeurogesX, Inc., San Carlos, CA, [2]Covance Laboratories, Vienna, VA and [3]Inveresk Research, Tranent, Scotland, United Kingdom.*

Both positive and negative effects have been found in classical genetic toxicology assays with capsaicin. However, the capsaicin tested in most studies has been derived from pepper plant extracts, which is likely to display varying degrees of purity and possibly diverse impurity profiles. Therefore, the objective of the series of studies reported here was to test the genotoxic potential of pure, synthetic *trans*-capsaicin (the only naturally- occurring stereoisomer of capsaicin), using four genotoxicity assays widely used to evaluate drug substances. These included the Ames, mouse lymphoma cell mutation, mouse *in vivo* bone marrow micronucleus and chromosomal aberration in human peripheral blood lymphocytes assays. In the Ames assay, pure capsaicin was not mutagenic to *Salmonella typhimurium* or *Escherichia coli* when dissolved in dimethylsulfoxide and tested at concentrations extending into the cytotoxic range. Capsaicin was weakly mutagenic in mouse lymphoma L5178Y cells, in the presence of S9 mix, when dissolved in dimethylsulfoxide and tested at concentrations extending into the cytotoxic range. Limited evidence for very weak activity was also obtained in the absence of S9 mix. Capsaicin did not induce micronuclei in bone marrow cells when tested to the maximum tolerated dose of 800 mg/kg/day in male and 200 mg/kg/day in female CD-1 mice using a 0 hr plus 24 hr oral dosing and 48 hr sampling regimen. Finally, capsaicin did not induce structural or numerical chromosomal aberration when evaluated for its ability to induce clastogenicity in blood lymphocytes. Taken together, these data suggest that the genotoxic potential of pure *trans*-capsaicin is very low, especially as the clinical significance of weak mutagenicity in the mouse lymphoma assay for catechol-moiety containing compounds is unclear. Moreover, the different genotoxicity profiles of pure *trans*- capsaicin and purified chili pepper extracts suggest that the purity and source of capsaicin should always be an important consideration for toxicological evaluations.

1429 GLYCOGEN SYNTHASE KINASE (GSK3) INHIBITORS STIMULATE CELLULAR PROLIFERATION VIA WNT SIGNALING PATHWAY *IN VITRO* AND *IN VIVO*.

C. E. Ruegg[1], B. R. Berridge[2], D. E. Watson[1], D. K. Monteith[1], B. Li[1], J. L. Blackbourne[1], S. W. Queener[3], C. M. Love[3], J. W. Ryder[3], R. A. Owens[3], T. E. Eessalu[3], E. A. Misener[5], T. A. Engler[4] and J. T. Brozinick[5]. *[1]Lead Optimization Toxicology, Lilly Research Labs, Greenfield, IN, [2]Lead Optimization/Investigative Pathology, Lilly Research Labs, Greenfield, IN, [3]Lead Optimization Biology, Lilly Research Labs, Indianapolis, IN, [4]Discovery Chemistry, Lilly Research Labs, Indianapolis, IN and [5]Discovery Biology, Lilly Research Labs, Indianapolis, IN.*

GSK3 is responsible for phosphorylating several protein substrates including glycogen synthase leading to its inactivation. Inhibitors of GSK3 could have therapeutic benefits in controlling blood glucose levels by allowing glycogen synthase to shunt glucose into glycogen. However, GSK3 is also integral to the Wnt signaling pathway where inhibition leads to stabilization and translocation of B-catenin into the nucleus resulting in transcription of several factors leading to cell proliferation. In these studies we show that selective small molecule inhibitors of GSK3 result in cellular proliferation both *in vitro* and *in vivo*. This proliferative effect correlates well with the inhibitory potency and exposure of the GSK3 inhibitors and has been associated with increases in B-catenin, cyclin D1, and mitotic figures within liver, kidney, adrenal, and bone tissues. These studies suggest that GSK3 inhibitors targeted to regulate glucose control may need to either avoid interaction with GSK3 in the Wnt signaling pathway, or have an adequate margin of safety relative to these proliferative effects.

1430 DRUG-INDUCED CARDIOMYOPATHY IN RATS: MORPHOGENISIS AND IDENTIFICATION OF POTENTIAL BIOMARKERS.

J. D. Moehlenkamp, W. A. Kelly, R. L. Kowalski, W. M. Peden, W. J. Saunders and G. D. Pilcher. *Drug Safety Evaluation, Bristol-Myers Squibb Company, Mt. Vernon, IN.*

Serum levels of cardiac troponins and lactate dehydrogenase isoenzymes have been shown to be increased after insult with cardiotoxicants (i.e., doxorubicin) and are used clinically as markers of cardiac damage. During 1-month toxicity studies with BMS-505130, cardiomyopathy was observed in male and female rats at 200 mg/kg/day. The cardiomyopathy, similar to the spontaneous change that commonly affects aged rats, was characterized by the presence of a combination of degenerate and/or necrotic muscle fibers, subacute inflammation, proliferation of muscle fiber nuclei, and/or muscle fiber regeneration. Studies were conducted to identify potential biomarkers and further characterize the dose- and time-dependence of cardiomyopathy in rats after BMS-505130 treatment. In the initial study, male and female rats were treated at 75 and 125 mg/kg/day for 1-month. Minimal to mild cardiomyopathy, similar to that observed on the previous study, was observed in females after the 1-month dosing period. Additional findings at 75 and 125 mg/kg/day included minimal to mild increases in lactate dehydrogenase (LDH) isoenzymes LDH-1 and LDH-2. The largest increases in LDH-1 and LDH-2 were in the high dose females where the cardiomyopathy was noted also. In a second timecourse study, females rats were administered BMS-505130 at 200 mg/kg/day and evaluated for changes in serum biomarkers and evidence of myocardial pathology on day 1 at 6 and 24 hours postdose, and on study days 4, 8, 15, and 29. Minimal to moderate cardiomyopathy was observed on day 8 with increasing severity over time. In correlation with the hisopathologic changes, time-dependent increases in troponin T and LDH-1 and LDH-2 were evident at day 8 and remained elevated for the duration of the study. The results of these studies demonstrate that the cardiomyopathy induced by BMS-505130 is both dose- and time-dependent and suggest that cardiac troponin T and LDH-1 and LDH-2 are potentially useful serum biomarkers of drug-induced myocardial damage.

1431 γ-SECRETASE INHIBITORS THAT MODULATE NOTCH PROCESSING CAUSE INTESTINAL GOBLET CELL METAPLASIA. PART II: GENE EXPRESSION.

J. Milano[1], S. Matis[2], F. Pognan[1] and P. Ciaccio[1]. *[1]Safety Assessment, AstraZeneca Pharmaceuticals, Wilmington, DE and [2]EST Infx, AstraZeneca Pharmaceuticals, Wilmington, DE.*

γ-Secretase (γ-sec) is an intramembranous multi-subunit aspartyl protease that catalyzes the final cleavage step of amyloid precursor protein (APP). The resulting extracellular domain, β-amyloid accumulates in the brain and is associated with cognitive impairment in Alzheimer's disease (AD). Inhibiting γ-sec may prevent the deposition of β-amyloid and is sought as a preventative therapy for AD. However, many γ-sec substrates have been identified, including the Notch receptor. Inhibitors of γ-sec effectively reduce the cleavage of such substrates. This relaxed substrate specificity may lead to deleterious mechanism-based effects. Signals trans-

duced from the intracellular domain of Notch play an essential role in cell fate decisions. Knock out (KO) studies of the transcriptional regulators Hes1 and Math1, which are downstream of the Notch signal, illustrate this pathway's role in the regulation of intestinal stem cell differentiation. A recent report showed that adipsin (ADN) may be under Hes1 control and is a potential biomarker for intestinal goblet cell metaplasia. (Ryan et al. JBC in Press). We dosed Han Wistar rats with 3 γ-sec inhibitors ip at 0, 10, 30 and 100 μmol/kg/bid for 5 days with a benzodiazepine (BD), and a dibenzazepine (DBZ) both known to interrupt the Notch signal, and an arylsulfonamide (AS) that shows weaker Notch signal interruption. Using gene expression profiling we detected several deregulated factors that are consistent with Hes-1 KO data. We confirmed the up-regulation of ADN mRNA in intestinal tissue by DBZ and BZ treatment but not AS treatment. However, the induction of Math1 was markedly higher than ADN at earlier time points and at lower dose with DBZ and BZ. This up-regulation preceded the appearance of goblet cell metaplasia in the crypts. Also, these elements showed a temporally distinct response to γ-sec inhibition. These data suggest an alternative control mechanism for ADN that is not limited to Notch signal interruption but may also be a product of cell differentiation in the metaplastic lesion.

1432 SOCIALIZATION AND ENVIRONMENTAL ENRICHMENT IN LONG-TERM TOXICITY STUDIES IN MICE.

L. Bonnet[1], J. Golfier[1], B. Heritier[1] and J. Descotes[2]. *[1]MDS Pharmacology Service, L'Arbresle, France and [2]Poison Center, Lyon, France.*

The forthcoming European regulations (GT 123) require that more appropriate housing conditions are used during toxicity studies. This study was performed to compare various housing conditions in groups of 12 male and 12 female B6C3F1 mice for 13 weeks. The reference group was housed singly in stainless-steel cages. The four other groups were housed in threes in enriched cages of a size compliant with the new European guidelines. Of these, two were kept in stainless-steel cages containing either a small plastic box or a mat of hemp fibres for nesting, and two in plastic cages with sawdust and either a small plastic box or a mat of hemp fibres. Food consumption was measured weekly. Hematology and clinical chemistry endpoints were assessed at the end of the study. Clinical observations were performed daily and included a careful examination of the animal behavior. Necropsy was performed at the end of the study. Marked wounds were observed in males housed in plastic cages, which led to the isolation of some animals and were associated with increased neutrophil counts. Body weight gain was higher and food consumption lower in males housed in groups, whereas both body weight gain and food consumption were lower in females housed in groups. The mean relative weight of adrenals in females housed in groups in metallic cages with a mat was statistically lower than in animals of the reference group. The mean relative thymus weight of females housed in groups in plastic cages was statistically higher than in animals of the reference group. In addition, serum corticosterone levels at the end of the study were statistically lower in animals housed in plastic cages. No other changes were noted. Overall, it appears that the most appropriate housing conditions for toxicity studies in mice are group-housed animals in metallic cages with a vegetable fibre mat as enrichment.

1433 BILIARY EXCRETION OF ^{14}C-DIAZEPAM IN MALE RATS AFTER PRETREATMENT WITH TACROLIMUS.

L. Faure[1], P. Vignand[1], A. Raynard[1], F. Pasello-Legrand[1] and J. Descotes[2]. *[1]MDS Pharmacology Services, L'Arbresle, France and [2]Poison Center, Lyon, France.*

The immunosuppressant tacrolimus is known to impair biliary excretion. As diazepam is extensively eliminated by this route in rats, the present study was conducted to determine whether acute or repeated administrations of tacrolimus influence the biliary excretion of ^{14}C diazepam in male Sprague-Dawley rats. Anesthetized rats were catheterized for subsequent bile collection as freely moving animals. One extremity of the catheter was inserted into the bile duct toward the liver and the other extremity into the duodenum. The catheter was tunneled from the neck of the animal with an exteriorized loop assuring a free flow of the bile flow during the post-operative recovery of the animals. On day 2 post-surgery, approximately 80% of the catheterized animals had liver enzymes within the normal range. Only these animals were included in the study. A group of 12 rats received an intravenous injection of 10 mg/kg ^{14}C-diazepam (corresponding to 1.11 MBq/kg) and the total and sequential amounts of diazepam excreted in the bile were measured over 72 hours. Diazepam biliary excretion accounted for 80% of elimination in these animals. On days 7 and 8, these rats were given an oral dose of 3 mg/kg tacrolimus followed by the same intravenous dose of ^{14}C-diazepam. Another group of 10 rats were treated with an oral daily dose of 3 mg/kg tacrolimus for 3 weeks and catheterized one week before receiving a single intravenous administration of ^{14}C-diazepam. No changes in diazepam biliary excretion were observed following either an acute or repeated-dose administration of tacrolimus. Nevertheless, this study demonstrates the feasibility of drug biliary excretion investigations under GLP conditions as a complement to regulatory acute or repeat dose toxicity studies.

1434 THE ACUTE SAFETY PHARMACOLOGY PROFILE OF CM-2, 239: A NEW THERAPEUTIC AGENT FOR THE TREATMENT OF MILD COGNITIVE IMPAIRMENT AND ATTENTION DEFICITS.

D. R. Helton[1], D. B. Fick[1], S. Nadjsombati[2], E. Pfadenhauer[1], M. Piacente[2], J. P. Sharp[1] and P. Mazur[2]. *[1]Cenomed, Inc., Lake Forest, CA and [2]Biological Test Center, Irvine, CA.*

CM-2, 239 (4-[3-(4-Oxo-4, 5, 6, 7-tetrahydroindol-1-yl)propionylamino]benzoic acid (ethyl ester) is a novel heterocyclic compound that is currently in pre-clinical development for the treatment of mild cognitive impairment and attention deficits in aging, and for the treatment of attention deficit hyperactivity disorder (ADHD) in children. CM-2, 239 has been shown to be efficacious in animal models of memory and attention, as well as in ADHD models. The purpose of the current series of studies was to characterize the acute secondary pharmacological activity (safety pharmacology) of CM-2, 239 in tests evaluating central nervous system (CNS), cardiovascular and respiratory function. The clinically effective ADHD drug, methylphenidate (Ritalin) was evaluated as a reference agent. Both compounds were administered orally in Sprague-Dawley rat or Beagle dogs. Methylphenidate (1, 3 and 10 mg/kg) produced significant stimulatory effects in CNS motor and reactivity measures in both rats and beagle dogs. Acute transient alterations were also seen in both cardiovascular (dogs) and respiratory endpoints (dogs and rats). In contrast, CM-2, 239 produced no significant alterations in CNS, cardiovascular, or respiratory function in these species at doses up to 100 mg/kg or 100 times the anticipated clinical dose. Evaluation of the safety of CM-2, 239 is currently ongoing in age appropriate models. These data support the safety of CM-2, 239 clinical use as a novel therapeutic agent for MCI, ADD and ADHD without the side effects and stimulatory properties of the currently prescribed medications.

1435 BIOAVAILABILITY OF INSULIN FOLLOWING PULMONARY ADMINISTRATION TO THE BEAGLE DOG VIA A SURGICALLY PREPARED TRACHEOSTOME.

G. Cow and P. McDonald. *Inhalation Toxicology, Inveresk Research, Edinburgh, United Kingdom.* Sponsor: R. Greenough.

Administration of drugs to experimental animals in inhalation studies results in a large proportion of the drug depositing in the oral cavity or upper respiratory tract. Where the absorption of drugs from the respiratory tract needs to be evaluated, a method of direct delivery to the lower respiratory tract is necessary to provide an estimation of pulmonary bioavailability. One method would be to dose Beagle dogs under general anaesthesia and administer *via* tracheal intubation. A more practical method (without anaesthesia) is surgical preparation of a permanent tracheostome to give access to the lower respiratory tract and allow direct pulmonary administration. This technique allows repeated use of the same animals for cross-over studies and testing of multiple drug formulations. The objective of this study was to investigate the bioavailability of 3 dry powder insulin formulations (A, B and C) following pulmonary administration to 4 male Beagle dogs *via* a surgically prepared tracheostome. Each formulation was administered *via* an endotracheal tube inserted into the tracheostome and the dry powder delivered as a bolus aerosol using a PennCenturyTM device. To demonstrate bioavailability of the pulmonary delivered insulin, endogenous insulin was suppressed over the experimental period by continuous intravenous administration of somatostatin (0.5 μg/kg/min after an initial loading bolus dose of 37.5 μg/kg). For each test formulation, systemic insulin and glucose levels were determined from serial blood samples taken over an approximate 15 hour period. Results indicated that pulmonary administration, *via* a tracheostome, of the 3 dry powder insulin formulations was well tolerated and produced no adverse effects. Assessment of plasma insulin showed that for all 3 formulations insulin profiles increased in a time dependent manner, peaking at 15-60 minutes post administration, correlating with a related reduction in blood glucose. The tracheostomed Beagle dog is therefore considered a suitable model for investigation of drugs intended for pulmonary delivery.

1436 NINETY DAY TOXICOLOGICAL EVALUATION OF THE ORAL TOXICITY OF GBR 12909 IN DOGS.

R. Krishnaraj[1], R. L. Morrissey[2] and B. S. Levine[1]. *[1]Toxicology Research Laboratory, University of Illinois at Chicago, Chicago, IL and [2]Pathology Associates, Chicago, IL.*

The toxicity of GBR 12909 (Vanorexine), a dopamine reuptake inhibitor, was evaluated in dogs during 13 weeks of daily oral (capsule) administration at doses of 0, 1, 5 and 20 mg/kg/day (control, low, mid and high dose groups, respectively; 4

dogs/group/sex) with a 4 week recovery period for high dose and control animals (2 additional dogs/group/sex). Animals were examined for mortality, clinical signs, changes in body weight, food consumption, clinical pathology, gross pathology, histopathology, organ weights, and ophthalmologic and electrocardiogram parameters. One high dose male showed neurological signs (reversed by haloperidol injection) and was moribund sacrificed on day 36. A second male was sacrificed on day 60 due to a non-treatment-related event. Increased (locomotor) activity was observed in high and mid dose group animals. At the high dose, other behavioral changes (head bobbing and weaving) were seen in 4/6 and 5/6 males, respectively, and in 2/6 and 6/6 females, respectively. In the mid dose group, head weaving was seen in 1/4 males and 1/4 females, and head bobbing seen in 1/4 males. Treatment-related diarrhea and emesis were seen in high and mid dose groups. These changes (behavioral, locomotor activity) were considered CNS pharmacologic effects of GBR 12909. Toxicological effects were also seen. Mild adverse effects on body weight gains and food consumption were noted in the high dose group, mostly the females. The weight gain reduction reversed during recovery period. Thrombocytosis was noted in the high dose group females. Mild serum electrolyte imbalance (hyperphosphatemia, hyperkalemia) in high dose males was not due to nephrotoxicity. No treatment-related histopathologic changes were seen. The No-Observed-Pharmacologic Effect-Level of GBR12909 was 1 mg/kg/day. Physical signs due to excessive CNS stimulation and reduced body weight gains noted at 20 mg/kg/day were considered signs of toxicity (Scientific consultation with Dr. James Terrill, NIDA is acknowledged; Supported by NIDA Contract No. NOIDA-9-8099).

1437 CONTINUOUS SUBCUTANEOUS INFUSION IN RODENTS.

M. Stilianesis, S. Groom and C. Copeman. *CTBR, Senneville, QC, Canada.* Sponsor: M. Vezina.

The subcutaneous route is often used for drug administration in humans. Continuous subcutaneous infusion offers an alternative to subcutaneous injection particularly to assess the potential biological effects of compounds having a short half-life. The use of implantable mini-pumps has become increasingly popular, and while these have been used in several applications at CTBR, their use does present several potential limitations in terms of infusion volumes and rates of infusion. Therefore, we have developed our own method of subcutaneous infusion for both large animals and rodents that allows higher infusion rates and volumes to be administered and a means to account for daily dosimetry. This poster presents data generated using this method in rodent studies ranging from 1 week to 13 weeks duration. Using saline as a vehicle, infusion rates as high as 1.0 mL/kg/h have been employed in studies of up to 28 days duration and rates of up to 0.4 mL/kg/h have been employed for up to 13 weeks. These rates correspond to daily dose volumes of 24 mL/kg and 10 mL/kg respectively. Our method of infusion has repeatedly been demonstrated to be both well-tolerated and without biologically significant effects upon hematology, clinical chemistry or tissue pathology. While some localized background tissue responses are inevitable with the presence of a small diameter silicone catheter in the subcutaneous space, these reactions are typically confined to a minimal cellulitis and/or fibrosis. Furthermore, this low level of tissue reactions appears to be less than that associated with the presence of an implantable pump. Therefore, we consider our method of infusion to provide a suitable means to evaluate the safety and kinetics of pharmaceutical products intended for subcutaneous administration.

1438 SAFETY EVALUATION OF XMP.629, A NOVEL PEPTIDE FOR ACNE TREATMENT.

R. Hawks[1], J. Secrest[2], S. Frantz[2], E. Serbinova[3], T. Merriman[4], D. Learn[5] and K. Meyer[1]. *[1]XOMA US, Berkeley, CA, [2]MPI Research, Mattawan, MI, [3]Dow Pharmaceuticals, Petaluma, CA, [4]Charles River Springborn, Spencerville, OH and [5]Charles River Argus, Horsham, PA.*

XOMA (US) LLC is currently developing a topical formulation of XMP.629 for the treatment of moderate to severe acne vulgaris. XMP.629 is a novel D-linked 9-amino acid peptide based on a variant of a natural antimicrobial protein. Safety evaluation studies have characterized the toxicological profile of formulated XMP.629, including genotoxicity studies, acute toxicity studies, photosafety studies, and 30-day repeat dose toxicity studies. Ames, mouse micronucleus, and chromosome aberration assays showed that XMP.629 was not genotoxic. An acute oral toxicity study in rats showed no toxic effects at doses tested up to 10 mg/mL XMP.629. Studies in rabbits determined that XMP.629 is neither a primary dermal nor ocular irritant. A photosafety study in guinea pigs showed that XMP.629 did not cause primary irritation, phototoxicity, contact hypersensitivity or photoallergy. In a 30-day study in the rat, XMP.629 was administered subcutaneously to maximize systemic exposure. The results showed no signs of systemic toxicity up to the highest dose tested. Findings considered secondary to inflammation arising at the injection site were noted in hematology, clinical chemistry and organ weight changes. The no-observed-effect level (NOEL) for systemic toxicity was determined to be 3.0 mg/kg administered subcutaneously, a dose level about 45-times higher than the anticipated clinical dose. A 30-day topical application study in the minipig showed no XMP.629-related toxicity in any of the parameters evaluated, and the NOEL for systemic and local toxicity was determined to be 700 μg/kg/day, a dose about 10-times higher than the anticipated clinical dose. The results from this evaluation support the use of this novel compound in clinical studies.

1439 MONITORING THE PRIMARY AND SECONDARY ANTIBODY RESPONSE TO KLH IN A DEVELOPMENTAL IMMUNOTOXICITY (DIT) STUDY.

G. Desilets, N. Rouleau, P. Louise and L. LeSauteur. *CTBR, Senneville, QC, Canada.* Sponsor: L. LeSauteur.

A T-cell dependent antibody response assay was used to monitor the primary and secondary antibody response in a DIT study. KLH was used as an antigen and a quantitative enzyme linked immunosorbent assay (ELISA) was used to monitor the anti-KLH IgM and anti-KLH IgG response. KLH (300 μg in 0.1 mL, or 150 μg in 0.05 mL) was administered intravenously *via* the tail vein. Blood samples were collected from Sprague-Dawley rats aged from 30 to 125 days. As expected, for Groups 1 and 2, no anti-KLH IgM or anti-KLH IgG antibodies were detected since animals were not immunized with KLH. Group 3 animals produced a substantial primary and secondary response, measured on Day 71 since KLH was administered on Days 57 and 64. Group 4 animals produced a substantial primary and secondary response on Day 125 since KLH was administered on Days 112 and 129. The primary response measured on Day 118 for Group 4 animals was lower than on Day 125. Group 5 animals produced a measurable but very low primary immune response since KLH was administered on Day 23. Group 6 animals were immunized with KLH twice (Days 16 and 23) and produced a greater primary antibody response on Day 30 than Group 5 animals. Group 6 animals were re-immunized with KLH a third time on Day 119 and a substantial primary and secondary immune response was observed on Day 125. In conclusion, the primary and secondary immune response can be measured in weanling Sprague-Dawley rats (30 days post-natal). However, the response is suboptimal. An intravenous KLH dose of 150 μg in 0.05 mL has shown to be adequate to induce a detectable immune response.

1440 PHARMACOKINETIC AND TOXICITY STUDIES OF GENTAMICIN IN AFRICAN GREEN MONKEYS.

D. J. Auyeung[1], S. Cyrek[1], J. Schindler-Horvat[2], L. Iyer[2], R. Swezey[2], Y. Li[2], J. Arezzo[3], K. Draper[1] and J. Mirsalis[2]. *[1]CRL DDS Sierra Division, Sparks, NV, [2]SRI International, Menlo Park, CA and [3]Albert Einstein College of Medicine, Bronx, NY.*

As part of the national biodefense program, the antibiotic gentamicin sulfate (GS) is being considered for treatment of aerosolized *Yersinia pestis*, the causative agent of pneumonic, bubonic and septicemic plague. We conducted pharmacokinetic and toxicity studies in African green (AG) monkeys, the efficacy model used for this disease. GS was administered to AG monkeys as a single intramuscular (IM) injection or 20-min intravenous (IV) infusion at doses of 3.0, 4.5, or 6.0 mg/kg or as twice daily IM or IV doses of 1.5, 2.25, or 3.0 mg/kg/dose for 14 days. There were no GS-related clinical signs or changes in food consumption, body weight, serum chemistry, hematology, coagulation or urinalysis parameters. There was no measurable auditory damage following multiple doses as determined by brainstem auditory evoked potential (BAEP). GS-related microscopic alterations were limited to persistent injection site edema and necrosis and reversible, minimal hyaline droplet formation in renal tubular epithelium following multiple IM doses. No adverse effects were seen following IV administration. Following a single dose, mean T_{max} values ranged from 0.33 to 0.4 hr for all dose levels *via* IV or IM administration. C_{max} values ranged from 12.9 to 49.6 μg/ml IM and 17.0 to 31.8 μg/ml IV at low to high doses. Dose-dependent increases in C_{max} and AUC values were linear after IM and IV doses, and apparent V_z values indicated distribution of GS to extravascular tissues. Bioavailability values indicate possible saturation of tissue binding sites at IM doses ≥ 4.5 mg/kg. No major differences in GS PK were observed between males and females. Toxicokinetic evaluations also showed a linear increase in C_{max} and AUC with dose on both Days 1 and 14 of dose administration; there were no differences in the PK parameters on these two days. In summary, GS is well tolerated in AG monkeys when administered IV or IM for up to 14 days. *Work supported by NIAID Contract N01-AI-05417.*

1441 SUBCHRONIC TOXICITY OF ZICONOTIDE ADMINISTERED BY CONTINUOUS INTRATHECAL INFUSION IN RAT AND DOG.

G. M. Shopp[1], M. J. Skov[1] and T. L. Yaksh[2]. *[1]Safety Evaluation, Elan Pharmaceuticals, Inc., South San Francisco, CA and [2]Anesthesiology, University of California, San Diego, La Jolla, CA.*

Ziconotide (PRIALT™) is the synthetic equivalent of a 25-amino acid, polybasic peptide found in the venom of the marine snail, Conus magus. Ziconotide is a potent and selective N-type calcium channel blocker being developed as an intrathecal (IT) analgesic agent for severe, chronic pain. The potential toxicity of ziconotide was evaluated by continuous IT infusion for 28 days at doses in rats up to 1500 ng/kg/hr and in dogs up to 1200 ng/kg/hr; at least 30-fold the highest expected dose in patients. Mean plasma drug levels measured in high-dose rats and dogs were approximately 3 and 10-fold greater than human, respectively, although overall systemic exposure to ziconotide was low (< 4 ng/mL). Expected treatment-related pharmacological effects on the CNS, consisting mainly of tremors, shaking-behavior and/or ataxia, were of sufficient severity in dogs to require moribund euthanasia in 4/16 animals at ≥ 600 ng/kg/hour. There were no treatment-related changes in body weight, food intake or clinical pathology parameters nor was any target organ toxicity identified in either species at termination. Neurohistopathologic examinations revealed spinal cord compression with associated chronic inflammation in control and ziconotide-treated rats and dogs, which was attributed to pressure exerted by the IT catheter. This finding was not exacerbated with ziconotide exposure and no other histopathologic changes were observed. In conclusion, behavioral and neurological effects observed in rats and dogs receiving 28-day continuous IT infusions of ziconotide appeared related to the pharmacological activity of ziconotide with no associated neurotoxicity or histopathology. Further, no granuloma formation at the catheter tip was observed in ziconotide-treated dogs when infusion site tissue sections were examined, as has been reported with IT morphine administration (Yaksh et al, *Anesthesiology* 2003; 99:174-87).

1442 FORMULATION OF POORLY WATER SOLUBLE DRUGS FOR PRE-CLINICAL TESTING.

B. Rabinow, J. Wong, M. Doty, M. Chaubal, J. Kipp, P. Papadopoulos and J. Kerzee. *Baxter Healthcare, Round Lake, IL.*

Purpose: A broadly applicable formulation approach for poorly water-soluble drugs is desirable, in order to overcome problems in pre-clinical testing. High drug loading, elimination of interfering solvents, and ability to use the same formulation for both PO and IV administration would optimize progress for lead drug candidates. A nanocrystalline formulation platform has been developed which accomplishes these objectives. Additional advantages include ability to control dissolution rates. Fast dissolution in plasma, results in conventional PK profiles and tissue distribution. Slow dissolution results in targeting of the monocyte phagocytic system, reducing systemic toxicity and enhancing efficacy in some cases, permitting salvage of lead drug candidates. Dissolution rates can be modified depending upon alteration of process parameters. This in turn affects plasma PK profiles and tissue distribution, thus altering efficacy. Experimental: A process was developed that would be broadly applicable to formulate poorly water-soluble drugs. Particle size distribution, stability, X-ray analysis, dissolution rates, pharmacokinetic profiles, tissue distributions and efficacy studies of various drug formulations involving flurbiprofen, budesonide, carbamazepine, tetracaine, and itraconazole were examined. Results: A combined process featuring microprecipitation of water insoluble drugs, followed by homogenization for annealing particle characteristics was refined and developed. Using this approach, the use of co-solvents was eliminated. Stable formulations, maintaining particle size distribution and crystallinity were obtained for the drugs studied at levels up to 10-15% w/v. Conclusions: A formulation platform generally applicable to the rapid formulation of poorly water-soluble drugs has been developed that overcomes many of the impediments to subsequent pre-clinical testing. The same formulation could be used both for IV and PO routes of administration. Novel features include the ability to tune the particle characteristics to modify pharmacokinetics for the purpose of salvaging lead candidates.

1443 PHIP PRODUCES DNA ADDUCTS FOLLOWING IN SITU PHASE I AND II METABOLISM IN MCF10A CELLS.

R. D. Thomas[1], M. R. Green[1], T. A. Kocarek[2] and M. Runge-Morris[2]. *[1]Basic Pharmaceutical Sciences, Florida A&M UNiversity, Tallahassee, FL and [2]Institute of Environmental Health Sciences, Wayne State University, Detroit, MI.*

Heterocyclic amines (HCAs) are formed when meat products such as beef, chicken, pork and fish are cooked at high temperatures. These amines cause colon, breast, and prostate cancer in rats; liver, lung, and forestomach cancer in mice and have been associated with increased risk of prostate and breast cancer development in humans. 2-amino-1-methyl-6-phenylimidazo[4, 5-b] pyridine (PhIP) is the most abundant and potent carcinogenic HCA found in food. PhIP produces DNA-adducts which lead to mutations and cancer. PhIP is hydroxylated by cytochrome P450 then conjugated by sulfur estrogen transferase to the ultimate carcinogen. Conventionally the first step (N-hydroxylation) occurs in liver and the second step (conjugation) occurs in the breast. We propose that PhIP is completely metabolized to the ultimate toxin in the breast tissue. To test this hypothesis MCF10A cells were treated with PhIP (100 mM) and N-OH-PhIP (100 mM) with and/or without a napthoflavone. DNA from treated cells was isolated and DNA adduct analysis was performed by 32P post-labeling analysis. Results from this study revealed that 100 mM PhIP produces DNA-adducts after 24 hours and N-OH-PhIP produces DNA-adducts as early as 3 hours. Treatment with a napthoflavone inhibited PhIP induced DNA-adducts by 49% but did not inhibit N-OH-PhIP induced DNA-adducts. This data demonstrates that the complete bioactivation of PhIP occurs in the breast epithelial cells. It also suggests that Cytochrome P450 1A1 is expressed in the breast epithelial cells at high enough concentrations to metabolize PhIP to N-OH-PhIP. Further studies are on going to determine the role of the sequential metobolism of PhIP in the induction of breast cancer.

1444 COMPARATIVE METABOLISM AND DISPOSITION OF 1-^{14}C- AND 2, 3-^{14}C-ACRYLAMIDE IN CYTOCHROME P450 2E1-NULL (KO) AND WILD-TYPE (WT) MICE.

L. El-Hadri and B. I. Ghanayem. *NIEHS/NIH, Research Triangle Park, NC.*

Acrylamide (AM) is a heavily produced industrial chemical, which is used to synthesize polyacrylamide for use in paper and textile industries, cosmetics, and in chromatography gels. Presence of AM in baked and fried carbohydrate-rich foods such as french fries, potato chips, bread, and cereals increased fears that human exposure to this chemical is more wide-spread than originally thought. AM is a known animal carcinogen, reproductive toxicant, and a potent neurotoxin. AM is metabolized *via* 2 competing pathways: conjugation with reduced glutathione and epoxidation to form glycidamide. Glycidamide formation is suspected to be responsible for the induction of toxicity and carcinogenicity by AM. Recent urinary metabolites identification studies demonstrated that CYP2E1 is the principal enzyme responsible for glycidamide formation. Current studies were undertaken to compare the metabolism and disposition of 1-^{14}C-AM and 2, 3-^{14}C-AM using KO and WT mice. AM was administered (IP) to mice at 50mg/5 ml saline/kg. Mice were immediately placed in metabolism cages that allowed for the quantification of expired ^{14}CO2, organic volatiles, urine, and feces. Animals were euthanized at 24 hr after dosing, and blood and tissues were collected. Preliminary data showed that urine is the primary route of AM elimination and accounted for 24-29 % of 1-^{14}C-AM dose vs. 46-49 % of 2, 3-^{14}C-AM dose regardless of the genotype of the animals. Elimination of AM-derived CO2 accounted for 2-5 % of the administered dose in 24 hr after the administration of either chemical to KO or WT mice. Elimination of AM-derived radioactivity in the feces and in the expired air, as organic volatiles, accounted for less than 1 and 2% of the administered dose in 24 hr, respectively. Further, the concentration of AM-derived radioactivity was significantly higher in the RBCs vs. plasma in all dose groups. Studies are currently in progress to compare the tissue distribution and to identify and quantitate the urinary metabolites of 1-^{14}C-AM and 2, 3-^{14}C-AM in KO and WT mice.

1445 METHACRYLONITRILE: EFFECT OF CAFFEINE AND ALCOHOL ON TOXICITY IN MALE SPRAGUE-DAWLEY RATS.

M. Farooqui and R. Ruiz. *Biology, University of Texas Pan American, Edinburg, TX.*

Effect of caffeine and alcohol pre-treatment was studied on the toxicity of methacrylonitrile (MeAN) in male Sprague-Dawley rats. One group of experimental rats received an oral dose of 6 % MeAN solution in corn oil (equivalent to 0.5 LD50). Other three groups of rats were pre-treated with alcohol (2 ml of 50 % solution in water), caffeine (1 ml of 2 % solution in water) or both alcohol and caffeine twelve hours before receiving MeAN dose. The rats were closely observed for mortality, cholinomimetic effects (salivation, lacrimation, vasodilation and diarrhea), central nervous system (CNS) effects (ataxia, trembling, convulsions and irregular breathing) and urinary dysfunction (urine retention in the bladder) for six hours. The cholinomimetic and CNS effects were graded on a scale of 0-4. Three, six and twenty four hours after the treatments the rats were sacrificed under ether anesthesia for collection of blood, liver, kidney and brain. None of the rats died in control group and the group pre-treated with caffeine alone. Three out of ten rats died with MeAN alone and 5 out of ten each died in rats pre-treated with alcohol alone and alcohol + caffeine. Alcohol and alcohol + caffeine pre-treatment caused significant increase (2-4 fold) in cholinomimetic, CNS and urinary dysfunction effects of MeAN. However, caffeine alone pre-treatment protected rats from any of these effects. These results suggest that alcohol pre-treatment enhanced metabolism of MeAN to cyanide resulting in a significant increase in CNS effects even in the presence of caffeine. This effect may be through enhancement of microsomal activity for the metabolism of MeAN to cyanide. Caffeine appears to provide protection

probably through inhibition of microsomal metabolism of MeAN to cyanide. (Supported by NIH South Texas Doctoral Bridge Program Grant # 2 R25GM50080).

1446 HYDROLYSIS OF ISOEUGENYL-ACETATE AND EUGENYL-ACETATE BY RAT AND HUMAN HEPATIC MICROSOMES.

D. J. Castro, C. J. Sweet, R. K. Kuester and G. Sipes. *Pharmacology and Center for Toxicology, University of Arizona, Tucson, AZ.*

Isoeugenyl-acetate (IA) and eugenyl-acetate (EA) are fragrance materials and flavoring agents that are also esters of the fragrance/flavor materials, isoeugenol and eugenol. If the hydrolysis of these esters is rapid and complete, the existing toxicity profiles for isoeugenol and eugenol should be appropriate for the safety assessment of IA and EA. This study quantified the rate of hydrolysis of these esters in rat (male SD) and human hepatic microsomes. Incubation of IA or EA (500 μM) with microsomal protein (0.0125 mg/ml) revealed that hydrolysis was complete within 15 min of incubation. Kinetic analysis of the hydrolytic reaction in hepatic microsomes (4.8 to 970 μM, 3.0 min incubation) yielded the following kinetic constants for IA: Vmax of 3822-(rat), 3795-(human male) and 3072-(human female) nmol/min/mg of protein; Km (μM) of 91-(rat), 72-(human male) and 51-(human female). For EA the kinetic values were: Vmax of 3829-(rat), 3656-(human male) and 2748-(human female) nmol/min/mg of protein; Km (μM) values were 97-(rat), 72-(human male) and 52-(human female). Preliminary results demonstrate that rat plasma and preparations of rat skin also readily hydrolyze these esters into their respective alcohols. This extensive hydrolysis of IA and EA by liver, plasma, and skin enzymes will minimize systemic exposure of these agents when used under appropriate conditions as fragrance/flavoring materials. This research was supported by the Research Institute for Fragrance Materials and the Southwest Environmental Health Sciences Center (ESO6694).

1447 EFFECTS OF ROXARSONE AND ITS METABOLITES ON CACO-2 CELL PROLIFERATION.

G. S. Bayse[1], W. G. Kirlin[2] and P. D. Kirkland[1]. *[1]Chemistry, Spelman College, Atlanta, GA and [2]Pharmacology & Toxicology, Morehouse School of Medicine, Atlanta, GA.*

Roxarsone (4-hydroxy-3-nitrobenzenearsonate) is a poultry feed additive used routinely since the 1960's with FDA approval, for prevention of microbial infections and for growth promotion. In excess of 90% of the additive is excreted unchanged and the massive amounts (>2 million lbs/year) added to waterways, especially from giant farm operations, have brought expressions of concern both from USGS and from USEPA. Literature reports indicate that reduced roxarsone (3-amino-4-hydroxybenzenearsonate;AHBA) is formed by microbial and geologic action, and is found in groundwater and in sediments; a proposed azobenzene derivative has been described, as well. The human intestinal cell line, Caco-2, was synchronized in culture prior to exposure to roxarsone, AHBA, and acetarsone (3-N-acetylamino-4-hydroxybenzene-arsonate), as well as to inorganic arsenite (AsIII) and arsenate (AsV). The cells' proliferative response to the arsenic compounds was determined by tritiated thymidine incorporation into DNA. At micromolar levels, except in the case of AsIII, proliferation of cells was increased significantly when compared to growth of controls. AsIII is a potent inhibitor of proliferation at the same level. These results indicate potential mutagenesis effects of the organoarsenicals and AsV, and toxicity of AsIII. Related *in vitro* studies in our laboratory have shown the N-acetylation of AHBA to acetarsone, catalyzed by human N-acetyl- transferase. The acetarsone product was detected in two independent HPLC systems by comparison with a commercial standard. In addition, preliminary experiments indicate that human CYP3A4 catalyzes oxidation of AHBA, as determined by NADPH disappearance and by product appearance in the 430nm spectral region. Thus, bioactivation of AHBA and related metabolites may be involved in the Caco-2 cell proliferation observed. (Support:RIMI RR11598;MBRS/RISE 1R25GM-6056601;RCMI RR03032;MBRS/GM-029248).

1448 EPOXIDATION OF COUMARIN IS THE MAJOR DETERMINANT OF COUMARIN-INDUCED CLARA CELL TOXICITY IN THE MOUSE.

J. D. Vassallo and G. P. Daston. *Central Product Safety, Procter & Gamble, Cincinnati, OH.*

Coumarin causes Clara cell toxicity in the mouse. We hypothesized that this effect is caused by metabolism of coumarin to coumarin 3, 4-epoxide (CE), which spontaneously rearranges to o-hydroxyphenylacetaldehyde (o-HPA), a cytotoxic metabolite. The purpose of the present work was to characterize the metabolism of coumarin in mouse (susceptible), rat (not susceptible) and human lung. In B6C3F1 mouse lung microsomes, coumarin was metabolized in the absence of glutathione to o-HPA with a Km of 155 μM and a Vmax of 7.3 nmol/min/mg of protein. In the F344 rat, where no toxicity is observed, the Km and Vmax were 2574 μM and 1.75 nmol/min/mg protein, respectively. Since the intrinsic clearance was 69-times higher in the mouse, the epoxidation was shown to correlate with species-sensitivity to toxicity. Metabolism of coumarin to o-HPA did not occur in sixteen different human lung microsomes indicating human lung is likely resistant to the toxic effect of coumarin. In order to determine whether detoxification reactions contribute to species differences in toxicity, the fate of CE and o-HPA were also determined. Detoxification of CE *via* conjugation with glutathione was examined in lung microsomes containing cytosol and the Km of this reaction was 827 μM and 751 μM in mice and rats, respectively. Detoxification of o-HPA *via* oxidation to o-hydroxyphenylacetic acid (o-HPAA) was also examined in lung cytosol and the Km of this reaction was 1.5 μM and 1.4 μM in mice and rats, respectively. Since the detoxification pathways of CE and o-HPA were similar between mice and rats these reactions do not predict species sensitivity to coumarin. Instead, lung toxicity correlates with the rate of epoxidation, and this is different from the liver in that detoxification pathways do not appear to play an important role in determining toxicity. Furthermore, human lung microsomes did not metabolize coumarin to the epoxide suggesting that humans are unlikely to show lung toxicity following human exposure.

1449 *IN VITRO* METABOLISM OF ERGOTAMINE BY MOUSE LIVER MICROSOMES FROM ENDOPHYTE SUSCEPTIBLE AND RESISTANT BREEDING LINES.

J. M. Duringer[1], R. M. Lewis[2], L. A. Kuehn[2], T. J. Fleischmann[1] and M. Craig[1]. *[1]Biomedical Sciences, Oregon State University, Corvallis, OR and [2]Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Fescue toxicosis causes significant economic losses to livestock production. Clinical signs include summer syndrome, fescue foot and fat necrosis. All disorders are associated with decreased average daily weight gains and reproductive efficiency. Research on livestock tall fescue toxicoses strongly implicates the ergopeptine alkaloids as the toxic moiety. Mice from lines previously selected for resistance or susceptibility to growth depression from endophyte-infected (E+) fescue seed diets were used in these experiments. Mice were fed a diet containing endophyte-free (E-) seed for two weeks followed by two weeks of E+ or E- seed. Metabolism of ergotamine, a representative ergot alkaloid, was analyzed by HPLC using liver microsome preparations from the experimental groups. During the first two weeks, gender differences were observed for the transformation of metabolites 1 (M1 and $M1_i$), 3, 4 and 5 and ergotamine (ET and ET_i). After challenge with the E+ diet, differences were seen for line and gender for metabolite 3 and for gender for metabolites 4, 5, and ergotamine (ET and ET_i). The finding of gender differences in metabolism of ergotamine has not been reported in this mouse line or in other species used to study ergot alkaloid metabolism. This adds a potential source of variation in the development of fescue toxicosis and would be of value to investigate further in terms of livestock management practices. Metabolites were then isolated by sequential chromatographic procedures and identified by mass spectroscopy (MS). Results of MS analysis showed a metabolite consistent with epimerization of ergotamine and four components consistent with hydroxylation of ergotamine. The site of hydroxylation was consistent with oxidation of the peptine portion of the molecule with no evidence of oxidative transformation of the lysergic acid half of the molecule.

1450 METABOLISM OF VINCLOZOLIN AND ITS METABOLITES IN RAT.

A. Sierra-Santoyo[1], R. A. Harrison[2], H. A. Barton[2] and M. F. Hughes[2]. *[1]TOXICOLOGY, CINVESTAV-IPN, Mexico City, D.F., Mexico and [2]ETD, NHEERL, ORD, , USEPA, Research Triangle Park, NC.*

Vinclozolin (V) is a fungicide used in agricultural settings. V administered to rats is hydrolyzed to 2-[[(3, 5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid (M1), 3'5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2), and 3, 5-dichloroaniline (M3). V, M1 and M2 have antiandrogenic properties by interacting with the androgen receptor. However, data on disposition of V, M1 and M2 is limited. Our objective was to study the metabolism of V, M1 and M2 *in vitro* and *in vivo*. Non-treated adult male Long-Evans rat liver microsomes (2 mg) were incubated (30 min, 37°C) in 0.1 M phosphate buffer (PB) pH 7.4, 5 mM $MgCl_2$, 2 mM NADPH with V (50 μM), M2 or M1 (200 μM). Rats were administered a single *po* dose of V (100 mg/Kg) in corn oil. Animals were sacrificed (24 h) and serum and liver were removed and analyzed for V and metabolites. Metabolites from enzyme reactions and serum and liver were extracted in acetonitrile after dilution of samples in PB pH 3.3 (1:4) and analyzed by HPLC/DAD/MS. M1 and M2 formation was evident from V chemical hydrolysis. Another product was observed

from V and M2 *in vitro* metabolism. This metabolite, in both cases had the same t_{ret} and using UV/Vis spectrum and negative ESI mass spectra ($[M]^-$ at *m/z* 294 and $[M-H]^-$ at *m/z* 293) was identified as 3', 5'-dichloro-2, 3, 4-trihydroxy-2-methylbutylanilide (M4). M3 was detected only from M2 but its formation was not NADPH-dependent. No metabolites from M1 were detected. Analysis of serum and liver extracts of treated rats showed the presence of M4, M1 and V. M4 levels were at least 5- and 9-fold higher in serum and liver, respectively, than M1 or V. These results indicate that metabolism of V is both enzymatic and non-enzymatic. M4 is an abundant metabolite of V, and may be used as an exposure biomarker for pharmacokinetic modeling of V. These results may clarify the relationship between toxicity and tissue dose of V and its metabolites. (Funded in part by NRC CR 828790. This abstract does not represent USEPA policy).

1451 *IN VITRO* METABOLISM OF CARBOFURAN BY HUMAN, MOUSE, AND RAT LIVER MICROSOMES, AND HUMAN CYTOCHROME P450 ISOFORMS.

K. A. Usmani, E. Hodgson and R. L. Rose. *Environmental & Molecular Toxicology, North Carolina State University, Raleigh, NC.*

Carbofuran is a widely used carbamate pesticide in agricultural practice throughout the world. Its effect as a pesticide is due to its ability to inhibit acetylcholinesterase activity. The present study was designed to investigate the *in vitro* metabolism of carbofuran by pooled human liver microsomes (HLM), rat liver microsomes (RLM), mouse liver microsomes (MLM), and human cytochrome P450 (CYP) isoforms. Carbofuran is metabolized by CYPs leading to the production of a major ring oxidation metabolite, 3-hydroxycarbofuran, and two minor metabolites. Pooled HLM have a significantly higher Km value (1950 μM) than RLM Km (210 μM) and MLM Km (550 μM) for metabolism of carbofuran to its major metabolite, 3-hydroxycarbofuran. Intrinsic clearance rate calculations indicate that HLM metabolize carbofuran to 3-hydroxycarbofuran almost 14-fold lower than RLM and MLM. Among 16 human cDNA-expressed CYP enzymes examined, CYP3A4 and 2C19 were the major isoforms responsible for carbofuran metabolism to 3-hydroxycarbofuran. Use of phenotyped HLM demonstrated that individuals with high levels of CYP3A4 and 2C19 have the greatest potential to metabolize carbofuran to its major metabolite. The variation in carbofuran metabolism among 17 single-donor HLM samples is over 5-fold and the best correlation between CYP isoform activity and carbofuran metabolism was observed with CYP3A4. (Supported by NIOSH grant OH07551-ECU)

1452 COMPARISON OF DETOXIFICATION AND BIOACTIVATION PATHWAYS FOR BROMODICHLOROMETHANE IN THE RAT.

M. K. Ross[1], C. R. Eklund[2] and R. A. Pegram[2]. *[1]Curriculum in Toxicology, UNC-CH, Chapel Hill, NC and [2]ETD, USEPA, Research Triangle Park, NC.*

Bromodichloromethane (BDCM) is metabolized *via* high-(CYP2E1) and low-(GSTT1) affinity pathways. The CYP2E1 pathway is thought to be a detoxification pathway at low concentrations. The GST pathway produces a labile genotoxic intermediate that is unlikely to escape from within cells where it is formed. Evidence for GSTT1-specific metabolism is supported by experiments which demonstrate that BDCM competitively inhibits GSTT1 activity toward the model substrate 1, 2-epoxy-3-(4-nitrophenoxy)propane (ENPP) (Ki = 4.2 mM). The flux of BDCM through the CYP2E1- and GSTT1-pathways has been investigated *in vitro* in non-target (liver) and cancer target tissues (kidney and large intestine) of the F344 rat. Compared to the liver, the intrinsic clearance (Vmax/Km) of BDCM *via* CYP-metabolism in target tissues was significantly reduced (8- and 16-fold for kidney and large intestine, respectively) while GST-metabolism in the same tissues was only slightly lower (~2-3-fold), suggesting less efficient detoxification particularly at high tissue concentrations of BDCM that saturate CYP2E1. The kinetic parameters of GSTT1 metabolism in liver and kidney have been incorporated into a previously published rat PBPK model for BDCM. Model simulations following constant inhaled exposures to BDCM indicate that CYP is the dominant pathway in liver and kidney following low inhaled concentrations; however, at high inhaled concentrations, the flux through CYP2E1 in kidney becomes saturated and the GSTT1 pathway becomes more pronounced than CYP in terms of total flux (at 3200 ppm, 235 mg BDCM metabolized by CYP2E1/liter per 6 h compared with 265 mg BDCM metabolized by GSTT1/liter per 6 h). Model simulations of oral doses in the dose range used in the cancer bioassay study do not result in saturation of hepatic or kidney CYP2E1 and thus flux through the GST pathway is limited. The model simulations suggest that greater genotoxic outcomes from BDCM exposure may occur from the inhalation route rather than the oral route. (Does not reflect USEPA policy. Supported by F32 ES11111-01).

1453 EFFECT OF DIMETHYL SULFOXIDE ON METABOLISM AND TOXICITY OF MODEL HEPATOTOXICANTS IN MICE.

M. Yoon[1, 2] and Y. Kim[2]. *[1]Research Associateship Program, National Research Council, Chapel Hill, NC and [2]College of Pharmacy, Seoul National University, Seoul, South Korea.*

Effect of dimethyl sulfoxide (DMSO) pretreatment on the metabolism and hepatotoxicity of several model hepatotoxicants including acetaminophen, chloroform and carbon tetrachloride was examined in adult mice. Administration of DMSO (2.5 ml/kg, ip) decreased acetaminophen and chloroform-induced hepatotoxicity, but not the hepatotoxicity of carbon tetrachloride as measured by elevation of serum alanine aminotransferase and sorbitol dehydrogenase activities. Hepatic microsomal metabolizing enzyme activities were depressed by DMSO, but hepatic CYP2E1 activity measured with p-nitrophenol as a substrate was increased to 144% of control. *In vitro* study using hepatic microsomes showed that the metabolic degradation of acetaminophen, dichloromethane or chloroform was all inhibited by addition of DMSO, however, metabolism of carbon tetrachloride was not affected. Lineweaver-Burk plot analysis of acetaminophen metabolism *in vitro* indicated that the inhibition pattern produced by addition of DMSO was competitive in nature. To explain the observation that DMSO decreased the hepatotoxicity of acetaminophen and chloroform despite significant induction of CYP2E1 activity, a reconstituted enzyme study was conducted employing a hepatic microsomal fraction reconstituted with a hepatic cytosolic fraction each prepared from DMSO-treated or control animals. The metabolic degradation of acetaminophen, dichloromethane and chloroform was greater in a system containing cytosolic fraction from control mice (DMSO-free) plus microsomes from DMSO-treated mice than a system with control microsomes. However, addition of DMSO-present cytosol to microsomes from DMSO-treated animals failed to increase the metabolic degradation of these chemicals. This result suggests that the direct inhibitory activity of DMSO on metabolism of model hepatotoxicants be responsible for the reduction of hepatotoxicity. Competition of DMSO and model hepatotoxicants for CYP2E1 appears to play an important role here.

1454 METABOLISM OF ORALLY ADMINISTERED N, N-DIMETHYL-P-TOLUIDINE (DMPT) IN F344 RATS AND B6C3F1 MICE.

K. Ghanbari, K. J. Dix, D. Kracko and J. McDonald. *Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM.*

DMPT is a high-production-volume chemical used in the manufacture of bone cements and dental materials, is found in industrial glues and artifical fingernail preparations, and is used as an intermediate in dye and pesticide synthesis. Human exposure to DMPT has resulted in methemoglobinemia and allergic responses; suspected from the formation of a toxic metabolite, p methylphenylhydroxylamine, an analog of the aniline metabolite phenylhydroxlyamine (thought to be responsible for methemoglobinemia from aniline exposure). We have previously reported results of disposition studies of DMPT in Fisher 344 (F344) rats and B6C3F1 mice after oral administration of [14C]DMPT. The majority of [14C]DMPT-derived radioactivity was excreted in urine by 24 hours. Here we report on the metabolism of orally administered DMPT. The profile of radiolabeled metabolites was determined by liquid chromatography (HPLC) using a C18 stationary phase and a mobile phase that transitioned from mostly water (polar) to mostly organic (neutral). The same four radiolabeled chromatographic peaks were observed in both rats and mice. Due to the limited volume of mouse urine, further work to elucidate the DMPT metabolite structures was performed with rat urine only. All four peaks have been isolated and preliminary structural elucidation by gas chromatography/mass spectrometry (GC/MS) and comparison to authentic standards confirmed excretion of the parent compound. We have tentatively identified a second peak by GC/MS as N-methyl-p-toluidine. A third peak had different HPLC and GC retention times than DMPT but an identical electron impact mass spectrum. Attempts at preliminary identification of the most polar metabolite have been unsuccessful. Further purification coupled with infrared and nuclear magenetic resonance spectroscopy will be employed along with incubation of isolated metabolites with glucuronidase and sulfatase enzymes (to assess conjugation) to complete structural elucidation/confirmation. Supported by NIEHS.

1455 SUBSTRATE SPECIFICITY OF THE INDIVIDUAL RAT UGT1A FAMILY OF ENZYMES.

L. J. Webb, F. K. Kessler and J. K. Ritter. *Pharmacology and Toxicology, Virginia Commonwealth University, Medical College of Virginia Campus, Richmond, VA.*

UDP-glucuronosyl transferases (UGT) are phase II enzymes that contribute to the elimination of xenobiotic substances and drugs. UGTs are expressed in a variety of tissues and play a major role in their metabolic activities. Due to the overlapping

substrate specificity of these enzymes, it is difficult to determine exactly which isoforms contribute to the glucuronidation of specific substrates. To this end, *in vitro* systems, adenovirus infected COS-7 cells or transfected HEK cells, individually expressing rat UGT1A1, UGT1A2, UGT1A3, UGT1A5, UGT1A6, UGT1A7 and UGT1A8 were developed. Using thin layer chromatography, the glucuronidating activity of each enzyme was examined for the following substrates: bisphenol A, naphthol, *p*-nitrophenol, hydroxybiphenyl, 4-methyl-umbelliferone, 7-hydroxycoumarin, phenol red (phenols), chrysin, quercetin, (-)epicatechin, dinitrocatechol (flavonoids/catechols), 16α-hydroxy-pregnenolone, 4-hydroxyestradiol, testosterone, estradiol, estriol, estrone, ethylnylestradiol (steroids), propofol, *t*-butylated hydroquinone, butylated hydroxytoluene (butylated), serotonin, buprenorphine, acetaminophen, and mycophenolic acid (drugs). In addition, a ^{14}C-UDPGA assay was utilized to determine the activity of these UGT forms towards bilirubin. It was found that most substrates were glucuronidated by multiple UGT isoforms. However, certain substrates were primarily glucuronidated by specific isoforms: bisphenol A, quercetin, (-)epicatechin, estriol, and propofol by UGT1A7; serotonin by UGT1A6; and bilirubin by UGT1A1. Several substrates, phenol red, testosterone and butylated hydroxytoluene, were not glucuronidated by any of the UGT1A isoforms studied. In conclusion, while many UGT1A isoforms have overlapping substrate specificities, unique substrate-isoform relationships can be determined. Further identification of these relationships could enable their use in *in vitro* and *in vivo* UGT1A inhibition and metabolic probe studies. (Supported by 1R01ES07763 from the NIEHS.)

1456 INVESTIGATION OF UDP-GLUCURONOSYLTRANSFERASES INVOLVED IN GLUCURONIDATION OF MYCOPHENOLIC ACID IN RATS.

K. Miles[1], S. Stern[2], F. Kessler[1], P. Smith[2] and J. Ritter[1]. *[1]Pharmacology and Toxicol., VCU, Richmond, VA and [2]Drug Deliv. and Dispos., UNC, Chapel Hill, NC.*

UDP-Glucuronosyltransferases (UGTs) glucuronidate a wide array of substrates. Mycophenolic acid (MPA), the active metabolite formed from Mycophenolate Mofetil, is primarily metabolized by glucuronidation and undergoes enterohepatic recycling. This may lead to increased intestinal exposure levels and associated gastrointestinal (GI)-toxicity. The mechanism responsible for this GI-toxicity is unknown. Intestinal UGT expression is hypothesized to modulate this phenomenon. The objectives of this study were to evaluate the UGT isoforms that are active in MPA metabolism in rats, and assess the potential roles of these forms in hepatic, renal, and intestinal MPA glucuronidation. Liver microsomes from homozygous Gunn j/j rats exhibited marked decreases in MPA glucuronidating activity. Recombinant rat UGT1A isoforms were expressed in HepG2 cells and membrane fractions were prepared and used for MPA activity assays. Rat UGT1A1, 1A6, and 1A7 but not 1A2, 1A3, 1A5, and 1A8 possessed MPA activity with Gunn rat liver microsomes exhibiting lower activity. The apparent Km (mM) values of: 0.446, 0.655, 0.511 and Vmax (mmol MPAG/min/mg) values of: 4.48 x 10^{-7}, 1.84 x 10^{-7}, and 1.1 x 10^{-6} were determined for 1A1, 1A6, and 1A7, respectively. Intrinsic clearance (CL_{int}) values (mL/min/mg) of 1.0, 0.28, and 2.15 were determined for 1A1, 1A6, and 1A7, respectively. CL_{int} for 1A7 was 2.2-fold higher than 1A1 and 7.7-fold higher than 1A6. Rat microsomes from liver, kidney, and intestine were analyzed for 1A1, 1A6, 1A7, and total UGT1A relative levels and MPA glucuronidating activity. All three isoforms were detected in each tissue. 1A1 levels were highest in liver whereas 1A7 was highest in intestine. In addition, MPA glucuronidating activity was highest in intestine when compared to the other tissues examined. These findings, suggest that multiple isoforms may be involved in modulating MPA-related GI-toxicity in rat. However, 1A7 may be particularly important in intestinal MPA glucuronidation. Supported by NIGMS grant 1R01GM61188.

1457 THYROID HORMONE METABOLISM IN SPRAGUE DAWLEY AND UGT2B2-DEFICIENT FISCHER 344 RATS.

T. A. Couch[2, 1] and C. D. Klaassen[2]. *[1]Pharmacology, Emory University, Atlanta, GA and [2]Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Microsomal enzyme inducers (MEI) that increase UDP-glucuronosyltransferases (UGTs) can significantly affect the hypothalamic-pituitary-thyroid axis and thyroid hormone (TH) homeostasis. Increased TH glucuronidation by UGTs can lead to reductions in serum TH, and an increase in thyroid stimulating hormone (TSH). Of the many UGT isoforms, UGT2B2 is thought to glucuronidate triiodothyronine (T3). The purpose of this research was to determine whether increased T3 glucuronidation mediates increased TSH observed after MEI treatment. Male Sprague-Dawley (SD) and UGT2B2-deficient Fischer 344 (F344) rats were fed control diet or diet containing pregnenolone-16a-carbonitrile (PCN; 800 ppm), 3-methylcholanthrene (3-MC; 200 ppm), or Aroclor 1254 (PCB; 100 ppm) for 7 days. Free and total thyroxine (T4), T3, TSH, androsterone/T4/T3 glucuronidation, and thyroid follicular cell proliferation were determined. PCN, 3-MC, and PCB treatments decreased serum T4, whereas serum T3 was generally maintained in both SD and F344 rats. T4 *in vitro* glucuronidation increased after MEI treatment: PCN (417 and 436 %), 3-MC (207 and 174 %), and PCB (327 and 324 %) in livers from SD and F344 rats, respectively, as compared to controls. Only PCN treatment significantly increased T3 *in vitro* glucuronidation (281 and 497 %) in livers from SD and F344 rats, respectively, despite little or no UGT2B2 activity present in F344 rats (as shown by androsterone glucuronidation). In both rat strains, PCN treatment increased serum TSH (160 and 379 %), and thyroid follicular cell proliferation (as shown by labeling index). These data demonstrate a strong correlation between increases in T3 glucuronidation, TSH, and follicular cell proliferation after PCN treatment. These data also suggest that, T3 is glucuronidated by other PCN-inducible UGTs in addition to UGT2B2. (Supported by NIEHS grants ES-08156 and ES-07079).

1458 *IN VITRO* CONJUGATION OF ETHANOLAMINE WITH FATTY ACIDS.

S. H. Khan, B. S. Khaphalia and G. Ansari. *Pathology, UTMB, Galveston, TX.*

Previous studies from our laboratory have shown the enzymatic formation of fatty conjugates of alcohols and amines. Present study describes the formation of fatty acid conjugates of a bifunctional compound, ethanolamine. The choice of ethanolamine is based on its endogenous presence and known formation of annandamide (a bioactive arachidonic acid amide of ethanolamine). Ethanolamine has the possibilities of conjugating with fatty acids through ester and/or amide bonds. [1-^{14}C]-Oleic acid was incubated with ethanolamine at 37oC in presence of rat liver post nuclear fraction. The resultant product(s) was separated by thin layer chromatography and the radioactivity corresponding to the relative flow of amide/ester was evaluated. The rate of formation of oleic acid conjugate(s) of ethanolamine was time- and dose-dependent. The optimum formation of ethanolamine conjugate was 16.4±1.7mol/hr/mg protein at 25mM ethanolamine and 1mM oleic acid. Formations of conjugates with other fatty acids (palmitic, stearic, linoleic, linolenic and arachidonic) were also found to be 6.25±0.09, 6.6±1.7, 5.9±0.93, 5.8±0.85 and 6.4±0.32 nmol/hr/mg protein, respectively, using different [1-^{14}C] fatty acids. These results indicate formation of fatty acid conjugates of ethanolamine. However, the structure of the compound formed needs to be established. Efforts to purify the product in sufficient quantities for spectral analysis and characterization of the enzyme responsible for the synthesis are under way.

1459 CHARACTERIZATION OF THE PHASE 2 METABOLITES OF RUTAECARPINE AS GLUCURONIDE/SULFATE CONJUGATES BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY.

S. Lee[1], D. Lee[1], J. Lee[2], D. Kim[2], E. Lee[1], Y. Jahng[1] and T. Jeong[1]. *[1]Pharmacy, Yeungnam University, Kyungsan, Kyungbuk, South Korea and [2]Bioanalysis and Biotransformation Research Center, KIST, Seoul, South Korea.*

From previous studies on the phase 1 metabolism of rutaecarpine, the metabolites formed were identified as products of hydroxylation on the aromatic rings. In the present studies, phase 2 metabolites of rutaecarpine *in vivo* and *in vitro* were studied in order to determine the possible metabolic fate of rutaecarpine by using high-performance liquid chromatography/electrospray ionization-tandem mass spectrometry. An *in vitro* incubation of rutaecarpine in isolated rat hepatocytes produced six main metabolites, corresponding to glucuronide/sulfate conjugation. When hepatocytes were incubated with 50 μM rutaecarpine for up to two hours, two metabolites (U1 and U2) formed were believed to be glucuronidated on the aromatic ring of the indole moiety. Other four metabolites (S1, S2, S3 and S4) were sulfate-conjugated on the aromatic ring of the indole moiety. Characteristic product ions for the identification of glucuronide/sulfate conjugates were observed at m/z 304, indicating the hydroxylated form of the aromatic ring of indole moiety. The main rutaecarpine metabolites *in vivo* were represented by two groups of compounds. One group was equal to the *in vitro* metabolism, i.e, glucuronidated conjugates (U1 and U2). The serum concentration of glucuronide conjugates reached a maximum two hour after the administration. The other group was identified as three sulfated conjugates (S'1, S'2 and S'3), but these were not equal to the *in vitro* metabolism, because they had different retention times from liquid chromatography. The serum concentration of this group reached a maximum one hour after administration. Therefore, it was concluded that the metabolism of rutaecarpine *in vivo* and in isolated hepatocytes would be somewhat different. (Supported by a grant from Korea Research Foundation for the Institute for Drug Research, Yeungnam University).

1460 SUBCELLULAR LOCALIZATION OF SOLUBLE EPOXIDE HYDROLASE IN HUMAN TISSUES USING CONFOCAL MICROSCOPY.

A. Enayetallah[1], D. F. Grant[1] and M. Barber[2]. *[1]Pharmaceutical sciences, University of Connecticut, Storrs, CT and [2]BIOTECHNOLOGY / BIOSERVICES CENTER, UNIVERSITY OF CONNECTICUT, AT STORRS, CT.*

Soluble epoxide hydrolase (sEH) is considered a phase I xenobiotic metabolizing enzyme, however, its physiological roles have not been clearly identified. Endogenous substrates include linoleic acid and arachidonic acid epoxides, some of which have been suggested to be involved in regulating blood pressure and inflammation. Following the documentation that sEH was not microsomal epoxide hydrolase (mEH), it was suggested to be mitochondrial. However, the enzyme was later shown to have a peroxisomal targeting sequence (PTS), and subsequent studies suggested that sEH was exclusively peroxisomal, or both cytosolic and peroxisomal. Previously, we studied the distribution of soluble epoxide hydrolase in normal and malignant human tissues using immunohistochemistry techniques. Interestingly, we found sEH in cells devoid of peroxisomes. In this study, we applied immunofluorescence and confocal microscopy techniques using markers for different subcellular compartments to evaluate sEH co-localization in an array of human tissues. Results showed that sEH is both cytosolic and peroxisomal in some tissues, and exclusively cytosolic in other tissues. We also show that it is not exclusively peroxisomal in any of the tissues included in this study. These data suggest that sEH contains a context dependent PTS, or there exists an sEH enzyme variant responsible for the dual localization.

1461 THE *IN VITRO* INHIBITION OF DIETHYLSTILBESTROL-DNA ADDUCTS BY DIALLYL SULFIDE: A POSSIBLE MECHANISM OF BREAST CANCER PREVENTION.

M. R. Green, C. L. Wilson, M. McCaskill and R. D. Thomas. *Basic Pharmaceutical Sciences, Florida A&M UNiversity, Tallahassee, FL.*

Estrogen related cancer is the second leading cause of death in women. The biotransformation of estrogen is a critical factor in the initiation of estrogen-induced cancer. It has been demonstrated that diethylstilbestrol (DES), a stilbene estrogen, is biotransformed to reactive chemicals that produce DNA Adducts. Diallyl sulfide (DAS), a potent chemopreventive compound found in garlic, has been shown to inhibit the biotransformtion of several procarcinogens such as benzo(a)pyrene and dimethylbenzsanthracine (DMBA). We propose that DAS will prevent DES induced breast cancer by inhibiting its biotransformation. This biotransformation leads to the formation of DNA adducts which results in cancer initiation. To test this hypothesis, we investigated the ability of DES to produce DNA adducts in the presence of microsomes, mitochondria, and nuclei *in vitro* metabolic systems. We also investigated the ability of DAS to inhibit DNA adduct formation. Microsomes, mitochondria, and nuclei were isolated from breast tissue of female ACI rats. These organelles were used to catalyze *in vitro* reactions containing DES and/or DAS, cumen hydroperoxide and 200mg DNA buffered with phosphate buffer at pH 7.4. The DNA was extracted and analyzed by P32 post labeling. We found that DES produced DNA adducts in all three systems. The relative adduct level was 2.2 x 10-4 in microsomal reactions, 6.2 x 10-6 in mitochondrial reactions, and 2.9 x 10-7 in nuclear reactions. DAS was able to inhibit DES induced DNA adducts in all three systems. The percent inhibition ranged from 86% in the microsomes to 93% in the nuclei. These findings suggest that DAS could inhibit DES induced breast cancer by inhibiting its metabolism. This inhibition may help explain the mechanism of the chemopreventive actions of DAS. Supported by DOD Grant # BC011376

1462 COMPARATIVE BIOSYNTHESIS OF FATTY ACID ETHYL ESTERS IN AR42J CELLS AND HEPG2 CELLS.

B. S. Kaphalia[1], H. Wu[1], D. L. Clemens[2], T. R. Jerrells[2], M. Khan[1] and G. Ansari[1]. *[1]Pathology, University of Texas Medical Branch, Galveston, TX and [2]Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE.*

Nonoxidative versus oxidative metabolism of ethanol is altered during chronic alcohol abuse due to inhibition of hepatic alcohol dehydrogenase (ADH, catalyzes the oxidation of alcohols). Previous studies have shown inhibition of ADH in chronic alcoholics and in rats subchronically exposed to ethanol, and significantly increased biosynthesis of fatty acid ethyl esters (FAEEs; nonoxidative metabolites of ethanol) in rats. Hepatic and pancreatic toxicity of some of these esters have also been reported *in vivo* as well as *in vitro*. Therefore, present study was undertaken to evaluate comparative biosynthesis of FAEEs 6 hr after 100-800 mg% ethanol exposure in rat pancreatic tumor (AR42J) cells and human hepatocellular carcinoma (HepG2) cells with or without ADH transfection. The FAEE levels in the AR42J cells (56 nmol/25 x 106 cells) were found to be significantly higher than those in HepG2 cells (45 nmol/25 x 106 cells). A three-fold increase of FAEE levels detected in HepG2 cells (ADH-deficient) as compared to HepG2 cells transfected with ADH suggests role of nonoxidative metabolism in disposition of ethanol during chronic alcoholism. HepG2 cells transfected with cytochrome 450 2E1 had comparable levels of FAEEs to those found in the HepG2 cells, indicating its limited role in oxidation of ethanol. The results of the present study suggest increased formation of FAEEs during chronic alcoholism and pancreas is a preferred organ system involved in such a metabolism. Although formation of FAEEs was found to be dose-dependent irrespective of cell types, further studies on quantitation of individual FAEEs in these cell lines and resultant toxicity will address the biological significance of FAEEs.

1463 THE UP-REGULATION OF STRESS RESPONSIVE GENES IN THE RAT KIDNEY GLOMERULUS FOLLOWING SINGLE INTRAVENOUS DOSE OF PUROMYCIN AMINONUCLEOSIDE (PAN).

T. Shimizu, N. Masutomi, T. Sakairi, Y. Inoue, N. Shimada, T. Hamano, J. Sugimoto and M. Mutai. *Mitsubishi Pharmacology Corporation, Kisarazu, Chiba, Japan.*

Puromycin aminonucleoside (PAN) is known to induce oxidative damage to glomerulus. In this study, we examined gene expression changes in glomerulus following the administration of PAN using DNA microarray to speculate the molecular mechanisms of its toxicity. Male SD(IGS) rats were intravenously injected with PAN at a dose of 100 mg/kg body weight or with saline. Animals were sacrificed 10 days after PAN injection. Histologically, a mild degree of hypertrophy was observed in glomerular epithelial cells. After glomeruli were collected from frozen section of kidney by laser microdissection, the extracted RNA was analyzed by Rat Toxicology U34 array (Affymetrix). Gene expression analysis revealed that genes belonging to the stress responsive MAPK cascade such as GADD45 and SAPK, oxidative stress responsive genes such as catalase and glutathione peroxidase and wide variety of cytochrome P450 (CYP) genes were up-regulated. When the RNA from renal cortex that dominantly contained the RNA from tubular cells was examined, the up-regulation of cell cycle and regeneration related genes was observed, while that of stress responsive genes was in the less extent. These results indicate that PAN induces oxidative stress and up-regulates various CYP genes in the glomerulus, when the tubular regeneration is in progress. Comparing the transcriptional profiles of glomelurus and renal cortex, laser microdissection was shown to be a powerful tool to investigate gene expression changes of the focused region of the heterologous tissue.

1464 ONTOGENY OF P-GLYCOPROTEIN (PGP) IN THE BRAIN AND GONADS 0F NEONATAL MALE AND FEMALE SPRAGUE-DAWLEY RATS.

S. J. Yavanhxay[1], J. T. Stevens[1], J. Eldridge[1], M. S. Chistian[2] and A. M. Hoberman[2]. *[1]Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC and [2]Charles River Laboratories, Horsham, PA.*

P-glycoprotein, a member of the ABC transporter superfamily, is a vital constituent of the blood-organ-barrier. It is expressed in capillary endothelial cells and acts as a transmembrane pump to facilitate removal of drugs and toxicants. We have assessed the development of PGP expression in female rat brain and ovaries, and brain, testes, and prostate of males, on PND 1, 5, 10, 15, 20 and 60. Using RT-PCR, PGP mRNA levels in female brain increased from 20% adult level at PND1 to 85% at PND20, and in males from 30% to 70%. A similar pattern was noted in the ovaries (10% on PND1 to 90% on PND20) and testes (15 to 75%) and prostate (25 to 80%). Using Western immunoblotting, measures of PGP protein in brain increased between PND 1-20 in a very similar pattern to PGP message: from 15 to 70% adult level in male and from 10 to 100% in females. Although expression of protein in testis and prostate failed to rise above 60% adult level by PND20, ovarian expression achieved 100% adult level at PND20. It can be concluded that the ontogeny of mRNA and protein expression of PGP, in the blood-brain barrier of the neonatal Sprague-Dawley rat, increases from a very low level at PND1 to nearly adult levels at PND20. PCR further suggests that the development of the blood-gonad-barriers and blood-prostate-barrier follows a similar pattern to development of the blood-brain-barrier, but the findings were not confirmed by immunoblotting techniques. It would appear from the data that the ontogeny of PGP in the Sprague-Dawley rat differs from that of human infants and children, which is completely developed from birth. It calls into serious question the validity of using neonatal rodent models to assess the potential toxic effects of natural hormones, pesticides, and environmental endocrine disrupting chemicals that interact with the P-glycoprotein pump, because rodent barriers remain far from completely developed at birth.

1465 MECHANISM OF FEMALE-PREDOMINANT OAT2 EXPRESSION.

S. C. Buist and C. D. Klaassen. *University of Kansas Medical Center, Kansas City, KS.*

Organic anion transporter 2 (Oat2) is highly expressed in kidney with female predominance in adult rats. In conjunction, gender divergent gene expression has been linked to endocrine factors, including gonadal steroids, growth hormone (GH) and thyroid hormones (TH). Thus, the goal of this study was to identify the mechanism of the Oat2 gender difference using gonadectomy, hypophysectomy (HX) and thyroidectomy (TX) as models of endocrine disruption. Additionally, Oat2 heteronuclear RNA (hnRNA) was measured to determine whether the gender difference is due to a transcriptional event. Oat2 mRNA and hnRNA levels were determined by branched DNA signal amplification and protein expression was qualitatively determined by immunohistochemistry. Oat2 mRNA was 4-fold higher in intact female kidney as compared with male kidney. Gonadectomy did not alter Oat2 mRNA levels in male or female kidney. Thus, gonadal steroids do not appear to regulate the female-predominant expression of Oat2 mRNA. After HX, female levels of Oat2 mRNA, hnRNA and protein decreased markedly to intact male levels, but male levels were not significantly changed. GH was administered to HX rats by injection (GH-IJ) or continuous infusion (GH-IF) to simulate the male or female GH secretion pattern, respectively. Oat2 mRNA, hnRNA, and protein increased after GH-IF (female pattern), but not after GH-IJ (male pattern). Therefore, the female GH secretion pattern may be partially responsible for the female-predominance of Oat2. TX produced a smaller decrease in Oat2 mRNA in kidney of female rats than after HX. However, TX did not change Oat2 mRNA levels in male rat kidney. Therefore, TH does not appear to directly effect Oat2 expression. In conclusion, this study indicates that the female GH secretion pattern is at least partially responsible for higher Oat2 levels in female kidney as compared with male kidney. Furthermore, elevated Oat2 hnRNA levels indicate that the gender difference occurs at the level of transcription. The effects of TH are minor, and may indicate an indirect role of thyroid hormones in basal expression of Oat2. (Supported by NIH grants ES-09716 and ES-07079)

1466 ALTERATIONS IN GENE EXPRESSION OF HEPATIC DRUG TRANSPORTERS BY THE LOSS OF THE TRANSCRIPTIONAL FACTOR HNF1α.

T. Callaghan[1], A. L. Slitt[1], J. M. Maher[1], C. Cheung[2], F. J. Gonzalez[2] and C. D. Klaassen[1]. *[1]University of Kansas Medical Center, Kansas City, KS and [2]National Cancer Institute, NIH, Bethesda, MD.*

Several transporters in liver are responsible for organic anion (OA) uptake and excretion. The organic anion transporting polypeptide (Oatp) family, along with the Na+-taurocholate cotransporting polypeptide (Ntcp), mediate bile salt and OA uptake into liver. In contrast, the multidrug resistance protein (Mrp) family and the bile salt export pump (Bsep) mediate bile salt and OA export from liver. The transcription factor HNF1α regulates the basal expression of many genes in liver. HNF1α increases the expression of several cytochrome P450s and decreases others. Therefore, it was hypothesized that HNF1α is important for the regulation of transporters in liver. To examine whether HNF1α regulates the basal expression of transporters, mRNA expression of eight Oatp and nine Mrp transporters, as well as Ntcp and Bsep, was quantified in liver of wild-type (WT) and HNF1α-knockout (KO) mice. The expression of four of the eight Oatp mRNAs in the livers of the KO mice was lower (Oatp1, 3, 4, and 9), with two almost completely absent (Oatp1 and 9), three increased (Oatp2, 5, and 14) and no changes in Oatp12 from WT mice. Only two of the export transporters (Bsep and Mrp6) were lower in the KO than in WT mice, whereas three were higher (Mrp4, 5 and 9) and five were similar in KO and WT mice. These data indicate that HNF1α is important in the regulation of many, but not all, hepatic transporters. As with cytochrome P450s, HNF1α does not have a consistent effect on transporter expression, as loss of HNF1α may increase, decrease, or have no effect on expression of transporters in liver. In general, the expression of the hepatic uptake transporters is affected more by lack of HNF1α than the expression of hepatic export transporters. (Supported by NIH grants ES-09716, ES-09649, and ES-07079, ASPET SURF Program)

1467 DISTRIBUTION OF THE MULTIDRUG RESISTANCE-ASSOCIATED PROTEINS (MRPS) IN TESTES, OVARY, AND PLACENTA OF MICE.

J. M. Maher, A. L. Slitt, T. M. Leazer and C. D. Klaassen. *Pharmacology, KU Medical Center, Kansas City, KS.*

Recent analysis of the genome has revealed that there are a minimum of nine Mrp transporters in mice. Many recently discovered mouse Mrps are orphan transporters, with no known substrates, and little is known about their tissue distribution. Mrp2 (abcc2), Mrp3 (abcc3), and Mrp4 (abcc4) are thought to transport bile acids and xenobiotics out of liver, whereas Mrp4 and Mrp5 (abcc5) have been shown to transport cyclic nucleotides into urine. To investigate the expression profile of Mrp1-9 in reproductive tissues, the mRNA expression in testes, placenta, and ovary was determined, and compared to expression in other major organs. Expression of Mrp1 (abcc1) was 7, 6, and 4-fold higher in testes, ovary, and placenta, respectively, than in stomach, the non-reproductive organ exhibiting the highest relative expression. Transporters known to be highly expressed in liver, namely Mrp2, Mrp3, and Mrp6 (abcc6), showed little expression in testes, ovary or placenta. The expression of Mrp4 and Mrp5 in reproductive organs is similar to that in most other tissues. Mrp7 (abcc10) was most highly expressed in testes, and its expression in ovary and placenta was similar to the non-reproductive organs (intestine and kidney) that exhibited the highest expression. The most pronounced result was with Mrp8 (abcc11), which appears to be expressed solely in testes. Similarly, Mrp9 (abcc12) was minimally expressed in most tissues, but was expressed about 20-fold higher in testes and ovary than any other organ. Mrps move chemicals out of cells, and have been demonstrated to transport estrogens and dehydroepiandrosterone. Thus, the Mrps highly expressed in ovary and testes may play a role in endocrine function. Alterations in Mrp expression could lead to changes in hormone levels, and thus may serve as targets for endocrine disrupting xenobiotics. In addition, Mrps may protect the ovary and testes from some xenobiotics. (Supported by NIH grants ES-09716 and ES-07079)

1468 REGULATION OF MOUSE ORGANIC ANION TRANSPORTING POLYPEPTIDES (OATPS) IN MOUSE LIVER BY CLASSES OF PROTOTYPICAL MICROSOMAL ENZYME INDUCERS THAT ACTIVATE VARIOUS TRANSCRIPTIONAL PATHWAYS.

X. Cheng and C. Klaassen. *University of Kansas Medical Center, Kansas City, KS.*

Phase I and phase II drug-metabolizing enzymes, as well as transporters are involved in the disposition of endogenous and exogenous compounds in the body. Phase I and II biotransformation enzymes can be up-regulated by classes of microsomal enzyme inducers (MEIs). For example: AhR ligands (TCDD, polychlorinated biphenyl 126, indole-3-carbinol and b-naphthoflavone) induce Cyp1A1/2; CAR ligands (phenobarbital, TCPOBOP, diallyl sulfide and trans-stilbene oxide) induce Cyp2B1/2; PXR ligands (PCN, spironolactone and dexamethasone) induce Cyp3A; PPARa ligands (clofibrate, ciprofibrate, diethylhexylphthalate and perfluorodecanoic acid) induce Cyp4A14; Nrf2 activators (oltipraz, ethoxyquin and butylated hydroxyanisole) induce quinone reductase; and isoniazid, aspirin and streptozotocin are Cyp2E1 inducers. Some chemicals need to be transported into the liver by Oatps for biotransformation. Therefore, it was hypothesized that the expression of drug-metabolizing enzymes and Oatps might be coordinately regulated in liver. However, there is little information concerning the effects of MEIs on the expression of Oatps in mouse liver. In the present study, the effects of the aforementioned MEI classes on the hepatic mRNA expression of 10 known mouse Oatps (Oatp1-5, 9, 11, 12, 14 and prostaglandin transporter (Pgt)) were determined by the branched DNA assay. Three of the four AhR ligands increased mRNA expression of Oatp9 and 11 in liver. CAR ligands down-regulated Oatp1 mRNA, but up-regulated Oatp2 mRNA. PXR and PPARa ligands decreased Oatp1, 4, 9 and Pgt mRNA levels in liver. Nrf2 activators down-regulated Oatp1 mRNA, but up-regulated Oatp2 and 9 mRNA. Cyp2E1 inducers increased Oatp2, 5 and 9 mRNA levels in liver. In conclusion, microsomal enzyme inducers up-regulate the expression of some Oatps in liver, but down-regulate others. These data also indicate that there is not always a coordinated induction of drug-metabolizing enzymes and Oatps in mice. (Supported by NIH grant ES-09649)

1469 REGULATION OF HEPATIC TRANSPORTERS DURING CHOLESTASIS IS INDEPENDENT OF TNFα, IL-1, AND IL-6 ACTIVITY.

A. J. Lickteig[1], A. L. Slitt[2], N. Li[2], C. D. Klaassen[2] and N. J. Cherrington[1]. *[1]University of Arizona, Tucson, AZ and [2]University of Kansas Medical Center, Kansas City, KS.*

Hepatic transporters play a critical role in the uptake and efflux of bile acids as an essential aspect of liver function. When the normal vectoral transport of bile acids by the basolateral uptake and canalicular excretion transporters is disrupted, cholestasis ensues, and the decreased flow of bile causes accumulation of toxic bile constituents and considerable hepatocellular damage. The purpose of this study was to assess how two models of cholestasis, lipopolysaccharide (LPS) administration and bile duct ligation (BDL) alter expression of transporters in mouse liver. Additionally, the potential involvement of cytokines was examined in knockout mouse strains lacking TNF-α-receptor, interleukin (IL)-1-receptor, and IL-6. Transporter mRNA levels in liver were determined using branched DNA signal amplification 16h following LPS administration or 3 days after bile duct ligation. LPS treatment tended to decrease mRNA levels of Bsep, Oat2, Ntcp, Oatp1, Mrp2 and

Mrp6 in comparison to saline treatment, whereas it increased Mrp1, Mrp3 and Mrp5 levels. Similar changes after LPS were observed in both wild type and knockout strains. Bile duct ligation did not cause a similar decrease in transporter expression as LPS administration, and only Oat2 expression was decreased. Like LPS treatment, bile duct ligation increased Mrp1, Mrp3, Mrp5, and Oatp2 expression in wild type and knockout strains. These data clearly reflect dissimilarity in hepatic transporter regulation in the bile duct ligation and LPS models of cholestasis. It is concluded that the differences in regulation of hepatic transporters during BDL and LPS-induced cholestasis are not due to the individual cytokine activity of TNFα, IL-1 and IL-6, respectively, but to the differences in the mechanism of cholestasis.

1470 XENOBIOTIC TRANSPORTER EXPRESSION IN THE BLOOD-TESTIS BARRIER.

N. J. Cherrington[1], R. J. Markelewicz[2] and K. Boekelheide[2]. *[1]University of Arizona, Tucson, AZ and [2]Brown University, Providence, RI.*

A major function of xenobiotic and endobiotic transporters is to move a wide range of organic substances across cell membranes. Sertoli cells play an important role in protecting developing germ cells by forming a physiological barrier, limiting exposure to potentially toxic substrates, or conversely, facilitating uptake of xenobiotics to the testis. The aim of this study was to quantitatively determine the constitutive expression of various transporters in isolated Sertoli cells from adult Sprague-Dawley rats. The mRNA levels of Mdr1a, 1b and 2; Mrp1, 2, 3, 4, 5, and 6; Oatp1, 2, 3, 4, 5, 9, and 12 and Pgt; Oat1, 2, 3, and K; Oct1, 2, 3, N1 and N2; Ntcp, Bsep, Ibat; Menke's and Wilson's transporters, Dmt1, Znt1; Pept1 and 2; Ent1 and 2; as well as Cnt1 and 2 were measured in isolated Sertoli cells by the branched DNA signal amplification method. The mRNA levels of these genes were also determined in liver, kidney, ileum and whole testis in order to provide a reference for determining relative expression levels. Ent1 and 2 were expressed in isolated Sertoli cells at higher levels than in liver, kidney or ileum, whereas Mrp1, 3 and 5, Oatp3, Oct3 and N2, Menke's and Wilson's transporters, Dmt1 and Znt1 were all significantly expressed in isolated Sertoli cells, but Sertoli cell expression was not the tissue of highest expression. The remaining transporters were expressed at low levels in isolated Sertoli cells. Additionally, xenobiotic transporter expression levels in whole testis were similar to levels observed in isolated Sertoli cell isolations. The constitutive expression levels of different transporters in isolated Sertoli cells may provide an insight into the range of xenobiotics that can potentially be transported by Sertoli cells and thereby provide a mechanistic understanding of blood-testis barrier function. (Supported by NIH grant P30 ES-06694 and R01 ES-05033)

1471 DEVELOPMENT OF GENE CHIPS FOR EARLY DETECTION OF WELDER'S PNEUMOCONIOSIS.

I. Yu[1], K. Park[2], K. Rim[1], J. Sung[1], B. Choi[1], Y. Chung[1] and J. Han[1]. *[1]Center for Occupational Toxicology, Occupatinal Safety & Health Research Institute, KOSHA, Daejeon, South Korea and [2]Pharmacogenechips, Chuncheon, South Korea.*

Welders with radiographic pneumoconiosis abnormalities have exhibited a gradual clearing of the X-ray identified effects following removal from exposure. In some cases, the pulmonary fibrosis associated with welding fumes appears in a more severe form in welders. Accordingly, for the early detection of the welding fume exposure-induced pulmonary fibrosis, we have attempted to develop gene chips. Rats were exposed to welding fume with concentrations of 107.82 ± 2.58 mg/m^3 of total suspended particulates for 2 hrs per day in an inhalation chamber for 30 days. After 30 days exposure, rats were sacrificed and mononuclear cells were prepared from peripheral blood. Total RNA and mRNA were prepared from the mononuclear cells. cDNA was synthesized from total RNA by SMART TM PCR cDNA method, then the subtractive hybridization was performed to select welding fume exposure regulated genes. The cDNAs identified by subtraction were sequenced after transformed into plasmid miniprep. The sequences were analyzed by the NCBI BLAST program and compared with the commercially available microarray data. Two hundred six genes were elevated from 2 to 13 fold and 742 genes were decreased from 0.1 to 0.5. These data demonstrated the differential gene regulation occurs following inhalation exposure of welding fumes. Some differentially regulated genes will be selected to develop gene chips to screen welder's pneumoconiosis.

1472 RENAL TOXICOLOGICAL GENE RESPONSE TO ANTI-HEPATITIS B PRODRUGS HEPAVIR B AND HEPSERA IN RATS.

C. Fang, R. Yan, C. Lim, D. Vitarella, P. Srivastava and C. Lin. *R & D Department, ICN Pharmaceuticals Inc., Costa Mesa, CA.*

Background: PMEA is an acyclic nucleoside phosphonate analog with potent anti-hepatitis B virus activity. However, it had low oral bioavailability and kidney toxicity. Hepsera (adefovir dipivoxil) is an approved, orally bioavailable prodrug of PMEA, whereas Hepavir B is a liver-targeting prodrug of PMEA. Aims: To profile renal toxicological gene expression and elucidate the molecular mechanism of PMEA-induced nephrotoxicity. Methods: 5 male rats per group received orally Hepsera (40 mg/kg/day) or Hepavir B (300 mg/kg/day) for 8 days and 28 days. RNA samples were isolated from the kidney tissue and Affymetrix Rat Toxicology Arrays were employed. Results: Significant increase of the mRNA level of Mdr1b (>20 fold), cyclin B and G, Mdm2 and c-jun (3-6 fold) could be detected as early as on the 8th day of treatment with either of these two prodrugs, before the appearance of any significant pathology in the kidney. Changes in expression of Mdr1b, cyclin B and G were further confirmed by RT-competitive PCR. Twenty-eight-day dosing with either of these two prodrugs changed the expression of additional 40 genes more than 3 fold, including the up-regulation of cell cycle regulators (i.e. p21 and cyclin D), signaling genes (c-myc and MEKK1), heat shock protein 27, apoptosis genes (Caspase-3 and -1), tissue regenerators (glycoprotein CD44 and Pro ?1 collagen type III) as well as down-regulation of CYP2C, 2D, 2E, 3A and 4A and antioxidant enzymes (GSTA3, GSHPx and catalase). Consistent with the gene expression, pathological examination showed renal proximal tubular inflammation and necrosis as well as scattered apoptosis detected only on day 28, not day 8. Conclusions: (1) microarrays can be a useful tool for early predictive toxicology. (2) Hepavir B at 300 mg/kg/day had a similar renal toxicological gene response as Hepsera at 40 mg/kg/day, suggesting better safety for Hepavir B than Hepsera at same dose. (3) PMEA-mediated nephrotoxicity appears to be involved in the development of apoptosis in proximal tubule cells.

1473 RAT LIVER TRANSCRIPTOMICS AFTER (COMBINATORIAL) 28-DAY EXPOSURE TO BENZENE AND TRICHLOROETHYLENE.

W. H. Heijne, M. J. Bart, D. Jonker, R. H. Stierum, B. van Ommen and J. Groten. *Biomolecular Sciences, TNO Nutrition and Food Research, ZEIST, Utrecht, Netherlands.*

The mechanisms of hepatotoxicity of Benzene (B) and Trichloroethylene (TCE), and combinatorial effects were studied at the transcriptome level in rat liver. Although liver is not the primary target organ for toxicity, after a 28 day exposure to B or T, (modest) toxic effects were observed in liver, though not at all three dose levels. Besides elaborate routine toxicological examinations, transcriptomics was performed to find more sensitive markers of effects, elucidate mechanisms of action and evaluate the use of gene transcription patterns in combinatorial toxicology. Additionally, metabolomics analyses revealed compound and dose specific metabolite profiles in urine. Since routine toxicological examinations did not markedly reveal hepatotoxicity, these could not be used to describe the combinatorial effects. On the contrary, transcriptome data revealed toxicant specific effects that enabled to build a mechanism-based hypothesis on combined action of B and TCE in rat liver. This hypothesis was tested in the second phase of the study, where actual combined exposures were analyzed. Two dose levels of the compounds were used in single and the 4 possible combined exposures, and individual rat livers were measured using oligonucleotide DNA arrays. Several drug-metabolizing enzymes, but also genes involved in other biological processes were identified to be induced or repressed by Benzene, TCE or by both. Bioinformatics methods like pattern recognition techniques were applied to compare the response between the different exposure groups and between individual rats. Concluding, transcriptomics provided a better way to deduce putative health risks of combined exposure to B and TCE, based on mechanistic insights rather than empirical evidence.

1474 BIOMARKER SETS THAT CHARACTERIZE BILE DUCT HYPERPLASIA.

K. Jarnagin, C. Pearson, A. Roter, A. Tolley, B. Eynon, B. Ganter, G. Natsoulis, G. Day, K. Kolaja, M. Fielden, M. Lee, R. Nair, S. Dunlea, J. Yang, L. Gong, S. Nicholson, S. Tugendreich, S. Fujimoto and S. Baumhueter. *Iconix Pharmaceuticals, Inc., Mountain View, CA.*

To improve compound selection during drug discovery we have developed a large library of biomarkers for important toxicities and mechanisms of action. These signatures come from a comprehensive database that marries ~13, 000 microarray gene expression results derived from treating rats with 580 drugs at two doses and four time points in triplicate with traditional measurements of toxicity, pathology and pharmacology. Bile duct hyperplasia (BDH) occurs when cholangiocytes that line the bile ducts proliferate in response to toxicants. A signature that indicates BDH induction was derived using gene expression results from experiments in which histologically confirmed BDH occurred. Compounds that caused BDH include 1-naphthyl isothiocyanate (ANIT), lomustine, carmustine, methylene dianiline, lipopolysaccharide, vinblastine, carvedilol and fenofibrate. Hyperplasia generally occurred concomitantly with other liver pathologies, such as necrosis, apoptosis, leukocytosis and hypertrophy. RNAs that changed during BDH include those involved in cell adhesion, morphology, motility, extracellular matrix, fibrosis

and immunity. These changes reflect the remodeling of the liver during BDH. Most RNAs that increase are expressed in relatively rare cells that are increasing in number, such as cholangiocytes, and those that are activated, such as liver macrophages. Most RNAs that decrease are expressed in cells that are dying or functionally impaired, such as hepatocytes. Many of the treatments that cause BDH induce RNAs that increase during fibrosis, although only ANIT caused histologically confirmed fibrosis. Thus, the BDH signature not only discriminates those compounds that cause BDH but may also predict if these compounds induce fibrosis later. These mechanistic and predictive findings may hasten and improve decison making during drug discovery, thus improving efficency.

1475 TRANSCRIPTIONAL PROFILE OF CIGARETTE SMOKE INDUCED EMPHYSEMA LUNGS.

K. H. Mai[1], T. Rangasamy[1], R. M. Tuder[2] and S. Biswal[1]. *[1]Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD and [2]Deapartment of Pathology, Johns Hopkins School of medicine, Baltimore, MD.*

Pulmonary emphysema is a major component of the morbidity and mortality of chronic obstructive pulmonary disease, that affects more than 14 million persons in the United States. Cigarette smoking is the main risk factor for the development of emphysema. This study investigates the gene expression pattern in the emphysematous lung tissues of A/J mice. The A/J mice (8 weeks old) were exposed to cigarette smoke (CS) - 7 h/day and 7 days/week for 6 months using a smoke machine. Lung alveolar measurements revealed 25 % and 30 % increase respectively, in the mean linear intercept and lung alveolar diameter of CS exposed A/J mice when compared to air exposed control mice. Fluorescent TUNEL and immunohistochmical analysis revealed the increased number of capase-3 positive, apoptotic cells and 8-OxodG positive cells in the lungs of CS exposed A/J mice. Differential cell count analysis showed 3 fold increase in the total inflammatory cells (80% macrophages) in the branchoalveolar lavage fluid of the CS exposed A/J mice. In order to identify the genes which are expressed in emphysematous lung tissues, oligonucleotide microarray analysis was carried out using Affimetrix chips (Mouse 430A chips) which contains 11, 400 genes. The data was analysed using MAS 5.0 and Data Mining Tool 3.0 program (Affymetryx, CA). Some of the selected genes from the microarray data were validated by Real Time PCR. Microarray analysis revealed the expression of numerous genes (expression of various proteases, genes involved in lipid metabolism, apoptosis associated genes, and inhibition of different genes encoding for various types of collagen, serine protease inhibitor, TGF beta 2, sodium channel protein, cell adhesion molecules and genes involved in signal transduction) which may play a critical role in development of emphysema. (This work was supported by grants: P50 CA058184-09 and NIEHS center grant P30 ES 03819).

1476 EFFECTS OF MULTIPLE CARDIAC APEX NECROSIS AGENTS ON GENOME WIDE EXPRESSION.

A. L. Castle, B. W. Higgs, C. G. Chang and D. L. Mendrick. *Toxicogenomics, Gene Logic Inc., Gaithersburg, MD.*

The differential gene expression and potential mechanisms of toxicity of several adrenergic agonists known or suspected to cause cardiac apex necrosis were compared to determine what mechanisms were in common or distinct among the compounds. One hundred and thirty four rats were treated once per day with either a high or low dose of mixed alpha/beta agonists epinephrine (EPI) and norepinephrine (NEP); beta agonist isoproterenol (ISO); atypical beta agonist clenbuterol (CLB) or time matched vehicle controls. High doses of 1.0, 0.5, 0.5, 4.0 mg/kg/day of EPI, NEP, ISO, or CLB respectively were previously determined to cause mild to moderate toxicity. Low doses were 1/10th the concentration of high doses. Animals were sacrificed at 3, 6, 24 hrs for EPI, ISO, and NEP. CLB animals were sacrificed at 6, 24, 120, 336 hrs post first administration. Affymetrix RGU34 GeneChip® and histopathology analyses were conducted on all samples. Mixed alpha/beta, beta, and atypical beta agonists had differential effects, albeit modest, on gene expression at both high and low doses. The diphtheria toxin receptor was significantly affected in EPI, NEP, and ISO with a corresponding trend in CLB. Gadd45a was upregulated to a greater extent in both EPI and NEP than ISO and CLB suggesting alpha mediated responses contributed to cellular damage. Transient up-regulation of Myc occurred with all treatments but the magnitude differed greatly at 24hrs suggesting a different duration or magnitude of effect. In addition, several genes linked to KEGG or BioCarta metabolic, inflammation, signal transduction and hypertrophy related pathways were affected. In general gene expression changes suggest common mechanisms among all compounds, with some differences that may allow both mechanism of toxicity and mechanism of action at the genome wide scale to be elucidated.

1477 MONITORING INDICATIONS OF LEAD EFFICACY AND TOXICITY WITH A MULTIPLEX GENE EXPRESSION ASSAY DURING EARLY DRUG DEVELOPMENT.

G. Vansant, P. Pezzoli, J. Monforte and F. Ferre. *eXpress Profiling, Althea Technologies, Inc., San Diego, CA.*

a) Many promising lead compounds are uncovered during the early high throughput screening process only to fail in preclinical safety studies. Out of 5000 compounds that are evaluated for safety in animals, approximately 5 are deemed safe enough to enter clinical trials. Animal studies cost 2-4 million dollars and can take 2 years to complete. Development of assays that can measure efficacy and safety indications simultaneously before long term and costly animal studies could substantially reduce drug development costs, and increase the productivity of toxicology labs. b) We have developed an RT-PCR based multiplex assay that simultaneously monitors the impact of compounds on three gene sets: lipid lowering genes controlled by the PPAR alpha receptor, genes that monitor a biological system's oxidative stress status, and selected metabolizing cytochromes. Rats were treated with various doses of clofibrate for 1 week. The expression of the genes shown in Table I was analyzed and compared to the expression in control rats. c) The impact of clofibrate on lipid lowering genes, stress related genes and the selected cytochrome genes was successfully monitored in a single reaction d) The results clearly illustrate that a compound's impact on a targeted pathway, as well as on toxicologically relevant pathways, can be monitored by multiplex gene expression analysis carried out in a single reaction. **Table I- Genes in Multiplex Assay Lipid metabolism/gluconeogenesis** Fatty acyl-CoA oxidase Apolipoprotein AIV Apolipoprotein CIII Pyruvate dehydrogenase kinase 4 Phosphoenolpyruvate carboxykinase PPARalpha **Oxidative stress** DNA topoisomerase IIA Heme oxygenase-1 Glutathione reductase p21 Thioredoxin reductase GCLM HSP70 HSPA8 Hsp27 NRF2 STIP1 **Cytochromes** CYP1A1 CYP3A1 **Controls** GAPDH beta-actin ext. control

1478 MITOCHONDRIAL TOXICITY OF 3'-AZIDO-3'-DEOXYTHYMIDINE (AZT) IN THE ABSENCE OF MITOCHONDRIAL DNA (MTDNA) DEPLETION.

K. C. Lund and K. B. Wallace. *Biochemistry and Molecular Biology, University of Minnesota School of Medicine, Duluth, MN.*

A growing concern in the treatment of HIV/AIDS is the increased incidence of hyperlacticacidemia and cardiac myopathy. Nucleoside analog reverse transcriptase inhibitors (NRTIs) have been implicated in these and a variety of other mitochondrial related pathologies believed to be precipitated by the inhibition of the native DNA polymerase γ. The objective of this study was to determine if the inhibition of polymerase γ in cardiac tissue is causally related to mitochondrial toxicity. Rats were treated with AZT in their drinking water (1 mg/mL) for 32 days. Serum lactate was monitored weekly, and mitochondrial function was determined at 32 days by measuring NADH-dehydrogenase, cytochrome oxidase, and F_1, F_0-ATPase enzyme activities. The possible inhibition of polymerase γ was assessed by the mtDNA copy number using quantitative PCR, and the expression of four mitochondrially encoded genes (ND1, COX1, ATPase6, and ND4) was measured using quantitative RT-PCR. The results indicate that mtDNA replication was not affected by long-term treatment with AZT; no difference in mtDNA copy number was observed between control and treated animals. There were no broad differences in mitochondrially encoded mRNAs or enzyme activities; with the exception of an elevated ND4 mRNA copy number and an increased activity of NADH-dehydrogenase in the AZT treatment group. AZT treatment caused an increase in serum lactate (22.9% compared to 12.5% for control) and a decrease in final body weight. A linear correlation was observed between cardiac NADH-dehydrogenase activity and serum lactate suggesting a compensatory response to AZT treatment. These data demonstrate that *in vivo* treatment with AZT induced cardiac mitochondrial dysfunction in the absence of mtDNA depletion, suggesting that AZT has a direct effect on mitochondrial targets independent of inhibition of mitochondrial DNA? polymerase γ. (Supported by HL-72715).

1479 PHTHALATES INFLUENCE THE EXPRESSION OF FATTY ACID HOMEOSTASIS REGULATING PROTEINS IN HRP-1 CELLS.

Y. Xu, P. Shah, T. J. Cook and G. T. Knipp. *Department of Pharmaceutics, Rutgers, The State University of New Jersey, Piscataway, NJ.* Sponsor: K. Reuhl.

Di(2-ethylhexyl)phthalate (DEHP) is a widely used plasticizer and ubiquitous environmental contaminant. The potential health hazards from exposure to DEHP and its metabolites, mono(2-ethylhexyl)phthalate (MEHP) and 2-ethylhexanoic acid (EHA), may be related to their role in the transactivation of peroxisome proliferator-activated receptors (PPAR) α and γ, established regulators of cellular fatty acid (FA) homeostasis. The maternal-to-fetal transfer of FAs is highly directional from

the mother to the fetus and plays a critical role in fetal development. Changes in placental FA homeostasis may result in abnormal fetal development. This study demonstrates the effects of DEHP, MEHP and EHA on the mRNA and protein expression of PPARα, β, and γ, and fatty acid transfer conferring proteins in a rat placental cell model (HRP-1). HRP-1 cells were grown as monolayers and treated with DEHP, MEHP and EHA in a dose and time dependent manner for RNA and protein isolation. RT-PCR were performed under optimized conditions and normalized to β-actin expression using gene specific primers for PPAR isoforms and fatty acid transporters including fatty acid transport protein 1 (FATP1), plasma membrane fatty acid binding protein (FABPpm) and heart cytoplasmic fatty acid binding protein (hFABP). Western and slot blotting was utilized to support the RT-PCR results. All mRNA and protein expressions of each target of interest were observed in the HRP-1 cell at baseline. In the DEHP, MEHP and EHA treated cells, the expression of PPARα and γ, FATP1, and hFABP were up regulated in a dose and time dependent manner. PPAR β, FABPpm demonstrated variable expression in these samples, consistent with literature reports. These results demonstrate that DEHP, MEHP and EHA can influence the expression of proteins involved in FA homeostasis in a rat placental cell line. This implies that these compounds may contribute to aberrant placental FA homeostasis through peroxisome proliferation and potentially result in abnormal fetal development. Financial support from NAAR.

1480 6-HYDROXYDOPAMINE-INDUCES OXIDATIVE STRESS AND TOXICITY IN HUMAN NEUROBLASTOMA CELLS.

C. hu, M. A. Tirmenstein, P. K. Narayanan, H. C. Thomas and L. W. Schwartz. *GlaxoSmithKline, King of Prussia, PA.*

6-Hydroxydopamine (6OHDA) has been implicated in the pathogenesis of Parkinson's disease (PD) and is frequently used in animal models of PD. Previous studies have demonstrated that 6OHDA is selectively toxic to catecholaminergic cells and is toxic to the human dopaminergic neuroblastoma cell line, SH-SY5Y, *in vitro*. Studies suggest that formation of free radicals by 6OHDA is an important causative factor in cellular toxicity in SH-SY5Y, but there is no direct evidence for 6OHDA-induced oxidative stress in SH-SY5Y cells. In our study, we examined cellular *via*bility, reduced to oxidized glutathione ratios, ATP levels, and mitochondrial membrane potential ($\Delta\Psi$m) in SH-SY5Y cells treated with 6OHDA at concentrations of 50 – 500 μM and 5 – 50 μM for 4 and 24 h, respectively. 6OHDA produced slight but significant increases in cell death after 4 h. After 24 h, a concentration-dependent increase in cell death was observed, but only the highest dose (50 μM) induced significant increases. After a 4 h incubation, 6OHDA decreased reduced glutathione (GSH) and increased oxidized glutathione (GSSG) levels in a concentration dependent manner resulting in substantial reductions of GSH/GSSG ratios. 6OHDA induced a dramatic (12-fold) concentration-dependent elevation of GSH at 24 h . Significant ATP depletion occurred in cells treated with 6OHDA at ≥ 100 μM for 4 h and at ≥ 25 μM for 24 h. The $\Delta\Psi$m of SH-SY5Y cells was also decreased by 6OHDA (≥ 50 μM) at 4 h. These findings demonstrate that 6OHDA induces mitochondrial dysfunction, oxidative stress and toxicity, in SH-SY5Y cells.

1481 A MODEL OF TOXICANT-INDUCED OXYGEN FREE RADICAL PRODUCTION FROM ISOLATED BOVINE RESPIRATORY CHAIN COMPLEX I.

J. E. Johnson[1, 2], K. Choksi[2] and W. R. Widger[2]. *[1]Optometry, University of Houston, Houston, TX and [2]Department Biology and Biochemistry, University of Houston, Houston, TX.* Sponsor: D. Fox.

The free radical theory of toxicant-induced injury and aging states that cell metabolism and function progressively decline in response to oxidative stress produced by reactive oxygen species (ROS). ROS production also is implicated in neurodegenerative disorders such as Parkinson's, Alzheimer's, and mitochondrial diseases. Multiple sites within the mitochondrial respiratory chain are hypothesized to produce ROS. The extent to which respiratory chain complex I contributes to ROS production is a topic of debate and clinical importance. Our aim was to determine whether ROS production co-isolated with respiratory complex I. Piericidin A, a potent inhibitor of complex I, and ubiquinone-1 (Q1) were used to stimulate ROS production from complex I. The ROS production was measured by two independent techniques: superoxide dismutase- (SOD) and catalase-sensitive oxygen consumption and SOD-sensitive cytochrome c reduction. Oxygen turnover and SOD-sensitive cytochrome c turnover were 150 - 550 per min. and 0 - 1000 per min., respectively. Q1-dependent ROS production was linear and non-saturating with increasing Q1 concentrations, suggesting that thie ROS production was largely nonspecific. Diphenyleneiodonium inhibited complex I ROS production, indicating that the site of ROS production was prior to the piericidin A block, possibly at one or more iron-sulfur centers and/or the flavin mononucleotide (FMN). Most importantly, the ROS production co-isolated with complex I FMN indicating that complex I is a potential site of mitochondrial ROS production. These results have mechanistic and therapeutic relevance for the diagnosis and treatment of free radical-induced injury in many tissues, especially those with high aerobic demand such as heart, kidney, and brain. Supported by the Welch Foundation Grant E-1381.

1482 LACK OF INHIBITION BY MELATONIN OF THE TOXIC AND PROLIFERATIVE EFFECTS OF DIETARY DIMETHYLARSINIC ACID ON RAT UROTHELIUM.

M. Wei, S. M. Cohen, M. Cano and L. L. Arnold. *Path/Micro, University of Nebraska Med. Ctr, Omaha, NE.*

Arsenic is a known human carcinogen of the skin, lung, bladder, and possibly other internal organs. Dimethylarsinic acid (DMA), a major organic metabolite of arsenic in most mammals including humans, has been shown to be a bladder carcinogen in rats when administered in the diet or in the drinking water. Oxidative stress has been suggested as a mechanism for DMA-induced carcinogenesis. Our recent results suggest that the induction of cytotoxicity with necrosis and consequent regenerative proliferation of the urothelium may be involved in the process of DMA-induced urinary bladder carcinogenesis. Dimethylarsinous acid (DMA^{III}) generated from reduction of DMA, probably contributes to the process. To determine whether an antioxidant, melatonin, could inhibit the cytotoxic and proliferative effects of DMA, female F344 rats were concurrently treated with 1000 ppm melatonin and 100 ppm DMA in the diet. Histopathology, bromodeoxyuridine (BrdU) immunohistochemistry and scanning electron microscopy (SEM) were used to evaluate proliferative and cytotoxic changes in the urothelium. Melatonin did not show significant inhibitory effects on urothelial proliferation when compared with DMA alone. The BrdU labeling index was significantly increased in the DMA-treated rats with or without melatonin and by light microscopy there was no difference between the DMA and the DMA plus melatonin groups. However, there was the suggestion of a slight protective effect against DMA-induced cytotoxicity based on SEM observations. These results show that melatonin had little inhibitory effect on DMA-induced urinary bladder toxicity or proliferation in the rat. In addition, melatonin (0.2 mM) did not inhibit DMA (0.6 mM) or DMA^{III} (0.4 μM)-induced cytotoxicity *in vitro*

1483 CARBON NANOTUBE EXPOSURE CAUSED FORMATION OF FREE RADICALS, INDUCTION OF OXIDATIVE STRESS AND CYTOTOXICITY IN HUMAN KERATINOCYTES AND BRONCHIAL EPITHELIAL CELLS.

E. Kisin[1], A. R. Murray[2], D. Schwegler-Berry[1], V. Z. Gandelsman[3], M. R. Ganther[2], V. Castranova[1, 2, 4] and A. A. Shvedova[1, 2]. *[1]PPRB, NIOSH, CDC, Morgantown, WV, [2]Physiology and Pharmacology, WVU, Morgantown, WV, [3]Advanced Technology Group, NASA-JSC/SAIC, Houston, TX and [4]GSPH, University of Pittsburgh, Pittsburgh, PA.*

Carbon nanotubes are new members of carbon allotropes similar to fullerenes and graphite. Because of their unique electrical, mechanical and thermal properties, carbon nanotubes are being evaluated for novel applications in the electronics, aerospace and computer industries. Exposure to graphite and carbon materials has been associated with increased incidence of skin and lung diseases, e.g., carbon fiber dermatitis, hyperkeratosis, naevi, asthma, COPD, pneumoconiosis and cancer. However, the potential toxicity of single wall carbon nanotubes (SWCNT) has yet to be completely evaluated. The present study investigated adverse effects of SWCNT using a cell culture of immortalized human epidermal keratinocytes (HaCaT) or bronchial epithelial cells (BEAS-2B). Exposure to SWCNT resulted in ultra-structural and morphological changes in cultured human cells. Treatment of keratinocytes and bronchial epithelial cells with SWCNT for 18 hr caused oxidative stress and cellular toxicity indicated by the formation of hydroxyl radicals, accumulation of peroxidative products, antioxidant depletion, loss of cell *via*bility and apoptosis. These data suggest that dermal and respiratory exposure to unrefined SWCNT may lead to dermal and pulmonary toxicity due to accelerated oxidative stress.

1484 GREEN CHEMISTRY CATALYST CAUSES DEPLETION OF GSH, OXIDATIVE STRESS AND CYTOTOXICITY IN KERATINOCYTES IN THE PRESENCE OF H_2O_2.

J. Kisin[1], Y. Y. Tyurina[2], E. Kisin[3], C. P. Horwitz[1], T. J. Collins[1], V. E. Kagan[2], V. Castranova[3, 4] and A. A. Shvedova[3, 4]. *[1]Carnegie Melon University, Pittsburgh, PA, [2]GSPH, University of Pittsburgh, Pittsburgh, PA, [3]PPRB, NIOSH, Morgantown, WV and [4]WVU, Morgantown, WV.*

The Pollution Prevention Act of 1990 (US CODE, 42) is a national policy to prevent or reduce pollution to protect human health and the environment. A practical, inexpensive, green chemical process for degrading environmental pollutants is

greatly needed, especially for persistent chlorinated pollutants. Chlorophenols (CP) have been widely used as components of pesticides, disinfectants, wood preservatives, personal care formulations, and many other products, and they are substantial by-products of wood pulp bleaching with chlorine. A newly developed chemical protocol for degradation of pentachorophenol (PCP) and 2, 4, 6-trichlorophenol (TCP) using iron complexes of tetraamidomacrocyclic ligand-Fe-TAML activators was successfully applied for rapid total destruction of CP. Introduction of novel Fe-TAML chemical catalysts into industry requires evaluation of their safety and understanding of the impact on the environment, biological species, and human health. The present study investigated cytotoxic effects of Fe-TAML using human keratinocytes (HaCaT). Free radical production was assessed by ESR-detectable ascorbate radicals. GSH content in cells was evaluated by measurements of its content in cells lysates using a specific maleimide reagent, ThioGlo-1^{TM}. Additionally, fluorescent imaging of GSH-ThioGlo-1 adducts in cells was performed using a Nikon TE-200 system. Cell *via*bility was estimated by Alamar Blue-based assay. After 8-18 hr of Fe-TAML exposure in the presence of H_2O_2, oxidant generation and cellular toxicity (as indicated by the formation of free radicals, accumulation of peroxidative products, antioxidant depletion, and loss of cell *via*bility) was detected. Exposure to Fe-TAML alone did not induce either oxidative stress or cytotoxicity in HaCaT cultured cells. These data indicated that exposure to Fe-TAML can cause accelerated oxidative stress and toxicity to skin only in the presence of H_2O_2.

1485 INDUCTION OF ENDOGENOUS GLUTATHIONE BY α-LIPOIC ACID IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS: PROTECTION AGAINST 4-HYDROXYNONENAL- AND PEROXYNITRITE-MEDIATED NEUROTOXICITY.

S. S. HALLUR, Z. CAO and Y. LI. *PHARMACEUTICAL SCIENCES, ST.JOHN'S UNIVERSITY, JAMAICA, NY.*

Oxidative stress is believed to play a key role in the pathogenesis of neurodegenerative diseases, such as Parkinson's disease (PD). Early depletion in the levels of thiol antioxidant glutathione (GSH) which may lead to generation of reactive oxygen species is an important biochemical feature of PD. Recent evidence suggests that 4-hydroxynonenal (HNE) and peroxynitrite, two cytotoxic compounds, significantly contribute to the pathogenesis of PD. This study was undertaken to determine whether α-lipoic acid (LA) treatment could increase endogenous antioxidant GSH and whether such increased cellular defense could afford protection against HNE- and peroxynitrite-induced toxicity in the human neuroblastoma SH-SY5Y cells. Incubation of SH-SY5Y cells with micromolar concentrations of LA (50-200μM) for 24, 48 and 72 h resulted in a significant 2-3-fold induction of cellular GSH in a concentration-dependent fashion. Induction of GSH by LA did not show a time-dependent manner; a maximal induction of GSH was observed 24 h after incubation with LA. To investigate the protective effects of the LA-induced cellular defense on toxic neuronal injury, SH-SY5Y cells were pre-treated with LA for 24 h and then exposed to HNE or peroxynitrite. We observed that LA pre-treatment of SH-SY5Y cells led to a significant protection against HNE- or peroxynitrite-induced neurotoxicity as determined by the MTT reduction assay. To further determine the role of cellular GSH in protecting against the above neurotoxicant-mediated cytotoxicity, buthionine sulfoximine (BSO) was used to inhibit cellular GSH biosynthesis. It was observed that depletion of cellular GSH by BSO led to a marked potentiation of peroxynitrite- or HNE-mediated cytotoxicity in SH-SY5Y cells. Taken together, our results demonstrate for the first time that LA potently induces cellular GSH biosynthesis, and that the LA-elevated cellular antioxidant defense appears to afford a marked protection against HNE- or peroxynitrite-induced toxicity in neuronal cells.

1486 MECHANISM OF GLUTATHIONE S-TRANSFERASE A4-4 IN PROTECTION AGAINST OXIDATIVE STRESS INDUCED APOPTOSIS IN ENDOTHELIUM.

Y. Yang[1], Y. Yang[2], M. B. Trent[1], S. D. Lick[3], P. Zimniak[4], Y. C. Awasthi[2] and P. Boor[1]. *[1]Pathology, University of Tex. Med. Branch, Galveston, TX, [2]2Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX, [3]3Department of Surgery, University of Texas Medical Branch, Galveston, TX and [4]4Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, and Central Arkansas Veterans Healthcare System, Little Rock, AR.*

Our previous studies demonstrated that glutathione-S-transferase (GSTs) play a protective role against oxidant damage in vascular smooth muscle cells and endothelium. In order to study the mechanism of GSTs protection of endothelium, we examined the intracellular signalling pathway and the protective role of a specific GST isozyme, mGSTA4-4. Stable transfection of mouse islet endothelial cells (MS1) with mGSTA4-4 was established; transfected cells demonstrated enzyme activity three times (toward 1-chloro-2, 4-dinitrobenzene, CDNB) and four times(toward 4-HNE) higher than wild-type MS1 cells or cells transfected with vector alone. Transfected cells expressed significantly increased resistance to cell apoptosis confirmed by TUNEL and caspase activation due to the cytotoxicity of allylamine, acrolein, 4-HNE, and H2O2. Immunoblotting studies of signal transduction pathway indicated that mGSTA4 transfection protected MS1 endothelial cells from oxidative stress induced apoptosis by inhibiting phosphorylation of c-Jun N-terminal kinases (p-JNK) and consequent activation of p53 and Bax. Application of JNK inhibitor blocked p53 and Bax activation in endothelium. Subcellular localization studies by immunolocalization and electronic microscope indicated that mGSTA4-4 localized in cell vesicles close to plasma membrane that may contain the major substrate(s) for this enzyme. Our results indicate that the mechanism of mGST A4-4 in protection of endothelial cells is through inhibiting the JNK activation pathway, most likely at cytoplasmic sites; thus GSTs may play a key role in protecting blood vessels against oxidative stress and are likely to be a critical defense against atherogens.

1487 MGSTA4-4 NULL (-/-) MICE ALTERS THE COURSE OF CCL4 INDUCED HEPATOTOXICITY.

S. Dwivedi[1], R. Sharma[2], A. Sharma[2], Y. C. Awasthi[2] and P. Boor[1]. *[1]Pathology, University of Tex. Med. Branch, Galveston, TX and [2]Human Biology and Genetics, University of Texas Medical Branch, Galveston, TX.*

Glutathione S-Transferases(GSTs) play a key role in cellular detoxification of environmental toxicants through their conjugation to glutathione(GSH). Recent studies have shown that the α-class GSTs also provide protection against oxidative stress and lipid peroxidation(LPO). GSTA4-4 is a member of a sub group of the α-class GSTs. It has been shown to metabolize 4-hydroxynonenal (4-HNE) with high catalytic efficiency through its conjugation to glutathione (GSH) and has been suggested to be a major component of defense mechanisms against toxic electrophiles such as 4-HNE generated during LPO. Since the hepatotoxicity of carbon tetrachloride (CCl4) has been suggested to be due to the generation of free radicals leading to membrane LPO, present studies were designed to compare hepatotoxicity of CCl4 in null (-/-) and wild type (+/+) mice. The results of these study showed that administration of a single dose of CCl4 (0.1ml/kg i.p) resulted in time dependent hepatotoxicity in both -/- and +/+ mice; the extent of cellular damage by serum enzymes suggested that progression was more rapid in -/- mice, although totals were similar by 24h. Histopathologic exam showed similar degrees of centrilobular necrosis but much greater surrounding degenerative change, including cellular swelling, disarray, and vacuolization, was observed in the liver of -/- mice. As expected -/- mice did not show any expression of mGSTA4-4 only slight compensatory increase in the activities of total GST activity was noted at 24h; major alterations in other antioxidant enzymes was not observed. 4-HNE levels in the liver of -/- mice were about four fold higher as compared to +/+ mice, suggesting a positive correlation between 4-HNE levels and the altered course of CCl4 hepatotoxicity. These studies suggest that GSTA4-4 may be an important component of defense mechanism against oxidative stress and LPO.

1488 MITOCHONDRIAL METABOLISM: THE ROOT OF HYPEROIXC CELL DAMAGE.

J. Li[1], X. Gao[2], M. Qian[2] and J. W. Eaton[2]. *[1]Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY and [2]Department of Medicine, University of Louisville, Louisville, KY.*

Exposure of mammals to hyperoxia causes pulmonary and ocular pathology. Hyperoxic damage and cell death may derive from enhanced intracellular formation of reactive oxygen species (ROS), probably of mitochondrial origin. There is, however, controversy on this point. When wild-type and respiration-deficient (rho0) HeLa cells were cultured in 80% O2, wild-type cells stopped growing after 5 days and died thereafter whereas rhoo cells survived and grew to confluence. This tolerance of rho0 cells to high oxygen was not associated with greater resistance to oxidants such as hydrogen peroxide and t-butyl hydroperoxide. Under both 20% and 80% O2, rho0 cells exhibited substantially decreased ROS production and, under 80% O2, rho0 cells showed no suppression of aconitase activity or mitochondrial protein carbonyl formation. Replacement of normal mitochondria in rho0 cells restored ROS production and susceptibility to hyperoxia. Two other strategies which diminish mitochondrial ROS generation also increased tolerance to hyperoxia. HeLa cells constantly exposed to the protonophoric uncoupler, carbonyl cyanide m-chlorophenylhydrazone (which enhances respiration but decreases ROS production), showed preferential survival under 80% O2 as did HeLa cells in the presence of chloramphenicol (which suppresses both respiration and mitochondrial ROS production). We conclude that interactions between respiring mitochondria and O2 are primarily responsible for hyperoxic cell damage.

1489 OXYGEN TOXICITY AND MITOCHONDRIAL FUNCTION.

E. C. Campian[1], J. Li[1], X. Gao[2], M. Qian[2], H. Joenje[3] and J. W. Eaton[2].
[1]Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY, [2]Department of Medicine, University of Louisville, Louisville, KY and [3]Department of Clinical Genetics and Human Genetics, VU University Medical Center, Amsterdam, Netherlands.

Oxygen is critical to aerobic metabolism, but excessive oxygen (hyperoxia) will injure most mammals. An oxygen tolerant strain of HeLa cells which proliferates even under 80% O2, termed "HeLa-80", was studied for clues to the nature of hyperoxic damage. These oxygen tolerant cells have wild-type (HeLa-20) levels of antioxidant defenses and are equally susceptible to peroxide killing. However, under both 20% and 80% O2, intracellular ROS production is >2-fold higher in wild-type vs. tolerant HeLa cells as assessed by (1) dihydrodicholorfluorescein oxidation, (2) dihydroethidium oxidation, (3) hyperoxia-mediated suppression of aconitase activity and (4) mitochondrial protein carbonyl content. The ROS generation is evidently mitochondrial because, in both HeLa-20 and HeLa-80 cells, the mitochondrial uncoupler carbonyl cyanide m-chloro phenylhydrazone almost totally inhibits ROS production as does the combination of rotenone and thenoyltrifluoroacetone (complex I and II inhibitors). Interestingly, the tolerant HeLa-80 cells have ~ 2-fold higher cytochrome c oxidase (COX) activity and preferential blockade of this terminal complex of mitochondrial electron transport chain, by treatment with n-methyl protoporphyrin which selectively diminishes synthesis of heme-aa3 in COX, abrogates the oxygen tolerance and increases ROS production in HeLa-80 cells under hyperoxia. In the oxygen tolerant HeLa cells, the elevated COX activity may be due to a >2-fold increase in COX Vb (a regulatory subunit of COX) whereas expression levels of other COX subunits (COX I, II and IV) are very close to wild-type. Overall, our results suggest that the oxygen tolerance may derive from tighter coupling of the electron transport chain in HeLa-80 cells which, in turn, depletes electron-rich intermediates within the chain and, thereby, diminishes the leak of ROS.

1490 PROTEIN MODIFICATION BY 4-HYDROXYNONENAL MODULATES 26S PROTEASOMAL DEGRADATION.

D. L. Carbone and D. R. Petersen. *Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO.*

Damaged or otherwise unwanted proteins are selected for degradation *via* the iterative attachment of ubiquitin to specific lysine residues. The α, β unsaturated aldehyde 4-hydroxynonenal (4-HNE) is a major product of lipid peroxidation, shown to readily form Michael addition products with multiple nucleophilic amino acids. Given the ability of 4-HNE to covalently modify lysine residues, we have hypothesized that protein modification by 4-HNE may lead to impaired ubiquitination, ultimately inhibiting degradation by the 26S proteasome. This hypothesis was tested using alcohol dehydrogenase (ADH), mitochondrial aldehyde dehydrogenase (ALDH2), and microsomal cytochrome P450 2E1 (CYP2E1) as model substrates. Model proteins were modified by 4-HNE, and adducts confirmed through immunoblot or MALDI-TOF-MS techniques. The effects of this modification on each protein were evaluated using either rabbit reticulocyte (RRL) or rat hepatocyte cytosol, supplemented with ATP, to provide the necessary components for polyubiquitination and 26S proteasomal degradation. Treatment of the proteins with excess concentrations of 4-HNE resulted in the predicted effect, in which degradation of the proteins was inhibited. Interestingly, exposure of ADH and ALDH2 to lower concentrations of the aldehyde have resulted in a 40% and 25% increase, respectively, in the rate of protein degradation. Involvement of the 26S proteasome was implicated through the use of the proteasome inhibitor MG-132 and non-hydrolyzable ATP analogues. Finally, purification of ubiquitinated proteins followed by immunoblotting demonstrated an enhanced rate of polyubiquitination following exposure to lower concentrations of the aldehyde. These data demonstrate a concentration-dependent ability of the lipid aldehyde 4-HNE to modulate ubiquitin-mediated proteasomal degradation. This work was supported by NIH/AA09300 (DRP) and NIH/AA1F31AA014308-01 (DLC).

1491 THE PROTECTIVE EFFECTS OF CHEMICALLY-INDUCED ENDOGENOUS GLUTATHIONE ON DOPAMINE AND 6-HYDROXYDOPAMINE-MEDIATED TOXICITY IN RAT PHECHROMOCYTOMA PC12 CELLS.

X. PENG and Y. LI. *Pharmaceutical Sciences, St. John's University, Jamaica, NY.*

Dopamine (DA) and 6-hydroxydopamine (6-OHDA) are well-recognized neurotoxins, which are also used to induce experimental Parkinson's disease (PD). Glutathione (GSH) depletion has been suggested to contribute to the pathogenesis of PD, since GSH levels are reduced in PD substantia nigra pars compacta. This study was undertaken to investigate the induction of endogenous GSH by the unique chemoprotective agent, 3H-1.2-dithiole-3-thione (D3T) and its protective effects against DA and 6-OHDA-mediated toxicity in rat pheochromocytoma PC12 cells. Incubation of PC12 cells with micromolar concentrations of D3T resulted in concentration-dependent elevation of GSH. To examine the protective effects of D3T-induced cellular GSH on DA and 6-OHDA-mediated toxicity, PC12 cells were pretreated with D3T for 24 h and then exposed to either DA or 6-OHDA for another 24 h. We found that D3T-pretreatment of PC12 cells led to a significant protection against DA and 6-OHDA-mediated toxicity, as assessed by 3-[4, 5-dimethythiazol-2yl]-2, 5-diphenyltetrazoliium bromide reduction and trypan blue exclusion assays. To further demonstrate the critical involvement of GSH in protecting against DA and 6-OHDA-mediated toxicity, buthionine sulfoximine (BSO) was used to inhibit cellular GSH biosynthesis. Depletion of cellular GSH by BSO caused a marked potentiation of DA and 6-OHDA-mediated toxicity. Co-treatment of cells with BSO was found to largely abolish the D3T-mediated GSH elevation and greatly reverse the protective effects of D3T on DA and 6-OHDA-mediated-toxicity. These data demonstrate for the first time that induction of endogenous GSH by D3T affords significant protection against DA and 6-OHDA-mediated toxicity in rat pheochromocytoma PC12 cells.

1492 DIFFERENTIAL EFFECTS OF CHRONIC ESTRADIOL TREATMENT ON INDUCTION OF PHASE II ANTIOXIDANT ENZYMES IN BRAIN AND LIVER OF ACI RATS.

T. M. Stakhiv, R. I. Sanchez and F. C. Kauffman. *Joint Graduate Program in Toxicology, Rutgers University/UMDNJ, Piscataway, NJ.*

Estrogen has been implicated in protecting against oxidative stress in certain neurological diseases (eg. Parkinson's Disease, Alzheimer's Disease); however, there is little information regarding the actions of estrogen on the levels or activities of antioxidant enzymes. Chronic exposure of ACI rats, which are highly sensitive to low doses of estradiol (E_2), to E_2 confers protection against oxidative stress in brain slices. The present study examines the action of chronic E_2 treatment of ACI rats on expression and activities of antioxidant enzymes including (NAD(P)H-quinone oxidoreductase (NQO1), glutathione S-transferase (GST), and phenol sulfotransferase 1A1 (SULT1A1)) in brain and liver. Male and female ACI rats were treated for 6 weeks with 3mg of E_2 implanted subcutaneously in a cholesterol pellet. Activities of these enzymes measured spectrophotometrically and radiochemically in the striatum, an area particularly sensitive to oxidative stress induced by catecholamine metabolism, were markedly increased (NQO1 +28% male, +7% female; GST +17% male, +7% female; SULT1A1 +110% male, +49% female) in rats treated with E_2. A tight correlation existed between blood E_2 levels and activities of all three enzymes in male striata ($p<0.05$). This relationship was not evident in female striata ($p>0.17$). Liver enzymes followed a different profile when compared to striatum where enzyme activities of NQO1 decreased in males (-70%) and increased in females (+260%). Amounts of enzyme protein in liver detected by immunoblotting correlated with measured activities of NQO1 and SULT1A1 activities in both genders treated with E_2. These results strongly suggest that enhanced expression of antioxidant enzymes including NQO1, GST, and SULT1A1 is a mechanism related to protection by E_2 against oxidative stress. Regulation of the expression of these enzymes in ACI rats treated chronically with E_2 varies in different tissues in a gender-specific manner. (Supported in part by NIEHS Training Grant No.ES-07148)

1493 ANILINE-INDUCED ACTIVATION OF REDOX-SENSITIVE TRANSCRIPTION FACTOR NF-KB IN THE RAT SPLEEN.

S. Kannan, J. Wang, H. Li and M. Khan. *Pathology, University of Texas medical branch, Galveston, TX.*

Exposure to aniline results in selective toxicity to the spleen, and fibrosis appears to be an important initiating preneoplastic lesion. However, the molecular mechanism(s) by which aniline leads to fibrogenic response in spleen is not well understood. Our previous studies have shown that aniline exposure causes iron overload in the spleen, resulting in oxidative stress and upregulation of several fibrogenic cytokines. These observations lead us to hypothesize that aniline-induced oxidative stress in the spleen induces transcriptional upregulation of fibrogenic cytokines *via* activation of redox-sensitive transcription factor, nuclear factor-kappa B (NF-kB). In order, to determine the activation of NF-kB in aniline-induced fibrogenic response of the spleen, male SD rats were given 0.5 mmol/kg/day aniline hydrochloride (AH) through drinking water for 30 days, and splenocytes were isolated from control and aniline-treated rats. For the detection of activated NF-kB, we chose to determine NF-kB p65, which represents the activated form of the heterodimer

complex (p65/p50) that translocates to nucleus, using an ELISA specific for NF-kB p65. Nuclear extracts obtained from cultured splenocytes of aniline-treated rats showed a two fold increase in NF-kB p65 level compared to the controls. The binding activity of NF-kB, as determined by electrophoretic mobility shift assay (EMSA) using NF-kB consensus motifs, clearly showed an increase in NF-kB binding in the nuclear extracts of the splenocytes from aniline-treated rats in comparison to controls. The specificity of NF-kB p65 binding for relevant DNA motifs was verified by competition EMSA assays, using an excess of unlabelled consensus and mutant oligonucleotides. Furthermore, super shift EMSA and Western blot analysis confirmed the specificity of NF-kB p65 binding activity. These observations thus provide an evidence for aniline-induced activation and increased binding activity of NF-kB, which could mediate upregulation of fibrogenic cytokines in the spleen.

1494 PHARMACOLOGIC SUPPRESSION OF OXIDATIVE DAMAGE AND DENDRITIC DEGENERATION FOLLOWING KAINIC ACID-INDUCED EXCITOTOXICITY IN MOUSE CEREBRUM.

D. Milatovic[1], S. Milatovic[1], R. C. Gupta[2] and T. J. Montine[1]. *[1]Pathology, University of Washington, Seattle, WA and [2]Toxicology, Murray State University, Hopkinsville, KY.*

Intense seizure activity associated with status epilepticus and excitatory amino acid application initiates neuronal injury in CA1 of the ventral hippocampus. Oxidative stress is an important participant in excitotoxicity. As a measure of oxidative stress, lipid peroxidation and nitric oxide synthase (NOS) activity, changes in F_2-isoprostanes (F_2-IsoPs), F_4-neuroprostanes (F_4-NeuroPs) and citrulline levels were studied in the brain of C57Bl/6 mice exposed to intracerebroventricular administration of kainic acid (KA, 1 nmol/5 μl). Hippocampal pyramidal neuron dendrites and spines were evaluated using rapid Golgi stains and Neurolucida system. KA produced severe seizures in mice immediately after administration and a significant increase in F_2-IsoPs (158%), F_4-NeuroPs (237%) and citrulline (249%) levels were seen 30 min following treatment compared to controls (100%). At the same time, pyramidal neurons in the hippocampus had 62 % and 37 % reduction in dendritic length and spine density, respectively. No significant change in neuronal dendrites and spine and F_2-IsoP, F_4-NeuroPs and citrulline levels were found in mice pretreated with α-tocopherol (100 mg/kg, i.p.) for 3 days or those exposed to indomethacin or ibuprofen (inhibitors of cyclooxygenase, COX, 14 μg/ml of drinking water) for 2 weeks prior to KA. These findings indicate novel interactions among free radical-induced generation of F_2-IsoPs and F_4-NeuroPs, the COX pathway, nitric oxide and dendritic degeneration and point to possible interventions to limit cerebral oxidative damage (Supported by AFAR, PD 02043 to D.M.).

1495 OVEREXPRESSION OF CYTOKINES IN SPLENIC FIBROGENIC RESPONSE TO ANILINE.

J. Wang, H. Li, S. Kannan, B. S. Kaphalia and M. Khan. *Pathology, University of Texas Medical Branch, Galveston, TX.*

Molecular mechanisms for aniline-induced fibrogenic response in spleen are not well understood. Previously we have shown that excessive iron deposition, increased lipid peroxidation (LPO) and formation of LPO-derived aldehydes are important events in the splenic toxicity of aniline. Increased steady-state production of LPO-derived aldehydes may upregulate the expression of fibrogenic cytokines leading to splenic fibrosis. The present study was aimed to determine transcriptional and post-transcriptional regulation of cytokines in aniline-induced fibrogenic response. To achieve this, male SD rats were orally administered with 1 mmol/kg/day aniline hydrochloride (AH) for 7 days. RNA from the spleens of control and aniline-treated rats was extracted, and mRNA for IL-1 alpha, IL-6 and TNF-alpha was quantitated by real-time PCR. Aniline treatment resulted in 4.8, 2.7 and 2.4 fold increase in mRNA level of IL-1 alpha, IL-6 and TNF-alpha, respectively, compared to the controls, indicating transcriptional upregulation of these cytokines. Cytokine protein levels, measured in the splenocyte culture supernatants by specific ELISAs, also showed significantly greater release of these cytokines in the aniline-treated rats. Furthermore, to determine the expression of these cytokines after a subchronic exposure (when splenic fibrosis is evident), rats were treated with 0.5 mmol/kg/day AH for 30 days. Spleens from aniline-treated rats showed 6.9, 2.9 and 2.6 fold increase in mRNA levels of IL-1 alpha, IL-6 and TNF-alpha, respectively, in comparison to the controls. The increases in mRNA levels were also associated with enhanced secretion of these cytokines in the splenocyte culture supernatants. Overexpression of these cytokines preceding fibrosis as well as during fibrosis suggests that these cytokines have an important regulatory role in aniline-induced splenic fibrosis.

1496 THE GENES INVOLVED IN THE ONSET OF PARAQUAT INJURY AND THE INDIVIDUAL DIFFERENCE OF THE TOXIC EFFECT.

M. Tomita[1], T. Okuyama[1] and T. Nohno[2]. *[1]Medical Toxicology, Kawasaki Medical School, Kurashiki, Japan and [2]Molecular Biology, Kawasaki Medical School, Kurashiki, Japan.* Sponsor: L. Birnbaum.

Paraquat (PQ), a quaternary nitrogen herbicide, is highly toxic to humans and animals and death due to accidental PQ exposure have been reported over the past few decades. In addition, it is known that PQ-treated animals have been divided into "minimally" and "severely" affected group. Excessive production of oxygen free radicals has been proposed to play an important role in the pulmonary pathology. The aim of the present work is to find out the genes that are involved in the onset of PQ injury in lungs and in the individual difference of the toxic effect of PQ. We extracted RNAs from rat lungs 3 hr after injection of PQ (20 mg / kg; i.p.), and hybridized to cDNA array membranes that contain 1, 176 rat genes (Clontech Toxicology 1.2 array). PQ exposure showed 29 genes differentially expressed (>1.5-fold) when compared with those in control. Some positive genes isolated in this screen were further validated and quantitated with real-time RT-PCR performed in an ABI Prism 7700 Sequence Detection System (Applied Biosystems). The expression of PDGF, thioredoxin, glutathione S-transferase, superoxide dismutase was significantly up-regulated, suggesting that the oxygen free radicals were major contributors to the onset of PQ poisoning. The up-regulation of cytochrome P450s 2C6 and 2C7 particularly drew our attention. As expected, the expressions of these two enzymes in normal lungs of control rats were not/little observed. On the contrary, the expressions in the lungs of 3h-PQ exposed rats were remarkably increased and also showed individual differences (from normal level to above 100-fold). This phenomenon was peculiar to lungs, and not to liver and kidneys. The genes were amplified about 2-fold in PQ-exposed liver, but not in kidneys. The up-regulation of the genes observed in lungs at 3h-PQ exposure declined remarkably at 24h after injection. These results suggest that cytochrome P450s 2C6 and 2C7 are involved in the onset of PQ-induced serious pulmonary injury and the individual difference of the toxic effect.

1497 HO-1 PROMOTER/ENHANCER LUCIFERASE ASSAY: A PROMISING *IN VITRO* TOOL FOR MEASURING CELLULAR OXIDATIVE STRESS.

C. Knoerr, S. Gebel and T. Mueller. *PHILIP MORRIS Research Laboratories GmbH, Cologne, Germany.* Sponsor: H. Haussmann.

Cigarette smoke (CS) harbors a strong oxidative stress potential, which broadly impacts exposed cells. A special feature of the cellular response to CS is a strong transcriptional up-regulation of antioxidant genes, among which heme oxygenase-1 (ho-1) was found to be the most strongly induced gene in *in vitro* and *in vivo* microarray studies conducted in our laboratories. In order to test CS-fractions or individual smoke constituents for their potential to induce oxidative stress, we generated a stable transfected NIH3T3 cell-line expressing the luciferase gene under the control of the ho-1 promoter/enhancer region. The efficiency of the system was checked by exposing cells to aqueous extracts of CS (smoke-bubbled (sb) PBS) and then comparing the expression of ectopic luciferase on mRNA and protein levels to the expression of endogenous ho-1. Results showed the regulation of the two genes to be the same with regard to both kinetics and dose response. Experiments with the particle phase of CS (total particulate matter (TPM)) revealed that sbPBS and TPM induced luciferase activity to a similar extent in this system. Testing of oxidants known to be present in sbPBS and TPM showed that acrolein and hydroquinone induced luciferase activity, but to a lesser extent than sbPBS or TPM, demonstrating that both sbPBS and TPM contain additional constituents that induce ho-1. These results suggest that this system may be a promising tool for helping to identify and quantify those constituents in CS that are responsible for oxidative stress.

1498 INDUCIBLE GLUTAMATE-CYSTEINE LIGASE TRANSGENIC MICE EXHIBIT PROTECTION AGAINST ACETAMINOPHEN INDUCED LIVER INJURY.

D. Botta, S. Shi, C. C. White, S. Chatterton-Kirchmeier, P. A. Vliet and T. J. Kavanagh. *Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.*

The antioxidant tripeptide glutathione (GSH) is important in xenobiotic detoxification, free radical scavenging, and maintaining cellular redox status. Glutamate-cysteine ligase (GCL) is the rate limiting enzyme in GSH synthesis and is composed of catalytic (GCLC) and modifier (GCLM) subunits. We have generated transgenic mice designed to conditionally overexpress both GCLC and GCLM

using the GeneSwitchTM system. This system employs a liver specific promoter (transthyretin) to drive expression of a chimeric progesterone receptor composed of a mutated ligand binding domain, the Gal4 protein DNA binding domain and the herpes simplex VP16 transactivator domain (GLVP). The GLVP transactivator protein dimerizes in the presence of mifepristone (RU486) and then binds to and transactivates target transgenes containing Gal4 DNA binding domain recognition sequences upstream of the transgene. Acetaminophen (APAP) is a known hepatotoxicant, and APAP overdose results in over 10, 000 cases being treated annually by US regional poison control centers. APAP is metabolized to the reactive intermediate NAPQI. GSH is known to scavenge NAPQI making APAP a good candidate drug to test the hypothesis that transgenic mice overexpressing GCL are protected against APAP-induced liver injury. The GCL trigenic mice were administered both RU486 and acetaminophen and showed reduced liver injury, as measured by serum ALT levels ($p<0.05$) and histopathology ($p<0.05$), when compared to either control mice (those carrying the GCL transgenes but not the transactivator GLVP), or trigenic mice administered only acetaminophen. This transgenic mouse system will be useful in investigating other hepatotoxicants known to deplete GSH or cause oxidative stress in the liver. Supported by NIH Grants ES10849-02, ES04696, and ES07033.

1499 QUANTITATION OF 4-HYDROXY-TRANS-2-NONENAL AND ITS METABOLITES BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY.

M. J. Meyer[1], M. Miyagi[2] and M. J. Picklo[1]. *[1]Pharmacology and Physiology, University of North Dakota, Grand Forks, ND and [2]Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND.*

A liquid chromatography tandem mass spectrometry (LC-MS-MS) method has been developed to quantify 4-hydroxy-trans-2-nonenal (HNE) and its metabolites. This method utilizes a high pressure liquid chromatography system (Agilent) coupled to an API 3000 triple quadrupole mass spectrometer (PerkinElmer-Sciex). Samples were prepared and run isocratically using 50:50 acetonitrile:water with 0.1% of acetic acid at 200 uL/min on a C8 reverse-phase column. Sample analysis times were under 4 minutes. Turbo ionspray was used at 400 C with a nebulizer gas flow rate of 6 L/min. Unique ion pairs were determined for each metabolite and the analogous deuterated internal standards. Due to the structure of the acid metabolite of HNE, 4-hydroxy-trans-2-nonenoate (HNEAcid), analysis under negative ion mode was more favorable than positive ion mode. The d11-HNEAcid was analyzed in the same manner as HNEAcid. All other metabolites, such as glutathione-HNE, were more amenable to analysis under positive ion mode. The mass spectrometer was capable of operating in negative and positive ion modes in the same sample analysis. Limits of detection were 250 fmol for the oxime-derivatized metabolite of HNE (HNEoxime) and for HNEAcid. Utilizing this HPLC-MS method, we were able to detect the formation of HNEAcid and the glutathione-HNE adduct in respiring rat brain mitochondria treated with HNE.

1500 MEMANTINE PROTECTS SKELETAL MUSCLES AGAINST OXIDATIVE STRESS.

R. C. Gupta[1], D. Milatovic[2], T. J. Montine[2] and W. D. Dettbarn[3]. *[1]Toxicology, Murry State University, Hopkinsville, KY, [2]Pathology, University Washington, Seattle, WA and [3]Pharmacology, Vanderbilt University, Nashville, TN.*

This study was undertaken to determine whether memantine pretreatment protects carbofuran-induced oxidative stress in slow (soleus) and fast (EDL) muscles of rats. Both muscles were analyzed for markers of lipid peroxidation/ROS (F_2-isoprostanes), RNS (citrulline, determinant of NO), high-energy phosphates (ATP and PCr), and AChE (target enzyme) to establish their time courses under the influence of acute carbofuran toxicity. Among the muscles of control rats, EDL contained greater levels of all the markers than the soleus, with the exception of citrulline, which was higher in the soleus. Rats acutely intoxicated with carbofuran (1.5mg/kg, sc) showed the onset of toxic signs within 5-10 min, and maximal severity (muscle fasiculations and convulsions) at 1 hr. At this time, carbofuran caused significant inhibition of AChE (39%), increases of F_2-isoprostanes (147-178%) and citrulline (267-304%), and decreases of ATP and TAN (54-57% and 57-58%, respectively) and PCr and TCC (54-56% and 57-59%, respectively). Rats pretreated with memantine HCl (MEM, 18mg/kg, sc) and atropine sulfate (ATS, 16mg/kg, sc), 45 min and 15 min prior to carbofuran administration, showed no signs of toxicity. In addition, MEM and ATS significantly protected muscles of both types against AChE inhibition, increases in F_2-isoprostanes and citrulline, and declines in energy phosphates. These findings indicated that MEM in combination with ATS protected the muscles from carbofuran-induced toxicity and oxidative stress by multiple mechanisms.

1501 ANTIOXIDANT EFFECTS ON ETHANOL-INDUCED OXIDATIVE STRESS AND HEPATOXICITY IN RATS FED VIA TOTAL ENTERAL NUTRITION.

T. M. Badger[1, 3], S. Korourian[2], M. Ferguson[3], B. Sampey[4], E. Albano[5], D. Petersen[4] and M. J. Ronis[6, 3]. *[1]Physiology, University of Arkansas for Medical Sciences, Little Rock, AR, [2]Pathology, University of Arkansas for Medical Sciences, Little Rock, AR, [3]Arkansas Children's Nutrition Center, Arkansas Children's Hospital, Little Rock, AR, [4]Pharmacy, University of Colorado Health Sciences Center, Denver, CO, [5]Medical Sciences, University "A Avogadro" of East Piedmont, Novara, Italy and [6]Pharmacology & Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR.*

We examined the effects of antioxidants N-acetyl cysteine (NAC) and butylated hydroxytoluene (BHT)in rats fed ethanol i.g. Groups of 8-13 rats were infused diets where ethanol (12-13 g/kg/d) replaced carbohydrates isocalorically for 45 d. Additional groups were treated with BHT at 150 mg/kg/d or NAC at 1.2 g/kg/d. Serum ALT and liver pathology were measured. Liver slices were stained with antisera against malondialdehyde (MDA) and hydroxynonenal (HNE) adducts and serum was assayed for antibodies directed towards hydroxyethyl radical-, MDA- and HNE-adducts. Liver microsomes were assayed for CYP2E1-associated CCl4-dependent lipid peroxidation and CYP2E1 apoprotein by Western blot. Ethanol treatment resulted in hepatotoxicity characterized by steatosis, macrophage infiltration, focal necrosis and elevated ALT ($p \leq 0.05$). Ethanol also induced hepatic CYP2E1 and oxidative stress as measured by presence of MDA and HNE adducts ($p \leq 0.05$) and induced antibody titers towards MDA-adducts ($p \leq 0.05$). NAC treatment effectively abolished hepatic adducts and reduced ALT ($p \leq 0.05$) in the absence of effects on CYP2E1 but resulted in no significant decrease in liver pathology score. In contrast BHT had no effect on liver adducts but did reduce CYP2E1 ($p \leq 0.05$) and abolished elevations in antibody titers against adducts without effects on ALT or liver pathology. The relative lack of protection afforded by either NAC or BHT suggests that CYP-2E1 mediated oxidative stress might not be the only mechanism underlying alcohol-induced hepatotoxicity. Supported in part by AA08645; AA12931 (TMB) and AA09300 (DRP).

1502 IMPAIRED TISSUE REPAIR IN THIOACETAMIDE TREATED DIABETIC RATS: NF-KB AS A RINGMASTER.

S. S. Devi and H. M. Mehendale. *Toxicology, The University of Louisiana at Monroe, Monroe, LA.*

A nonlethal dose of thioacetamide (TA) causes 90 % mortality in diabetic (DB) rats due to impaired tissue repair. The objective of this study was to test our hypothesis that down regulation of NF-kB/DNA-binding in DB liver leads to down regulation of cell proliferative genes transcribed by NF-kB and is responsible for inhibited tissue repair in DB group. Gel shift assay revealed lower NF-kB/DNA binding in the DB liver, due to lower nuclear translocation of NF-kB consequent to lower degradation of I-kBα, the endogenous cytoplasmic inhibitor of NF-kB. Lower degradation of I-kBα is due to down regulated IKK enzyme. Investigation of EGFR/MAPKs pathway revealed down regulation of EGFR, and MAPKs (ERK1 and ERK2) in the DB rats, which may further explain lower degradative dissociation of I-kBα. In contrast, in the NDB group receiving the same dose of TA, up regulation of EGFR/MAPK pathway stimulates prompt and efficient degradation of I-kBα, facilitating nuclear translocation and higher NF-kB/DNA binding. NF-kB/DNA binding transcribes antiapoptotic as well as cell proliferation genes. Lower NF-kB/DNA-binding in the DB liver, indicates lower transcription of antiapoptotic genes, explaining higher apoptosis in DB liver (histology: H & E staining and Tunnel assay) as compared to NDB liver. NF-kB also transcribes cyclin D1, which in association with cdk4 and cdk6 clears hepatocytes through the G1 to S-phase checkpoint to progress through the cell cycle. Immunoblotting showed that cyclin D1, cdk4 and cdk6 were down regulated in DB rats. Real time RT-PCR analysis confirmed that not only cyclin D1 expression but also the expression of ICAM1 and VCAM1, cell proliferative genes transcribed by NF-kB was also decreased in TA-treated DB rats, whereas in the NDB group, higher NF-kB/DNA binding leads to higher cell proliferative gene expression (cyclin D1, ICAM1 and VCAM1). These findings suggest that lower NF-kB/DNA binding in DB group underlies the down regulation of cell proliferative genes, explaining impaired tissue repair in DB rats. (Supported by Kitty DeGree Endowed Chair and LBRSF).

1503 ROLE OF CALPAIN IN ENDOTOXIN-MEDIATED HEPATIC INJURY.

University. M. Apte, R. McRee, J. Nguyen and S. K. Ramaiah. *Department of Pathobiology, Texas A&M University, College Station, TX.*

Calpain, a Ca^{2+} dependent cytosolic cystein protease is known to be involved in a variety of pathological conditions including anoxia-induced liver injury. It has recently been reported that inhibition of calpain protects from progressive injury induced by hepatotoxicants such as acetaminophen and CCl_4. However, the role of

calpain in endotoxin-mediated hepatic injury is unknown. Bacterial endotoxin (LPS) is a potent hepatotoxicant which stimulates hepatic neutrophilic infiltration resulting in hepatocyte death by apoptosis and necrosis. The hypothesized role of calpain in endotoxin-mediated hepatic injury was tested using a specific calpain inhibitor in this study. Male Sprague-Dawley rats were treated with 20 mg/kg LPS (*E. coli* 0111:B4) followed by a single dose of a specific inhibitor of calpain, CBZ-VAL-PHE methyl ester (CBZ, 60 mg/kg) 1 hr after LPS administration. All rats were sacrificed 24 hrs following LPS and assessed for liver injury by plasma transaminase activities and histopathology. LPS treatment resulted in a significant increase in plasma ALT and AST activities which was corroborated by extensive hepatocellular necrosis as evaluated in H&E sections. Extensive hepatocyte apoptosis as evaluated by TUNEL assay and neutrophil infiltration was also noted in the LPS-treated rats. Calpain inhibition with CBZ significantly decreased LPS-mediated liver injury as evidenced by decreased plasma transaminase activity, necrosis and neutrophilic infiltration. Degradation of α-fodrin, the cellular substrate of calpain evaluated by Western blot analysis indicated lower calpain activity in LPS+ CBZ treated rats compared to LPS alone group. Although the number of apoptotic cells decreased in CBZ treated rats, it was not completely abolished. These data suggest the likely role of calpain in endotoxin-mediated hepatic necrosis. Although calpain inhibition, partly mitigated LPS-induced apoptosis, a calpain independent pathway of apoptosis seems to be operational as evidenced by persistent apoptosis in the LPS+ CBZ treated rats.

1504 OSTEOPONTIN-MEDIATED INDUCTION OF MATRIX METALLOPROTEINASE-9 ACTIVITY VIA NF-κB IN ALCOHOLIC STEATOSIS.

S. K. Ramaiah, R. McRee, J. Nguyen, S. J. Smith, M. Garza, E. Wellberg and University. M. Apte. *Department of Pathobiology, Texas A&M University, College Station, TX.*

Alcoholic liver disease (ALD) is a major complication of heavy alcohol (EtOH) consumption characterized by four sequential pathologic stages namely steatosis (fat accumulation), steatohepatitis, fibrosis and cirrhosis. Although the steatosis stage is considered benign and reversible, it is known that the biochemical abnormalities in fatty liver leads to hepatic inflammation and steatohepatitis. The overall objective of this study is to investigate the role of osteopontin (OPN), an important matricellular protein in the progression of liver pathology from alcoholic steatosis to steatohepatitis. The hypothesized role of OPN in NF-κB-mediated induction of matrix metalloproteinases (MMP) during steatosis stage of ALD was tested in this study. Male Sprague-Dawley rats fed Lieber-DeCarli diet containing either EtOH or maltose-dextrin for 5 weeks, resulted in panlobular steatosis with mild increase in liver injury as measured by plasma transaminase activities and histopathology. A significant induction of OPN was observed in the EtOH treated rats by week 1, which further increased and reached a peak at week 2 following EtOH treatment. OPN induction correlated with higher NF-κB DNA binding. Hepatic MMP-9 activity as measured by collagen zymography indicated a temporal increase with a peak activity at week 5 following EtOH ingestion. Higher MMP-9 activity significantly correlated with hepatic fibronectin degradation, as measured by Western blot analysis. These data strongly suggest the role of OPN in MMP induction, possibly mediated *via* NF-κB. Significant induction of MMP-9 together with hepatic fibronectin degradation in steatotic liver, may likely predispose fatty liver to steatohepatitis by facilitating neutrophil migration into hepatic parenchyma (SUPPORTED IN PART BY ES09106).

1505 OVERACTIVATION OF POLY(ADP-RIBOSE) POLYMERASE-1 ACCOMPANIES CARBON TETRACHLORIDE-INDUCED CENTRILOBULAR NECROSIS.

M. Banasik[1, 2], T. Stedeford[1, 2], C. Muro-Cacho[3] and R. D. Harbison[1]. *[1]Department of Environmental and Occupational Health, University of South Florida, Tampa, FL, [2]Laboratory of Toxicology and Risk Assessment, Polish Academy of Sciences, Gliwice, Poland and [3]Department of Interdisciplinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.*

Overactivation of poly(ADP-ribose) polymerase-1 (PARP-1) in response to oxidative stress has been shown to contribute to necrotic cell death by consuming NAD+ and ATP. In the present study, PARP-1 overactivation was determined by identifying the distribution and accumulation of poly(ADP-ribose) in mouse liver tissue following intraperitoneal administration of a hepatotoxic dose of carbon tetrachloride (572 mg/kg). Treated animals exhibited lipid peroxide levels 16.5-fold higher than controls. Serum activities of glutamic pyruvic transaminase and glutamic oxalacetic transaminase were increased by 6.1-fold and 22.8-fold, respectively. Lactate dehydrogenase activity was significantly increased by 1.2-fold. Histopathological analyses revealed severe necrosis and increased poly(ADP-ribosylation) of cells in the centrilobular region of treated animals versus saline controls. These results demonstrate the role of PARP-1 overactivation in chemical-induced pathologies and suggest the potential role of PARP-1 inhibitors at preventing toxicity.

1506 STRESS-RESPONSIVE MAP KINASES REGULATE RUBRATOXIN B-INDUCED CYTOKINE SECRETION IN HEPG2 CELLS.

H. Nagashima, K. Nakamura and T. Goto. *National Food Research Institute, Tsukuba, Ibaraki, Japan.* Sponsor: M. Fukayama.

Rubratoxin B is a potent hepatotoxic mycotoxin produced by various Penicillium fungi. Last year, we reported that rubratoxin B induced the levels of IL-8, M-CSF and GM-CSF in the culture supernatants of the human hepatoma cell lines HepG2 and HuH-7. To our knowledge, ours was the first report that an exogenous stimulus induced the secretion of M-CSF and GM-CSF in hepatocyte-derived cells, suggesting that rubratoxin B is an excellent model compound for studying the mechanisms of M-CSF and GM-CSF secretion. This year, we investigated the effects of the stress-responsive MAP kinases on rubratoxin B-induced cytokine secretion. We first measured the amounts of the stress-responsive MAP kinases JNK and p38. While treatment of cells with rubratoxin B did not change the total amounts of both kinases, the amounts of the activated (phosphorylated) forms of both kinases significantly increased. These results indicate the possibility that JNK and p38 play pivotal roles in exerting the toxicity of rubratoxin B. According to results from ELISA, treatment with the JNK inhibitor SP600125 decreased the rubratoxin B-induced release of IL-8, M-CSF and GM-CSF. In addition, the p38 inhibitor SB203580 decreased rubratoxin B-induced IL-8 and M-CSF secretion. On the other hand, SB203580 augmented rubratoxin B-induced GM-CSF secretion. To identify the step(s) crucial for regulations by JNK and p38, we used RT-PCR to measure the amounts of cytokine mRNAs. The RT-PCR results for cells treated with both rubratoxin B and the inhibitors of the stress-responsive MAP kinases showed similar tendencies to ELISA. However, the inhibitor-associated effects were much lower than ELISA, suggesting that the amounts of the secreted proteins were chiefly determined by post-transcriptional step(s). Our results suggest that A) the stress-responsive MAP kinases JNK and p38 regulate the rubratoxin B-induced secretion of IL-8, M-CSF and GM-CSF, B) these regulations mainly occur at the post-transcriptional level, and C) the mechanism of regulation of GM-CSF secretion differ from those of the other two cytokines.

1507 INHIBITION OF PHAGOCYTOSIS IN PRIMARY RAT KUPFFER CELLS BY METHYL PALMITATE.

P. Cai, B. S. Kaphalia and G. Ansari. *Pathology, UTMB, Galveston, TX.*

Kupffer cells play an important role in the early events of liver injury by releasing biologically active mediators. Methyl palmitate (MP) is known to modulate Kupffer cell functions (phagocytosis and release of chemokines). In order to evaluate the efficacy of MP towards Kupffer cell function, primary Kupffer cells were isolated from the rat liver and the cells cultured in RPMI 1640 medium. After 48h incubation, cells were treated with MP and phagocytic activity was measured by their capacity to phagocytize latex beads after 2 and 24h post incubation. A dose-dependent decrease (71%, 64% and 50% for 2h post incubation; 48%, 33%, and 12% for 24h and with LPS treatment for 6h) was observed at 0.25mM, 0.5mM and 1mM, respectively. The phagocytosis at 0.5mM MP was also found to be time-dependent with a maximum decrease of 49% at 6h (control 77%). TNF-a release from these treated cells was decreased to 1567pg/ml at 1mM, as compare to control (2532pg/ml) with lipopolysaccharide (LPS) stimulation. IL-10 release was also decreased (76 pg/ml vs. 555pg/ml). Similarly MP, reduced nitric oxide production in the supernatant was found to be 0.35, 0.33, and 0.30uM at 0.25mM, 0.5mM and 1.0mM respectively, 24h post incubation, as compared to control (0.53uM). These results indicate that MP inhibits phagocytosis and mediates the release of several chemokines from the Kupffer cells. The mechanism of such inhibition and down regulation of chemokines by MP needs to be thoroughly investigated.

1508 DIETARY ZINC SUPPLEMENTATION ATTENUATES CHRONIC ALCOHOL-INDUCED LIVER INJURY IN MICE THROUGH INHIBITION OF OXIDATIVE STRESS.

Z. Zhou[1], L. Wang[1], J. T. Saari[2], C. J. McClain[1] and Y. Kang[1]. *[1]University of Louisville, Louisville, KY and [2]Human Nutrition Research Center, USDA, Grant Forks, ND.*

Zinc depletion in the liver has long been noted in alcoholic liver disease. However, there is limited information about the role of zinc in the pathophysiology of alcohol-induced liver injury. The present study was undertaken to determine whether dietary zinc supplementation confers resistance to chronic alcoholic liver injury.

Because metallothionein (MT) may participate in the regulation of cellular zinc homeostasis, MT-knockout (MT-KO) mice along with their wild-type (WT) 129/Sv controls were used and fed Lieber-DeCarli liquid diets following a 2x2 factorial design; ethanol x zinc. Zinc was supplemented at a level of 100 mg zinc ion/l. An improved feeding protocol was established as follows: 1) The alcohol content (%, w/v) in the diet started from 3.2, with an increase of 0.2 every week, reached 5.0 at the end, and 2) an one-day-stop on the last day of each week was introduced in the alcohol feeding schedule. Both WT and MT-KO mice fed ad libitum for 10 weeks showed significant liver injury, as indicated by increased plasma alanine aminotransferase activity, pathological changes, and hepatocyte apoptosis. Zinc supplementation attenuated alcohol-induced liver injury in both WT and MT-KO mice. Zinc supplementation also abrogated ethanol-induced oxidative injury including lipid peroxidation and protein oxidation. Dihydroethidine fluorescent microscopy demonstrated that zinc suppressed ethanol-induce superoxide accumulation in the liver. Zinc also inhibited ethanol-induced increases in hepatic CYP2E1 and xanthine oxidase, and the decrease in hepatic glutathione peroxidase. Furthermore, ethanol-induced depletion of hepatic zinc concentration was repleted by zinc supplementation. These results thus demonstrate that zinc is a potent hepatoprotective agent against alcoholic liver injury, whose action is related to depressed oxidative stress. (Supported in part by NIH grants AA13601, AA01762, and HL59225)

1509 CHENODEOXYCHOLIC ACID-MEDIATES INDUCTION OF THE MITOCHONDRIAL PERMEABILITY TRANSITION THROUGH ALTERED MEMBRANE FLUIDITY.

A. P. Rolo, P. J. Oliveira, A. J. Moreno and C. M. Palmeira. *Center for Neurosciences and Cell Biology of Coimbra, Department of Zoology, University of Coimbra, Coimbra, Portugal.*

Chenodeoxycholic acid (CDCA), a primary bile acid mostly implicated in cholestatic liver injury, is toxic to hepatocytes by mechanisms involving mitochondrial dysfunction. In this study we have investigated the involvement of membrane fluidity and cytochrome c release in CDCA-induced mitochondrial permeability transition (MPT), and the preventive role of carvedilol. Within this context, mitochondrial swelling, membrane potential, and mitochondrial calcium fluxes were monitored. The involvement of alterations on membrane fluidity, as probed by membrane labelling with fluorescent dyes, and cytochrome c release in CDCA-induced MPT was also studied. Treatment of calcium-loaded hepatic mitochondria with CDCA was found to cause osmotic swelling and release of cytochrome c, associated with an increase in membrane fluidity, in both surface and membrane core regions. Carvedilol and cyclosporine A (CyA) reduced both cytochrome c release and alterations in membrane fluidity induced by CDCA. The hydroxylated metabolite of carvedilol, BM-910228, had no effect. Thus, modulation of membrane fluidity, play an important role in MPT pore opening promoted by CDCA. As a result, we have delineated a pathway for the preventive role of carvedilol in mitochondrial dysfunction induced by CDCA. (Supported by PRAXIS XXI/21454/99 and Portuguese Research Council (POCTI/CBO/42486/2001) FCT)

1510 *IN VITRO* DEVELOPMENT OF BIOMARKERS FOR ACETAMINOPHEN TOXICITY IN PRIMARY MOUSE HEPATOCYTES.

J. N. Mayes[1, 2], R. Edmondson[3], R. Jones[3] and Y. P. Dragan[2, 1]. [1]*Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR,* [2]*Hepatic Toxicology, NCTR, Jefferson, AR and* [3]*Chemistry, NCTR, Jefferson, AR.*

Hepatotoxicity is an important concern in drug development, as well as a primary reason for drug recall. Therefore, biomarkers that detect the potential for agents to induce adverse liver effects are of great need. Acetaminophen (APAP) is a known toxin to mouse and human liver and is responsible for a large percentage of acute liver failure cases in this country. The availability of early, specific and selective biomarkers that are accessible in biofluids could permit the detection of liver damage prior to the presence of morphological evidence of damage. Clinically, serum enzyme levels are used as an indicator of liver function; we hypothesize that proteins released into cell culture media following toxin exposure are equivalent indicators of liver damage and a proteomic analysis of these proteins can be used to identify these potential biomarkers. Our studies used primary hepatocytes isolated from 5 male C57BL/6 mice to perform a dose response and time course analysis of APAP and its stereoisomer, 3'-hydroxyacetanilide (AMAP). Hepatocytes were isolated and cultured on collagen coated plates. ALT activities increased as a function of dose (0, 0.3, 1, 3, and 10mM) and time (6, 12, 18, and 24 hr) with both APAP and AMAP administration. Morphological evidence of hepatic damage preceded the increase in alanine aminotransferase (ALT) serum enzyme levels. The cell culture media from APAP treated hepatocytes was concentrated with deoxycholic acid / trichloroacetic acid (DOC/TCA), normalized for protein content and solublized for two-dimensional gel electrophoresis (2DGE). Proteins that were altered as a function of dose or time in a manner suggestive of correlation with toxicity were excised, digested with trypsin, and subjected to LC/MS/MS analysis. Several differentially expressed proteins were identified using this approach, but their relationship to the induction or progression of toxicity is unclear.

1511 PREDICTION FOR HEPATOTOXINS IN A PRIMARY HEPATOCYTE TEST SYSTEM WITH TRANSCRIPTIONAL PROFILING OF TOXICITY RELATED GENES.

P. Eddy, J. Lin, E. Fedyk, S. Badola, V. Sasseville and C. L. Alden. *Drug Safety and Disposition, Millennium Pharmacology., Cambridge, MA.*

The prediction of development limiting toxicity in lead optimization using 5 day repeat dose toxicity studies in rat and higher species has significantly reduced development attrition. Repeat dose *in vivo* toxicity testing engenders significant cost for synthesis of test agent. The opportunity for the toxicology discipline to create a discontinuity in the efficiency of the drug discovery process exists through leveraging molecular technology in cell based systems. Cell based toxicity testing using biochemical or functional endpoints are not useful in predicitng for systemic toxicity. Cell based systems using microarrays have been demonstrated to predict successfully for pharmacology specific patterns of gene expression. Only a few companies have focused on toxicity related patterns of gene expression in cell based assays. Although debate continues on the value of microarray data versus select subsets of genes for predicting systemic toxicity potential, a small number of genes specific to toxicologic responses in cell based systems represents a more practical approach for screening at early stages in lead optimization. Using transcriptional profiling of a few stress and cell cycle related genes in a cryopreserved human hepatocyte test system to predict for human hepatotoxicity enables reliable differentiation of over one half of the hepatotoxins *via* robust upregulation of several of the toxicity genes. While none of the agents predicted to be toxic were nontoxic, an additional subset of hepatotoxins without modest upregulation of toxicity genes can not be differentiated from certain nontoxins, specifically nontoxins causing upregulation of p450 microsommal enzymes such as phenobarbital. Over 40 drugs of known toxicity potential including drugs causing idiosyncratic injury have been evaluated in this testing paradigm. Predicting for a significant proportion of hepatotoxins utilizing miniarrays in a human cell test system will create substantial cost benefit applied antecedent to repeat dose *in vivo* preclinical efficacy testing.

1512 VISUALIZATION AND QUANTITATION OF PEROXISOMES USING QUANTUM DOTS™: DETECTION IN THE LIVER FOLLOWING TREATMENT OF RATS AND MONKEYS WITH FIBRATES.

G. Falls[1], H. M. Colton[1], H. Ni[1], P. Kwanyuen[1], D. R. Creech[1], N. F. Cariello[1] and G. Hamilton[2]. [1]*Safety Assessment, GlaxoSmithKline, Research Triangle Park, NC and* [2]*Hepatotech Inc., Pittsboro, NC.*

Peroxisome proliferation has been well documented in rodents following treatment with hypolipidemic drugs such as fibrates, and extensive research has focused on the induction of peroxisome proliferation by compounds in drug development. Levels of peroxisome proliferation have been estimated by measuring gene expression and enzyme activity as well as with immunolabeling and direct counting using electron microscopy. Although each method has its benefit, high levels of cost, labor, and sample manipulation require a more efficient and reproducible method for peroxisome visualization. To address this issue, we developed a method for immunofluorescent staining of peroxisomes using a polyclonal antibody to the 70kDal peroxisomal membrane protein (PMP70) and fluorescent cadmium selenide-based nanocrystals called Quantum Dots™. The unique composition and structure of these nanocrystals lead to highly desirable optical properties including quantum yields of over 50%, narrow emission peaks, remarkable photostability, and large Stokes shifts provided by the exceptionally broad excitation range. Using streptavidin-coated Quantum Dots™ and a PMP70 antibody, we were able to stain and visualize peroxisomes in standard formalin-fixed paraffin-embedded rat and monkey liver after *in vivo* treatment with fenofibrate and ciprofibrate, respectively. A dose-dependent increase in peroxisome number and area compared to controls was observed in cynomolgus monkeys given 3, 30, 150, and 400 mg/kg/day of ciprofibrate. This finding correlated with an increase in peroxisome count determined by electron microscopy. In liver sections from male Wistar rats treated for 10 days with fenofibrate (0, 10, 20, or 200 mg/kg/day), there was a dose-dependent increase in PMP70 staining, indicative of peroxisome proliferation. We have also utilized this method to detect peroxisomes in sections from human liver and rat soleus muscle, and in rat primary hepatocytes.

1513 GENOMIC ANALYSIS OF SUSCEPTIBILITY FACTORS ASSOCIATED WITH HALOTHANE-INDUCED LIVER INJURY IN GUINEA PIGS.

M. Holt[1], M. Bourdi[1], A. Elkahloun[2], D. Erias[1] and L. Pohl[1]. *[1]Melecular and Cellular Toxicology Section/LMI, NHLBI/NIH/DHHS, Bethesda, MD and [2]Cancer Genetics Branch, NHGRI/NIH/DHHS, Bethesda, MD.*

Halothane causes a mild form of liver injury in guinea pigs that appears to model the hepatotoxicity seen in approximately 20% of patients treated with this drug. Although the increased susceptibility of some outbred guinea pigs to halothane-induced liver injury (HILI) has been associated with elevated levels of trifluoroacetylated (TFA) protein adducts in the liver, the role of other susceptibility factors in this toxicity remains to be more thoroughly explored. In the current study, we have addressed this issue by comparing the hepatic gene expression of susceptible guinea pigs (SGP) to those of resistant guinea pigs (RGP) following halothane treatment using a cDNA microarray that can detect the expression of over 30, 000 genes and ESTs. It was found that several genes that encode for antioxidant and stress proteins were underexpressed in the liver of SGP relative to those of RGP including catalase, peroxiredoxins, glutathione S-transferase family members, ferritin heavy chain, selenoprotein P, and heat shock proteins as were genes involved in lipid synthesis and regulation including 3-hydroxy-3-methylglutaryl-CoA synthase 2, apolipoprotein A-IV, fatty acid synthase, low density lipoprotein receptor-related protein 1, and sterol carrier protein 2. These finding suggest that the increased susceptibility of a subset of guinea pigs to HILI is not only due to the increased formation of TFA-protein adducts but also to deficiencies in the expression of multiple hepatoprotective factors and proteins involved in lipid homeostasis. Similar factors may have a role in predisposing patients to HILI and to liver injury caused by other drugs.

1514 EFFECTS OF FLAVONOID TREATMENT ON THE PERMEABILITY OF CYCLOSPORIN A ACROSS CACO-2 CELL MONOLAYERS.

J. E. Mata[1], R. Rodriguez-Proteau[1, 2], C. L. Miranda[2], D. R. Buhler[2] and J. Brown[1]. *[1]Pharmaceutical Sciences, Oregon State University, Corvallis, OR and [2]Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Flavonoids present in plant materials have demonstrated activity in cancer chemoprevention, cancer treatment paradigms, modulation of signal transduction pathways and drug transport processes. The ability of specific flavonoids such as xanthohumol, quercetin, epigallocatechin gallate (EGCG), epicatechin gallate (ECG), genestein, naringenin, genestin, and naringin to modulate the permeability of cyclosporin A (CSA), a P-glycoprotein substrate, across Caco-2 cell monolayers was evaluated. Flavonoid concentrations of 100 μM were used in the transport experiments with the exception of xanthohumol, EGCG and ECG which were at a concentration of 30 μM. Experiments were performed in sink conditions from basolateral (BL) to apical (AP) direction and in non-sink conditions from AP → BL with ^{3}H-CSA concentrations of 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μM. The aglycone form of the flavonoids tested reduced the permeability (P_e) of CSA to a greater extent than the glycosylated form with xanthohumol producing the greatest overall effect. Thirty μM xanthohumol reduced the P_e of CSA from $7.2 \times 10^{-6} \pm 4.8 \times 10^{-7}$ cm/sec to $6.6 \times 10^{-7} \pm 3.99 \times 10^{-7}$ cm/sec from the BL → AP direction. ^{3}H-Xanthohumol demonstrated a saturable efflux from the BL → AP compartments with significant inhibition at 5 μM, $p < 0.05$. These studies suggest that flavonoids are capable of reducing the efflux of CSA across Caco-2 cell monolayers with differences in potency. Xanthohumol is an effective inhibitor of CSA permeability and is capable of limiting its own efflux. Supported by NIH grant AT00853.

1515 ABSENCE OF LIVER CANALICULAR MRP2 TRANSPORTER SHIFTS METHYLENE DIANILINE INJURY FROM BILIARY EPITHELIAL CELLS TO HEPATOCYTES.

M. F. Kanz[1], F. Nayeem[1], V. Santa Cruz[1], T. R. Dugas[2] and M. Treinen-Moslen[1]. *[1]Pathology, University of Texas Medical Branch, Galveston, TX and [2]Pharmacology, LSU Health Sciences Center, Shreveport, LA.*

Background. Methylene dianiline (4, 4'-diaminodiphenylmethane, DAPM), a compound used in the production of polyurethanes and epoxy resins, initially injures biliary epithelial cells (BEC) *in vivo. In vitro* studies indicate that BEC are injured by proximate toxicant(s) in bile. Two biliary DAPM metabolites have been preliminarily identified as glutathione and cysteinyl-glycine conjugates of DAPM. Mutant transport-deficient (TR$^-$) rats lack expression of the canalicular multispecific organic anion transporter (cMOAT or MRP2) and have deficient biliary excretion of organic anions, GSH and GSH conjugates. We hypothesized that absence of the MRP2 transporter would modulate DAPM-induced injury of BEC. **Methods.** Time course (25 mg/kg) and dose response (25 to 100 mg/kg) studies were conducted in male or female TR$^-$ rats (16-20 wks old). DAPM was given po in 35% ethanol and tissues and blood were analyzed for injury. **Results.** *Time Course:* Liver weights were increased in female rats at 24, 48, 72, 168 hr but only at 24 and 48 hr in male rats. Spleen weights increased in females at 48 and 72 hr but only at 72 hr in males. Serum bilirubin was elevated in male rats at 24, 48, 72 hr while serum indices were highly variable in both treated and control TR$^-$ female rats. Scattered, small foci of necrosis and inflammation were observed in centrolobular to midzonal regions in both genders at 24 hr with peak injury at 48 hr. Recover occurred faster in male than female rats. *Dose-Response:* Significant centrolobular necrosis with bridging between lobules was apparent at 48 hr after 50 mg/kg. At 100 mg/kg, 50% of the females died before 48 hr. In the remaining animals, massive liver congestion was observed with 70-90% of the hepatic parenchyma destroyed. Only minimal injury to BEC was seen at the highest dose. **Conclusion.** The MRP2 transporter is involved in excretion of DAPM metabolites into bile that injure BEC. (Supported by NIH grants ES06348; DK56494; T32-ES07254)

1516 MULTIPLE DRUG RESISTANCE GENE REGULATION IN MICE.

J. M. Brady, X. G. Cheng, J. M. Maher and C. D. Klaassen. *University of Kansas Medical Center, Kansas City, KS.*

Multiple drug resistance (Mdr) genes encode P-glycoprotein, which is responsible for resistance to some cancer chemotherapeutic drugs and efflux of xenobiotics out of cells. Thus, Mdr can protect organs from xenobiotic toxicity. There are two Mdr1 gene products in mice capable of xenobiotic transport, Mdr1a (Abcb1a) and Mdr1b (Abcb1b). A third related gene product, Mdr2 (Abcb4), is associated with phospolipid transport into bile. The aims of the present study were to determine 1) the tissue distribution of mouse Mdr mRNAs, 2) the ontogeny of Mdr mRNAs, and 3) whether microsomal enzyme inducers that increase phase I and II drug-metabolizing enzymes coordinately regulate Mdr genes. Mdr1a mRNA expression was high in small intestine; moderate in large intestine and brain; and low in liver and kidney. The highest expression of Mdr1b mRNA was in kidney, where the concentration was about 3-fold higher than most other tissues. In brain, Mdr1b mRNA levels were higher in males than females. The highest Mdr2 mRNA level was observed in liver, with female-predominance. Ontogeny studies showed that in liver, Mdr1a mRNA increased from day 10 through day 35. Mdr1b mRNA expression showed a slight increase after birth through day 15. Male-predominant expression of Mdr1b mRNA was observed from days 5-35, but there was no gender difference in adults. Mdr2 mRNA levels were highest at birth with a decrease throughout days 5-30. The induction of Mdr mRNA transcripts in liver by treatment with 22 chemicals representing AhR ligands, CAR ligands, PXR ligands, PPARa ligands, Nrf2 activators, and Cyp4502E1 inducers was also assessed. Mdr mRNA transcripts in liver were not significantly altered by treatment of mice with any of these classes of inducers. In conclusion, the primary expression of mouse Mdr1a gene is in the gastrointestinal tract where it functions to decrease absorption of some xenobiotics. Mouse Mdr gene expression is not readily increased by microsomal enzyme inducers through coordinate mechanisms with phase I and II drug-metabolizing enzymes. (Supported by NIH Grants ES-09716 and ES-07079)

1517 TISSUE DISTRIBUTION AND HORMONAL REGULATION OF THE BREAST CANCER RESISTANCE PROTEIN (BCRP/ABCG2) IN RATS AND MICE.

Y. Tanaka, A. L. Slitt, J. M. Maher, T. M. Leazer and C. D. Klaassen. *University of Kansas Medical Center, Kansas City, KS.*

Breast cancer resistance protein (Bcrp/Abcg2), also known as the mitoxantrone resistance protein or the placental ABC transporter, is a member of the ABC transporter family. The purpose of this study was to quantify Bcrp mRNA in rat and mouse tissues, and to determine whether there are gender differences in Bcrp mRNA expression by the branched DNA assay. Rat Bcrp mRNA levels were high in intestine and kidney; intermediate in testes; and low in other tissues, including liver. Bcrp expression in mouse was highest in kidney, followed by liver, ileum and testes. BCRP mRNA expression is known to be very high in human placenta, but its expression in rat and mouse placenta was much lower than that in other tissues. Male-predominant expression of Bcrp was observed in rat kidney, and mouse liver. Female-predominant expression of Bcrp was observed in rat brain. Furthermore, gonadectomy and hypophysectomy (HX) experiments were conducted to determine whether sex steroids and/or growth hormone (GH) are responsible for Bcrp gender-divergent expression patterns in rat kidney and mouse liver. Ovariectomy increased Bcrp expression in female rat kidney, but Bcrp expression in male rat kidney was unaffected by castration. HX had no significant effect on Bcrp mRNA levels in male or female rat kidney. In mice, gonadectomy reduced Bcrp expression markedly in male liver, but only slightly in female liver. Administration of 5α-dihydroxytestosterone or 17β-estradiol, to castrated males and ovariectomized females respectively, resulted in an increase in Bcrp mRNA by testosterone but not estra-

diol. In summary, species differences exist in the tissue distribution of Bcrp, especially with regard to intestinal expression. Gender differences were observed within both species. Male-predominant expression of Bcrp in rat kidney appears to be due to the suppressive effect of estradiol. Male-predominant expression of Bcrp in mouse liver appears to be due to the inductive effect of testosterone. (Supported by NIH grants ES-09714 and ES-07079)

1518 LIPOPOLYSACCHARIDE-MEDIATED DOWN-REGULATION OF ORGANIC ANION TRANSPORTING POLYPEPTIDE 4 (OATP4; SLC21A10) IS INDEPENDENT OF TUMOR NECROSIS FACTOR-α, INTERLEUKIN-1β, INTERLEUKIN-6, OR INDUCIBLE NITRIC OXIDE SYNTHASE.

N. Li and C. D. Klaassen. *University of Kansas Medical Center, Kansas City, KS.*

Organic anion transporting polypeptide 4 (Oatp4) is expressed almost exclusively in liver, where it mediates uptake of a variety of anionic compounds, including bile acids, across hepatic sinusoidal membranes in a Na+-independent manner. Lipopolysaccharide (LPS) has been shown to decrease Oatp4 mRNA concentrations in a dose- and time-dependent manner in Toll-like receptor 4 (TLR4) normal (C3H/OuJ) mice, but not in TLR4-mutant (C3H/HeJ) mice. Moreover, after LPS administration, serum concentrations of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) are markedly lower in TLR-4 mutant mice than in TLR-4 normal mice. Thus, TLR4 is considered an upstream mediator of LPS-induced decrease in mouse Oatp4 mRNA and increase in some serum cytokines. LPS is thought to alter liver gene expression, including that of hepatic transport proteins, through TLR4-mediated TNF-α, IL-1β, IL-6, or nitric oxide (NO) release. TNF receptor p55 (TNFRp55) and type I IL-1 receptor (IL-1RI) mediate the biological functions of TNF-α and IL-1β, respectively. Therefore, to determine whether endogenous cytokines or NO are mediators of LPS-induced down-regulation of mouse Oatp4 mRNA, Oatp4 mRNA levels were determined in mice deficient in the TNFRp55, IL-1RI, IL-6, or inducible nitric oxide synthase (iNOS) after LPS administration. Mice homozygous for a targeted deletion in genes for TNFRp55, IL-1RI, IL-6, or iNOS exhibited similar decreases in Oatp4 mRNA levels as wild-type mice after LPS administration. Moreover, in mouse hepatoma cells, treatment with TNF-α, IL-1β, or IL-6 individually or in combination did not suppress activity of a mouse Oatp4 promoter construct (-4.8kb/+30). Therefore, LPS-induced down-regulation of Oatp4 appears to be independent of TNF-α, IL-1β, IL-6 or iNOS. (Supported by NIH grant ES-09649)

1519 ROLE OF NAD(P)H:QUINONE OXIDOREDUCTASE 1 IN CLOFIBRATE MEDIATED HEPATOPROTECTION FROM ACETAMINOPHEN TOXICITY.

J. Moffit[1], M. Kardas[1], L. M. Aleksunes[1], A. M. Slitt[2], C. D. Klaassen[2] and J. E. Manautou[1]. *[1]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT and [2]Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Acetaminophen (APAP) is an analgesic and antipyretic agent that produces liver toxicity at high doses. The toxicity of APAP is highly dependent on hepatic bioactivation and detoxification pathways. Pretreatment with the hypolipidemic drug clofibrate (CFB) reduces APAP hepatotoxicity significantly in male CD-1 mice. However, the mechanism of this resistance is not entirely known. Previous studies showed that CFB reduces APAP binding to hepatic proteins and depletion of cellular glutathione independently of changes in bioactivation and conjugative pathways for APAP. These results suggest that CFB pretreatment enhances the detoxification of the APAP reactive intermediate, N-acetyl-p-benzoquinone imine (NAPQI). Since NAD(P)H quinone oxidoreductase 1 (NQO1) is involved in the reduction of cellular quinones, we investigated the effect of CFB on the expression and activity of this detoxifying enzyme. Administration of CFB (500 mg/kg, i.p.) to male CD-1 mice for 5 or 10 days resulted in a modest induction of NQO1 activity, which was only significant for the 5 day dosing regimen. Branched DNA signal amplification and western blot analysis showed that CFB treatment does not significantly increase NQO1 gene transcription or protein levels. Additionally, inclusion of human recombinant NQO1 does not alter the amount of NAPQI generated by an *in vitro* microsomal activating system. In conclusion, CFB treatment does not appear to alter NQO1 expression or function in a manner consistent with its hepatoprotective capacity. The lack of NQO1 induction coupled with the inability of recombinant NQO1 to alter NAPQI availability, suggests that the detoxification of APAP active metabolites by quinone reduction is not a mechanism involved in hepatoprotection afforded by CFB. Supported by NIH ES10093 and the University of Connecticut Research Foundation.

1520 DIFFERENTIAL GENE EXPRESSION OF MEMBRANE TRANSPORT AND DETOXIFICATION PROTEINS DURING HEPATIC INJURY.

L. Aleksunes[1], A. Slitt[2], M. Thibodeau[1], C. Klaassen[2] and J. Manautou[1]. *[1]Pharmaceutical Sciences, University of Connecticut, Storrs, CT and [2]Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Drug metabolizing enzymes and membrane transporters are responsible for the detoxification and elimination of xenobiotics from the liver. Following acute chemical injury, hepatocytes are generally more resistant to toxicant re-exposure. Alterations in the production of transport and cellular defense proteins may contribute to this resistance. Characterization of transcriptional changes is one approach for studying regulation of detoxification and elimination pathways. The goal of this study was to identify alterations in mRNA levels of transport and detoxification proteins as a result of pro-oxidant liver injury. These included the multidrug resistance-associated proteins (Mrp 1-4), organic anion transport proteins (Oatp 1, 2 and 4) and sodium taurocholate co-transporting protein (Ntcp), in addition to heme oxygenase (HO), quinone reductase (QR) and epoxide hydrolase (EH). For this purpose, groups of male C57BL/6J mice received acetaminophen (APAP, 200, 300 or 400 mg/kg, i.p.) or carbon tetrachloride (CCl_4, 10 or 25 ul/kg, i.p.). Liver samples were collected at 6, 24 and 48 hrs for total RNA isolation and histopathological analysis of injury. Gene expression was determined using branched DNA signal amplification. Although EH expression was unaltered, APAP and CCl_4 increased HO and QR levels by 21- and 2.5-fold, respectively. A uniform reduction in transcription of Oatp1, 2, 4 and Ntcp was observed with CCl_4 treatment. By contrast, expression of Mrps 1-4 was increased differentially by APAP and CCl_4. Notably, a marked elevation of Mrp4 was observed 24 hrs after APAP (5-fold) and CCl_4 (37-fold). Collectively, these expression patterns suggest a coordinated regulation of both transport and detoxification genes during liver injury. This reduction in expression of uptake transporters and enhanced transcription of detoxification enzymes and export transporters may contribute to the resistance of hepatocytes to subsequent toxicant exposure.

1521 TRANSPORT CHARACTERISTICS OF 3, 3′-DIINDOLYLMETHANE USING HUMAN DERIVED INTESTINAL CELLS, CACO-2 AND P27.7 CELLS.

A. J. Rodriguez[1], R. Rodriguez-Proteau[1, 2], S. C. Tilton[2], J. Chen[1], K. P. Hall[1], J. E. Mata[1] and D. E. Williams[2, 3, 4]. *[1]Pharmaceutical Sciences, Oregon State University, Corvallis, OR, [2]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, [3]Linus Pauling Institute, Oregon State University, Corvallis, OR and [4]Marine and Freshwater Biomedical Science Center, Oregon State University, Corvallis, OR.*

Indole-3-carbinol (I3C) is a major component of cruciferous vegetables that has been shown to be chemoprotective in animal studies and is under evaluation for chemoprevention of breast cancer in women. I3C undergoes acid condensation in the stomach to numerous products such as 3, 3′-diindolylymethane (DIM). DIM has been shown to be antiestrogenic in MCF-7 cells and presumably interacts with the aryl hydrocarbon receptor (AHR) to inhibit tumor development. This study evaluated the transport characteristics of DIM using the *in vitro* intestinal drug transport/metabolism models, Caco-2 cells and P27.7 cells. The transport of ^{3}H-DIM (0.1, 0.5, 0.75, 1, and 10 μM) over 3 hrs was investigated using sink conditions from the apical (A) to basolateral (B) direction and vise versa, B → A, in Caco-2 and P27.7 cells. The permeability (Pe) of DIM was slightly greater from the A → B direction than the B → A with the Caco-2 cells at all concentrations evaluated. There were no differences seen with the P27.7 cells (expressing CYP3A4), in either direction without and with 100 μM verapamil, a P-glycoprotein (P-gp) inhibitor. ^{3}H-DIM demonstrated a saturable efflux from the B → A compartments without and with verapamil with significance seen between the 0.75 μM and 1.0 μM, Pe from $1.54 \times 10^{-5} \pm 2.91 \times 10^{-6}$ cm/sec to $8.13 \times 10^{-6} \pm 1.51 \times 10^{-6}$ cm/sec and $1.69 \times 10^{-5} \pm 1.30 \times 10^{-6}$ cm/sec to $9.48 \times 10^{-6} \pm 9.74 \times 10^{-7}$ cm/sec, respectively, $p < 0.05$. These studies suggest that DIM is not a substrate for P-gp; however, DIM can limit its own efflux. Also, in the clone of Caco-2 cells, P27.7 cells, Pe of DIM was increased in both directions without and with verapamil when compared to the parent cell line; thus, the transport characteristics of DIM was altered compared to the Caco-2 cells. Supported by NIH grants ES007316 and CA90890.

1522 EFFECT OF TROGLITAZONE (TGZ) ON BASOLATERAL AND CANALICULAR TRANSPORT OF MODEL ORGANIC ANIONS.

D. C. Kemp[1] and K. L. Brouwer[2, 1]. *[1]Curriculum in Toxicology, Univeristy of North Carolina, Chapel Hill, NC and [2]Drug Disposition and Delivery, School of Pharmacy, University of North Carolina, Chapel Hill, NC.*

Studies in this laboratory have shown that TGZ decreased taurocholate (TC) accumulation in sandwich-cultured rat hepatocytes (SCRH). The primary objective of this study was to determine whether TGZ modulates basolateral transport protein

function; a secondary objective was to determine whether TGZ alters Mrp2-mediated transport. Initial substrate uptake (15-60 sec) in freshly isolated male Wistar rat hepatocytes was evaluated. In addition, substrate uptake and biliary excretion were examined in SCRH. Suspended hepatocytes were isolated from male Wistar rats in Dulbecco's Modified Eagle's Medium (DMEM), and uptake determined using standard methodology. Isolated hepatocytes from male Wistar rats were cultured for 4 days between two layers of gelled collagen. Culture media consisted of DMEM, supplemented with 5% fetal bovine serum and insulin (4 mg/L) for the first 24 hrs; subsequently DMEM was supplemented with 1% ITS (insulin, transferring, selenium, linoleic acid) and 0.1 μM dexamethasone, replaced every 24 hrs. The TC (1 μM) uptake rate in suspended hepatocytes was decreased ~3-fold by TGZ (10 μM) over a 60 sec time range. To determine whether this effect was due to impaired Oatp-mediated TC uptake, the effect of TGZ on estradiol-17β-D-glucuronide (E217G) uptake was examined. TGZ inhibited E217G (1 μM) uptake in isolated hepatocytes ~2-fold (0.06±0.02 to 0.03±0.004 nmol/mg protein/60 sec, mean±S.E.M). In SCRH, TGZ (10 μM) also decreased E217G 10-min accumulation ~2-fold (150±19 vs. 68±11 pmol/mg protein, mean±S.E.M) in standard HBSS (Hanks balanced salt solution) (cell + bile canaliculi). TGZ decreased accumulation of carboxydichlorofluorescein, an Mrp2 substrate, in standard HBSS, but differences were not statistically significant (733±91 vs. 566±98, mean±S.E.M). These data suggest that 10 μM TGZ primarily alters basolateral uptake with modest alterations in Mrp2-mediated transport of organic anions in hepatocytes. Supported by 5-T32-ESO7126 and NIH GM41935.

1523 AUTOPROTECTION: SUBCHRONIC LOW DOSE ADMINISTRATION OF CHLOROFORM RENDERS RESISTANCE TO A LETHAL DOSE OF CHLOROFORM.

H. M. Mehendale[1], S. S. Anand[1], P. S. Palkar[1] and M. M. Mumtaz[2].
[1]Department of Toxicology, University of Louisiana, Monroe, monroe, LA and [2]ATSDR, Atlanta, GA.

The aim of the study was to develop a subchronic autoprotection model for $CHCl_3$ and to investigate the role of toxicokinetics and tissue repair. Swiss-Webster mice were treated with 150 and 300 mg $CHCl_3$/kg *via* aqueous gavage for 30 d before challenging with a LD90 dose (750 mg $CHCl_3$/kg) resulted in 100% survival of mice. Another set of mice receiving only the LD90 dose died within 3 d. Liver and kidney injury were measured by ALT and BUN, respectively, and by histopathology. Tissue repair was assessed by (^{3}H-T) thymidine incorporation into hepato- and nephro-nuclear DNA and by PCNA. These were measured from 24 to 96 h after the lethal dose. Blood, liver and kidney levels of $CHCl_3$ were quantified over 15 to 360 min. Only the high dose caused 20% mortality during the 30 d dosing regimen. Blood $CHCl_3$ levels in 150 and 300 mg/kg groups were 40 and 60% lower, respectively, than the mice receiving only 750 mg $CHCl_3$/kg. Without the priming treatment, 750 mg $CHCl_3$/kg caused significant hepatic and renal injury at 24 h, peaking at 48 h. While liver injury resolved, kidney injury was still evident at 72 h. Mortality occurred between 48 to 96 h. In the absence of priming, lethal dose inhibited tissue repair in liver and kidney. Tissue repair was significantly higher in autoprotected mice at 48 and 72 h. Liver and kidney injury were remarkably lower in the primed mice. The biochemical findings were supported by histopathology and PCNA. Significantly larger numbers of cells entered S-phase in liver and kidney of pretreated mice. Presence of the resistant new cells from ongoing tissue repair and lower blood $CHCl_3$ levels could be the reason for the lower infliction of injury. Next wave of tissue repair occurring at 48 and 72 h offered second line of defense and stops the progression of injury. These findings suggest that ongoing tissue repair initiated by the priming doses in addition to altered toxicokinetics of $CHCl_3$ is critical and vital to the subchronic $CHCl_3$ autoprotection (supported by ATSDR U61/ATD 681482).

1524 ANALYSIS OF GENOMIC AND PROTEOMIC DATA FROM TRANSGENIC RAT LIVER NEOPLASMS.

Y. Dragan[1], F. Hong[2], W. Tong[2], J. Ward[3], S. Yim[4], R. Perez[5], L. Zhang[5] and M. Freitas[5]. *[1]Hepatic Toxicology, NCTR, Jefferson, AR, [2]Bioinformatics, NCTR, Jefferson, AR, [3]Pathology, NCI, Frederick, MD, [4]Metabolism, NCI, Bethesda, MD and [5]Chemistry, Ohio State University, Columbus, OH.*

The development of liver neoplasms in the rat is of concern for analysis of two year bioassays since there is a low spontaneous occurrence. Albumin SV40-T antigen transgenic rats (TG+) spontaneously develop neoplasms within 6-9 months of age and can be used as a genetic model to understand the genetic changes that accompany rat liver neoplasm induction and progression. Neoplasms (N=4) from TG+ were examined by microarray analysis using the NCI Incyte Mouse Chip. RNA was isolated from laser capture microdissected tissue and amplified prior to gene expression analysis. Initial clustering and gene expression analysis was performed with Spotfire and ArrayTrack. Numerous genes had expression differences including those important in cell cycle control, cell proliferation, transgene specific effects, and tumor maintenance and progression. Previous studies indicated that specific cytogenetic changes are associated with the induction and progression of rat liver neoplasms and the microarray data was organized by chromosomal location. Real time PCR, dot blot, or Northern analysis was used to confirm altered expression of selected genes. In addition, 2D gel analysis was performed for whole cell lysates. The whole cell lysates were run on tube gels or IEF strips (ampholytes pH 3-10) in the first dimension and on 12% SDS gels in the second dimension. Gels were stained with Coomassie blue or Sypro Ruby and proteins differing between a concurrently run control and neoplasm were handpicked, digested with trypsin, and analyzed by Maldi in ten neoplasms from these rats. Protein identification was confirmed by ESI-MS/MS fragmentation patterns. For example, calregulin was consistently decreased at the message and protein level.

1525 QUANTITATIVE RNA INVADER ANALYSIS AS A FAST METHOD TO SCREEN FOR INDUCTION POTENTIAL OF DRUGS USING PRIMARY CULTURES OF HUMAN AND RAT HEPATOCYTES.

V. Kostrubsky, S. Kulkarni, J. Hanson and S. Duddy. *Safety Sciences, Pfizer, Ann Arbor, MI.*

Using primary cultures of human and rat hepatocytes we investigated whether Invader mRNA (Third Wave Technology, Inc.) analysis can be used as a fast method to study induction potential of drugs and whether changes in mRNA expression would associate with changes in appropriate enzyme activity and protein expression. Cultured hepatocytes were treated with beta-naphthoflavone, dexamethasone (Dex), rifampicin, phenobarbital (PB) and chenodeoxycholic (CDCA) acid for 48 h, and mRNA expression of CYP1A2, CYP3A4, CYP3A, CYP2B1/2, and biliary transporters, BSEP and MRP2, were analyzed. Corresponding enzymatic activities and protein expression were also measured. Different quantities of total RNA isolated from induced and control cells, as well as positive control mRNA, produced linear responses as detected by Invader. The specificity of detection was validated *via* exposing cells to positive or negative control inducers as well as in cells transfected with genes of interest. Increases of 8-, 12-, 6-, 30-, 30- and 3-fold in CYP3A4, rat CYP3A (Dex), rat CYP3A (PB), CYP2B1/2, CYP1A2 and human BSEP mRNA, respectively were detected. Increases in mRNAs correlated with increases in enzymatic activities and protein expression, though the increases in enzyme activities were generally smaller magnitude relative to corresponding mRNAs suggesting that the sensitivity of mRNA detection is greater than changes in activities. These data suggest that the mRNA Invader assay provides fast (3 h, 96-well plate), quantitative and sensitive method for evaluation of different mRNA expression profiles.

1526 DOES FUNDING SOURCE INFLUENCE RESEARCH INTEGRITY.

S. G. Gilbert[1], B. J. Kelman[2], L. A. Bero[3], R. L. Brent[4] and J. I. Goodman[5].
[1]INND, Seattle, WA, [2]GlobalTox, Seattle, WA, [3]Clinical Pharmacy, University of California, San Francisco, CA, [4]Pediatrics, Radiology and Pathology, duPont Hospital for Children and Thomas Jefferson University, Wilmington, DE and [5]Pharmacology and Toxicology, Michigan State University, East Lansing, MI.

The integrity of research and its subsequent interpretation, presentation, and publication influences the development of new drugs, control of environmental contaminants, regulatory policy, and litigation. Often these decisions have significant public health and economic consequences. The purpose of this session is to examine the subtler aspects of the question *Does funding source influence research integrity?* All sponsors (including NIH) have expectations of those conducting research on their behalf. GLP guidelines were adopted to try to ensure the quality of data submitted to regulatory agencies, e.g. USFDA and EPA. In contract research or litigation the client may be an industry sponsor with a direct financial stake in the outcome. The client may wish to influence study design or interpretation of results. In academia expectations or pressures can take different forms. Researchers may desire certain results to ensure continued funding or the generation of significant publications for tenure. The controversy engendered by a funding source is exemplified by tobacco industry support of research. Some researchers and even institutions have decided not to accept funds from specific groups such as the tobacco industry. Others have argued that research can and should be judged on it merits and not on the funding source. Increasingly, authors and researchers are asked to disclose the source of funding or support related to their work, along with other potential conflicts of interest. Our panelists represent a range of views and professional experiences. The primary goal of this Roundtable is to generate discussion and explore the real, imagined and/or perceived expectations and conflicts related to the source of research funding. This Roundtable was supported in part by a grant from the US Department of Health and Human Services, Office of Research Integrity.

1527 COMPARISON OF THRESHOLD DOSE-RESPONSE METHODS FOR ENTIRE DATA SETS: COPPER AS A CASE STUDY.

S. Baker. *International Copper Association, New York, NY.*

Copper is an essential element with a high redox potential, and therefore can be highly toxic. The complete range of physiologic response (toxicity of deficiency, essentiality, and toxicity of excess) was considered in a dose-response assessment of copper. The data set on the biological effects of copper is difficult to interpret because the cascade of effects occurs over a very narrow dose range, making it hard to define the critical effect and dose. Dose-response data from the entire database on health effects of copper (observations on acute human poisoning, chronic toxicity, nutritional essentiality/homeostasis, animal nutrition and toxicity, and molecular/genetic mechanisms of copper control and action) were subjected to biological and mathematical modeling to better understand the human response. This was particularly important to better define the regions of marginal deficiency and excess, where adverse effects are subtle and where individuals with mild inborn errors of metabolism are positioned on the University- shaped dose-response curve. The purposes of this approach to dose- response analysis are to better define quantitative ranges for marginal deficiency and excess, bound the limits of essentiality and uncertainty, account for broad interindividual variability, and use the power of the entire database to define the human response to copper exposure. The comparative analysis of NOAEL/LOAEL, Benchmark Dose, and Categorical Regression approaches to understanding how copper behaves in the body and the thresholds for effects will applicable to other essential trace elements, in developing more precise RDAs and regulatory/guidance limits for metals in food, diet, and water.

1528 THE ESSENTIALITY AND TOXICITY OF COPPER: IMPLICATIONS FOR DOSE-RESPONSE ASSESSMENT.

C. Keen. *Department of Nutrition, University of California, Davis, CA.* Sponsor: L. Plunkett.

Copper plays a role in several critical processes in the body, including energy production, immune function, oxidative defense, iron mobilization and trafficking, red and white blood cell maturation, neuropeptide synthesis, vessel and bone integrity, and myocardial contractility. In the United States a significant proportion of the population consume diets that contain less than the RDA for copper. In addition to low dietary intakes (primary deficiency), copper deficiency can arise secondary to genetic abnormalities, nutritional interactions, drug interactions, and disease-induced alterations in copper metabolism. Copper deficiency during pregnancy can result in early embryonic death, and gross structural abnormalities of the skeletal, pulmonary, cardiovascular, and central nervous systems. The teratogenicity of copper deficiency can be attributed in part to excessive oxidative damage, altered angiogenesis, altered composition of extracellular matrix, and low activities of several copper enzymes. Perinatal copper deficiency can result in persistent immunological and behavioral abnormalities even after the correction of the deficiency. Copper deficiency in adulthood increases the risk for bone and cardiovascular disease. At any stage of life, copper deficiency can impair iron metabolism, thus signs of iron deficiency can also occur in copper deficient individuals. Potential mechanisms that underlie the above copper deficiency induced pathologies will be discussed. An excess of copper can also pose significant health risks, as excessive tissue copper can trigger the production of reactive oxygen and reactive nitrogen species. In contrast to copper deficiency, dietary copper toxicity is rare. However, certain genetic disorders can result in copper toxicosis. The relative risks for copper deficiency and copper toxicity will be discussed, with an emphasis on how these relative risks impact the dose-response assessment, its interpretation, risk assessment, and public health policy.

1529 QUALITATIVE INTERPRETATION OF COMPLEX AND DISPARATE DATA SETS FOR DOSE-RESPONSE ASSESSMENT OF ESSENTIAL TRACE ELEMENTS: COPPER AS A CASE STUDY.

L. M. Plunkett. *Integrative Biostrategies, LLC, Houston, TX.*

In order to perform a dose-response assessment for essential trace elements such as copper it is necessary to identify bounds on both essentiality and excess and then to delineate the interface of the two conditions, resulting in what has been labeled a University-shaped dose-response curve. No single study is able to define the complete dose-response curve for an essential element. Thus, the first step was to systematically organize and categorize the available data to determine what would be useful for defining the likely human responses to copper deficiency as well as excess. This presentation will describe how the large amount of disparate data on copper deficiency and excess was systematically screened and then categorized in order to provide a framework for the dose-response assessment. The copper data set for toxicity, data concerning both deficiency and excess, was considered as a whole. The goal of the screening exercise was to identify the bounds around the homeostatic range wherein toxicity is not evident, as well as identify levels associated with adaptive changes in physiological systems, and finally levels associated with frank toxicity in a variety of organ systems. Data was categorized based on affected systems (e.g., enzyme systems, hematopoietic parameters, gut, liver, cardiovascular system, kidney) and then according to outcome (e.g., adaptive changes, biochemical derangement, architectural disruption, histopathological changes). Data were weighted in the assessment according to standard approaches to screening such as number of doses tested or administered in a study, number of test subjects or animals in a study, existence of multiple outcomes, whether data were from humans versus animals versus cells, and adequacy of data reporting by the authors. The studies identified through this screening process were then used as the basis for a quantitative dose-response assessment of copper deficiency and excess. This integrative approach to examining the entire databases for copper will be applicable to other essential trace elements.

1530 A CRITICAL COMPARISON OF DOSE-RESPONSE ASSESSMENT APPROACHES FOR COPPER, AN ESSENTIAL TRACE ELEMENT.

T. B. Starr. *TBS Associates, Raleigh, NC.*

The University-shaped dose-response curve for copper toxicity arises from the multiple, distinct toxicologic processes that are triggered by varying degrees of copper deficiency and excess, with adaptive responses such as dose-dependent absorption contributing to a flattening of the dose-response relationship in the intermediate homeostatic range. Three different empirical techniques are used to characterize the shape of the dose-response curves for copper toxicity in laboratory animals and humans: 1) a graphical approach based upon NOAELs and LOAELs as obtained from toxicity individual studies; 2) equivalent Benchmark Dose analyses of all data from the same studies; and 3) an integrated approach that utilizes categorical regression to simultaneously analyze the data from multiple studies. Four strategic questions are addressed: 1) are the existing data sufficient to characterize the limits of the homeostatic range, 2) what is the best analytical approach to defining this range, 3) what critical data gaps should be filled by future studies, and 4) how should those future studies be designed to be optimally useful in better-defining the shape of the dose-response curve for copper toxicity.

1531 DOSE-RESPONSE MODELS FOR COPPER: IMPLICATIONS FOR RISK ASSESSMENT.

D. Krewski. *McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Ottawa, ON, Canada.* Sponsor: L. Plunkett.

The establishment of exposure guidelines for substances that present risks to human health requires careful consideration of dose-response relationships indicating how risk varies with dose. Whereas most chemicals demonstrate monotonically increasing dose-response curves, essential elements such as copper exhibit University-shaped dose-response relationships as a consequence of the adverse effects of both deficiency and excess. The establishment of a homoeostatic region of acceptable copper intake therefore requires the application of dose-response models that take into account both of these effects. Additional factors of importance in modelling copper dose-response include inter-individual variability due to genetic and other factors, and the biologically competitive interplay between copper, zinc, and iron. With the extensive database on copper toxicity, including a large amount of information on copper toxicity in humans, analyses of combined data from multiple sources may be used to better define the region of copper essentiality. These and other factors involved in characterizing copper dose-response are examined, and their implications for copper risk assessment evaluated.

1532 SUMMARY: SELECTING AND ASSESSING BIOLOGICAL DATA IN TOXICITY, DOSE-RESPONSE, AND RISK ASSESSMENTS FOR ESSENTIAL TRACE ELEMENTS: USE IN SETTING SAFE LIMITS.

M. L. Dourson. *Toxicology Excellence for Risk Assessment, Cincinnati, OH.*

This presentation will tie the conclusions of the previous four presentations together, exploring (1) the impact of inclusion or exclusion of biological data on the downstream assessments of the safety of essential trace elements, (2) the advantages and disadvantages of each of the three dose-response methods examined, and (3) options for considering different dose-response methods in a single assessment of toxicity and risk for copper. The presentation will also describe the path forward to creating stronger data sets for characterizing the dose-response assessment for essential trace elements, with greater certainty and accountability for marginal effects, human variability, and sensitive populations

1533 ENVIRONMENTAL POLLUTION AND THE IMMUNE SYSTEM: MECHANISMS OF IMMUNOTOXICITY ACROSS PHYLA.

B. Luebke[1] and D. Germolec[2]. [1]*Immunotoxicology Branch, USEPA, ORD, NHEERL, ETD, Research Triangle Park, NC and* [2]*Environmental Immunology, NIEHS, Research Triangle Park, NC.*

Our current understanding of immunotoxicology comes largely from studies done in rodents or using *in vitro* systems, as surrogates for potential human effects. However, innate and adaptive immune responses are remarkably similar across a broad range of species: innate immunity, which appeared billions of years ago, is evident at lower levels of biological complexity and adaptive immune responses made an evolutionary appearance 400 million years ago in cartilaginous fish. Recent evidence indicates that plant responses to environmental factors depend on a family of pattern-recognition receptors that are homologous to Toll-like receptors that in animals are critical to both innate and adaptive responses. This phylogenetic conservation of effector mechanisms suggests that a given xenobiotic may cause immunotoxicity in a range of species extending beyond vertebrates. Immunotoxicology studies in plants and wildlife support this concept, with reported adverse effects following exposure to oxidant gases, various hydrocarbons, metals or endocrine disruptors. It is important for mammalian immunotoxicologists to develop an understanding of immune system organization and homeostasis across a broad phylogenetic scale, as well as an appreciation for the effects that similar xenobiotics have on immune system health across species. Field studies of populations in polluted environments, which are unable to escape exposure, provide insight into the risk xenobiotics pose to biologically diverse members of the ecosystem, from plants to mammals. This symposium will address shared and unique features of immune system organization and responses by selected species at diverse levels of biological complexity and immune system sophistication, and the mechanisms whereby selected xenobiotics alter immune function.

1534 DISEASE RESISTANCE AND INNATE IMMUNITY IN PLANTS.

K. R. Davis and R. V. Mulpuri. *Paradigm Genetics, Inc., Research Triangle Park, NC.* Sponsor: R. Luebke.

Disease resistance in plants is mediated by a complex surveillance system that detects potential pathogens and activates a resistance reaction- the hypersensitive response (HR). HR can be induced by at least two distinct signaling pathways and includes activation of programmed cell death (PCD) and the accumulation of antimicrobial compounds. There are similarities between the activation of HR in plants and innate immunity in animals. A key similarity is the role of plant Resistance (R) genes important for pathogen-specific recognition that belong to the Toll/interleukin receptor (TIR) class of genes. Plant R genes containing TIR domains also contain a unique nucleotide binding site (NBS) and a leucine-rich repeat (LRR) domain of variable length. Current data suggest plant TIR-NBS-LRR R proteins perform signaling functions similar to the structurally related Toll-like receptor (TLR) proteins in animals. Besides the similarities of pathogen recognition in plants and animals, there are also some similarities in downstream signaling events. These include the importance of an oxidative burst, nitric oxide production, and the involvement of lipases and lipids as signals for the activation of resistance mechanisms. Another aspect of plant responses to pathogens that may have relevance to animals is that abiotic stresses can induce resistance mechanisms using the same signaling mechanisms employed during pathogen resistance. Responses to ozone represent one such example. Ozone induces PCD *via* an oxidative burst and the activation of the same signaling pathways utilized during HR. It appears that plant cell damage caused by ozone may be due to the inappropriate activation of a defense response that evolved to limit pathogen growth. Recent studies of animal responses to ozone exposure suggest that cell damage may be caused by a similar inappropriate activation of PCD. Taken together, it is clear that there are some significant similarities in how plants and animals respond to biotic and abiotic signals, and that there is significant potential for researchers investigating resistance mechanisms in plants and animals to learn from one another.

1535 CHEMICAL TOXICITY AND HOST DEFENSE IN INVERTEBRATES: AN EARTHWORM MODEL FOR IMMUNOTOXICOLOGY.

A. Goven and L. Fitzpatrick. *Department of Biological Sciences, University of North Texas, Denton, TX.* Sponsor: B. Luebke.

Prediction of chemical-induced immunotoxic risk across key wildlife taxa and to ecosystems requires use of sentinel and surrogate species. Sufficient basic and comparative information exits on the immune systems of several invertebrate taxa to support using their immune function to assess the sublethal immunotoxicity of environmentally relevant chemicals. Measurements of innate immune responses that are homologous across taxa such as immunohistology, phagocytosis and associated biochemical responses involved in ingestion and destruction of foreign material, and host resistance to challenge infection show the most promise for predicting risks to other wildlife, including mammals. Earthworms, Lumbricus terrestris and Eisenia fetida, have emerged as standard invertebrate organisms for modeling the chemical-induced immunotoxic potential of xenobiotics in terrestrial ecosystems. Assessment of innate immune function using protocols modified for earthworms demonstrate immunomodulatory effects of metals (Cu, Cd, Cr) and organic chemicals (polychlorinated biphenyls, chlordane, pentachlorophenol, DDT) on earthworm lysozyme activity; cytology as total and differential coelomocyte counts and *via*bility; and phagocytosis as activation, ingestion, oxidative burst, and bacterial killing. General acceptance of invertebrate or earthworm immune components as biomarkers of eco-immunotoxicity in risk assessment will ultimately depend upon results of future research focusing on normalization of exposure metrics across taxon-specific toxicokinetics, consideration of and control for natural variability in immune marker performance, and affirmative link of immune markers to endpoints having ecological significance.

1536 ENVIRONMENTAL CHEMICALS AND AMPHIBIAN IMMUNITY: WHAT ARE THE RISKS TO AMPHIBIAN HEALTH.

L. A. Rollins-Smith. *Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN.* Sponsor: L. Rollins-Smith.

The amphibian immune system is very similar to that of mammalian species. The adaptive immune system is characterized by organs and cells that serve similar functions as those of mammals. Amphibians have T and B lymphocytes that express rearranging T cell receptors (TCR) and immunoglobulin (Ig) receptors. They have a major histocompatibility complex (MHC) encoding classical class I and class II molecules. Amphibians express several Ig isotypes, and they can produce leukocyte-derived cytokines. Their innate immune system includes phagocytic cells, a complement system, and natural killer (NK) cells. In addition, amphibians possess granular glands in the dermis of the skin that release a variety of bioactive peptides including antimicrobial peptides. The immune system may be harmed during embryonic, larval, or adult stages by contamination from xenobiotic chemicals in the water. It may be especially at risk during embryonic life and during metamorphic transition. Chemicals in the water may affect adult amphibians by altering the function of antigen presenting cells in the skin or by inhibiting synthesis of antimicrobial peptides produced in granular glands of the skin. Environmental chemicals that contribute to ozone depletion may result in increased UV-B exposure. The loss of effective skin defenses may predispose amphibians to infection by skin pathogens such as the chytrid fungus, Batrachochytrium dendrobatidis, associated with global population declines. Recent studies illustrate the possible negative effects of xenobiotic chemicals or increased UV-B on the development of embryonic components of the immune system, development of larval allograft rejection responses, and the production and secretion of antimicrobial peptides by young postmetamorphic frogs.

1537 FISH AS EXPERIMENTAL MODELS IN IMMUNOTOXICOLOGY: EVOLUTIONARY CONSERVED MECHANISMS OF ACTION.

C. D. Rice. *Biological Sciences, Clemson University, Clemson, SC.*

Teleost fish represent the lowest group of vertebrates with immune systems that are "mammalian-like". Both RAG-1 and RAG-2 genes, as well as T-cells and B-cells are present. In terms of innate immunity, the toll-like receptors (TLR) and NF-kappa beta signaling have been demonstrated in fish, and the expression of these activation- and pro-inflammatory-associated genes and proteins follow patterns that are mammalian-like. Using Tier-I and Tier-II immunotoxicity testing protocols modified for fish, environmental immunotoxicologists show that fish respond to a particular contaminant in a manner similar to rodents. A few species of fish have emerged as standard models. Notably, the rainbow trout, channel catfish, and medaka are now fairly standard fresh water fish models in immunotoxicology. The mummichog, a common marsh killifish, as emerged as a standard representative of estuarine ecosystems. As a case in point, certain populations of the mummichog along their east coast North America range exhibit characteristics of resistance or adaptation to high levels of priority pollutants. One such population inhabits the upper Elizabeth River sub-estuary in Virginia at the Atlantic Wood site (AW). Atlantic Wood is a heavily contaminated Superfund site containing a mixture of PAHs from creosote. Although PAHs are known to be immunotoxic, the resident mummichog population seems to thrive. Mummichogs from the AW site and from

a reference location at Kings Creek (KC) Virginia were examined for circulating antibody titers against several ubiquitous marine bacteria. The expression of inducible innate immune proteins were also examined. Compared to high responses in reference fish, AW mummichogs had very low plasma immunoglobulins and very low antibodies against all bacteria examined, but they expressed higher circulating lysozyme. Lymphoid cells in the AW mummichogs also expressed more lysozyme and COX 2, which may indicate a state of activation and possible resistance. Taken together, these and other findings suggest that exposed animals may compensate for this loss with higher innate immune responses.

1538 ANALYSIS OF NEUROTOXICITY IN VESICULAR MONOAMINE TRANSPORTER KNOCKOUT MICE.

G. W. Miller. *Emory University, Atlanta, GA.*

Numerous diseases and toxic agents target the brain monoamine systems. Disruption of the normal handling of these neurotransmitters can have devastating consequences on the function of these neuronal systems. The uptake, storage, and release of dopamine, serotonin, and norepinephrine are mediated by the vesicular monoamine transporter (VMAT2). Given the critical nature of VMAT2 in monoamine function, we have created a mouse model of enhanced neurotoxicity by decreasing the expression of VMAT2. Homozygote knockout mice die within a few days after birth, while heterozygote VMAT2 knockout mice (VMAT2 +/-) survive into adulthood and appear healthy. We have subjected the VMAT2+/- mice to various neurotoxicants and assessed vulnerability to toxicity. The classical parkinsonian-inducing toxin, MPTP, was more toxic to the mice with reduced VMAT2 and this was attributed to the decreased ability to sequester to toxic metabolite of MPTP. Methamphetamine was also found to be more toxic in VMAT2+/- mice, which was attributed to an inability to handle elevated intracellular dopamine. There has been some question as to whether the administration of L-DOPA (a dopamine precursor) to patients with Parkinsons disease may exacerbate dopamine neuron cell death. Since the VMAT2+/- mice have an impaired ability to handle dopamine, we hypothesized that they would be vulnerable to L-DOPA-induced toxicity. We subjected the VMAT2+/- mice to L-DOPA three times a day for 28 days and found no enhanced toxicity. Indeed, the VMAT2+/- and wild type did not exhibit any toxicity to a dose of L-DOPA 10x higher than that used in humans. In addition to the generation of animal model of neurotoxicity, our lab has been working on more sensitive assays for detecting damage to the nigrostriatal dopamine system, including the development of novel behavioral tests to assess dopaminergic toxicity in mice and the use of laser capture microdissection and quantitative PCR to analyze gene expression in dopamine neurons. This work has been supported by NIEHS and NINDS.

1539 USE OF MOLECULAR APPROACHES TO EXAMINE MECHANISMS OF NEUROTOXICITY.

W. D. Atchison. *Mich State University, East Lansing, MI.*

Genomic and molecular biological techniques provide powerful tools to study perturbations in cellular processes which occur normally, or in pathophysiological conditions. At the whole animal level these modifications include knock-in, upregulation or knock-out of protein function. Use of small interfering RNAs permits protein function to be impaired temporarily, while heterologous expression of recombinant protein permits study of chemical modification on specific protein functions in isolation. Moreover, with "chimeric" proteins, or "site-directed mutants" the role of specific regions of the protein in neurotoxicity can be probed. Thus these techniques permit the study of mechanisms of chemical action or disease progression in the nervous system at the intact animal to isolated proteins. This symposium will present results of recent studies using three levels of genomic modification to study mechanisms of neurotoxicity. The use of chimeric proteins and site-directed mutagenesis to understand how distinct sites of interaction of pyrethroid insecticides with voltage-sensitive Na^+ channels relate to insecticide resistance will be discussed. Heterologous expression of chimeric Ca^{2+} channels as a tool to understand differences in sites of action of Pb^{2+} and Hg^{2+} will be discussed. The use of small interfering mRNAs to downregulate calnexin, or mutations in Ca^{2+} release channels of the SER to prevent necrotic neuronal cell death in *C. elegans* will be described. The ability of Bcl-xL overexpression in mice to block apoptosis of rod photoreceptors in response to lead exposure will be described, as will the role that genetic deletion in mice of the vesicular monoamine transporter (VMAT2) has to increase vulnerability to dopaminergic damage. This symposium provides the opportunity for investigators with interests in mechanistic neurotoxicity to consider new and powerful approaches to enhance understanding of initiation of disease processes or chemical-induced damage to the nervous system.

1540 THE USE OF BCL-XL OVEREXPRESSING TRANSGENIC MICE HELPS DECIPHER THE MITOCHONDRIALLY-MEDIATED MECHANISM OF LEAD-INDUCED ROD PHOTORECEPTOR APOPTOSIS.

D. A. Fox. *College of Optometry, University of Houston, Houston, TX.*

Transgenic animal models are an integral component in determining the molecular mechanisms that underlie normal physiological processes and toxicant-induced pathophysiology. Photoreceptor apoptosis and resultant visual deficits occur in humans and animals with inherited and chemical-, disease- and injury-induced retinal degeneration. A clinically-relevant mouse model of progressive rod photoreceptor-selective apoptosis was produced by low-level developmental lead exposure. To enhance our mechanistic knowledge, our lead model was studied in combination with transgenic mice overexpressing Bcl-xL only in the photoreceptors. A multiparametric analysis of rod apoptosis and mitochondrial structure-function was performed. Mitochondrial cristae topography and connectivity, matrix volume and contact sites were examined using three-dimensional electron tomography. The lead-induced rod-selective apoptosis was accompanied by rod calcium and lead overload, rhodopsin loss, translocation of Bax from the rod cytosol to the mitochondria, decreased rod mitochondrial respiration and membrane potential, rod mitochondrial cytochrome c release, caspase-3 activation and an increase in the number of rod mitochondrial contact sites. These effects occurred without mitochondrial matrix swelling or outer membrane rupture, caspase-8 activation or Bid cleavage. Bcl-xL overexpression completely blocked all apoptotic events, except the calcium and lead overload, and maintained normal rod mitochondrial function throughout adulthood. These findings extend our *in vitro* retinal studies with lead and calcium and suggest that developmental lead exposure produced rod-selective apoptosis without mitochondrial swelling by translocating cytosolic Bax to the mitochondria. The translocation of Bax likely sensitized the calcium and lead overloaded rod mitochondria to release their intermembrane cytochrome c. The results have relevance for therapies in a wide variety of progressive retinal and neuronal degenerations where calcium overload, lead exposure and/or mitochondrial dysfunction occur.

1541 MOLECULAR APPROACHES TO DEFINE NECROTIC-LIKE NEURONAL DEATH AND STRATEGIES OF PHYSIOLOGICAL DEATH SUPPRESSION IN *C. ELEGANS*.

M. Driscoll. *Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ.*

Neuronal death is the catastrophic outcome of stroke, ischemia, physical injury and devastating neurodegenerative diseases. Although apoptosis has been implicated in these conditions, considerable evidence now supports that alternative death mechanisms are activated in parallel to apoptosis. One critical component in neuronal injury and disease is necrotic cell death. In *C. elegans* we have characterized a necrotic-like death prototype, distinct from apoptosis, associated with neuronal swelling and death. The necrotic paradigm we study involves death initiation by hyper-activated ion channels and requires elevation of intracellular Ca^{+2}. This Ca^{+2} rise activates calpain and cathepsin proteases. Several features of the basic pathway are shared with mammalian necrosis, supporting that, like apoptosis, necrotic death mechanisms are conserved from nematodes to humans. Using approaches uniquely applied in *C. elegans*, such as genome-wide RNA interference and genetic screens, we are elaborating molecular mechanisms of a necrotic-like death and defining physiological death suppression strategies.

1542 MOLECULAR BASIS OF DIFFERENTIAL SENSITIVITIES OF INSECT SODIUM CHANNELS TO PYRETHROID INSECTICIDES.

K. Dong[1, 2]. [1]*Entomology, Michigan State University, East Lansing, MI and* [2]*Neuroscience Program, Michigan State University, East Lansing, MI.* Sponsor: B. Atchison.

The voltage-gated sodium channel is the primary target-site of pyrethroid insecticides. Pyrethroids induce prolonged sodium current by inhibiting the inactivation and deactivation of sodium channels. To understand the interaction between insect sodium channels and pyrethroids at the molecular level, we cloned and sequenced cDNAs of the cockroach sodium channel gene $para^{CSMA}$ from a pyrethroid-susceptible and several pyrethroid-resistant German cockroach strains. Three point mutations, E434K, C764R and L993F, were found to be associated with pyrethroid resistance. E434K and C764R are located in the intracellular linker connecting domains I and II, and L993F is located in the segment 6 of domain II. The wild-type and pyrethroid-resistant sodium channels carrying single, double or triple mutations were expressed in *Xenopus* oocytes and the channel properties and channel sensitivity to pyrethroids were examined using two-electrode voltage-clamp. The

L993F mutation reduces the channel sensitivity to deltamethin, a type II pyrethroid, by 5-fold, whereas E434K or C764R alone had no affect. However, E434K or C764R combined with L993F further reduces the channel sensitivity to deltamethrin by 100-fold. Furthermore, the channel carrying the triple mutations was almost completely resistant to deltamethrin. Theses results demonstrate that amino acid residues, E434, C764 and L993, are critical for the action of pyrethroids.

1543 EFFECTS OF NEUROTOXIC METALS ON HUMAN RECOMBINANT CALCIUM CHANNELS- ROLE OF THE α_1 SUBUNIT.

W. D. Atchison. *Department Pharmacology/Toxicology, Mich State University, East Lansing, MI.*

Voltage-gated Ca^{2+} channels are targets of a number of naturally occurring toxins, as well as certain environmental toxicants including Pb and Hg. These metals reduce Ca^{2+} influx through the channel when applied acutely, and also apparently use the Ca^{2+} channel as a path to enter the cell. Ca^{2+} channels are multimeric proteins which typically consist of 3 subunits-α_1, β and $\alpha 2\delta$. The $\alpha 1$ subunit is the largest, and forms the ion conducting pore. It also is the primary determinant of the phenotypic identity of the channel; different types of Ca^{2+} channels have distinct α_1 subunits. To date 6 subtypes of Ca^{2+}channels and 10 α_1 subunits have been identified. In many cells and most neurons, multiple subtypes of Ca^{2+} channels coexist; this permits precise regulation of Ca^{2+} entry to match the specific cellular function. However, it makes analysis of the effects of neurotoxic agents on function of specific types of Ca^{2+} channel extremely difficult. By use of recombinant channels of known identity, and expressed heterologously in a system which normally does not contain these channels, the effects of metals on distinct types of Ca^{2+} channels can be examined in isolation. Additionally, by constructing chimeric channels, in which known combinations of channel subunits are co-expressed, specific effects of a toxicant on a particular portion of the protein can be examined. We used transient heterologous expression of recombinant α_{1B}, α_{1C}, and α_{1E} subunits - comprising respectively, N-, L- and R-type Ca^{2+} channels of human origin in the HEK 293 expression system to compare the actions of Pb, Hg and methylmercury using whole cell current recording methods. A constant β and $\alpha 2\delta$ subunits were used; only the $\alpha 1$ subunit was varied. In comparing these various metals, differences in potency of blocking action, reversibility, and kinetics of block were observed among the different subtypes of Ca^{2+} channels to a given metal and of a given channel subtype to different metals. Thus differences in the α_1 subunit of Ca^{2+} channels confer distinct responses to neurotoxic metals. Supported by NIEHS grants ES03299 and ES05822.

1544 BIOMARKERS: DEVELOPMENT, EVALUATION AND USE.

R. Roberts[1] and T. Monticello[2]. *[1]Drug Safety Evaluation, Aventis, Vitry sur Seine, France and [2]Drug Safety Evaluation, Aventis, Bridgewater, NJ.*

Interest in biomarkers continues to grow at a rapid pace. In part, this interest is attributed to the potential utilization of biomarkers in the many aspects of toxicology. Biomarkers of toxicity are characteristics that can be measured and evaluated as indicators of adverse or pathological responses to a drug or xenobiotic. This workshop will feature four stimulating, state-of-the-art presentations on the different aspects of biomarker development, evaluation and use in the field of toxicology. Topics to be covered will include the utility of biomarkers for nonclinical toxicity during drug development, including current applications, validation procedures and regulatory interest, and an evaluation of the newer genomic and proteomic technologies that can contribute to the discovery of potential novel biomarkers of toxicity. In addition, biomarkers for rodent nongenotoxic carcinogenesis will be described and evaluated in the context of screening assays. Finally, the development and utilization of biomarkers for environmental exposure to toxicants and human risk assessment will be addressed in the session. This workshop will suit those who wish to update and extend their knowledge in this rapidly moving field. It is of general interest to all toxicologists, particularly those concerned with drug development, carcinogenesis and risk assessment.

1545 BIOMARKERS OF NONCLINICAL TOXICITY.

T. M. Monticello. *Drug Safety Evaluation, Aventis, Bridgewater, NJ.*

Over the last 5 years there have been tremendous advances in the identification and potential utilization of novel biomarkers of cell and tissue integrity that would allow the monitoring of pathological processes. The advent of newer "omic" technologies, such as proteomics and metabonomics, has also resulted in the discovery of potential novel biomarkers of toxicity. This presentation will introduce the session on biomarkers by first discussing definitions of interest in the field and acceptable criteria of an adequate biomarker. Requirements for establishing a biomarker including the characterization and validation process will be discussed. Current and specific examples of biochemical and bioimaging biomarkers of target organ and cell toxicity will also be presented. Finally, regulatory acceptance and interest in biomarkers that improve predictivity of nonclinical studies and provide an enhanced interface between nonclinical and clinical studies will be discussed.

1546 BIOMARKERS FOR NONGENOTOXIC CARCINOGENS.

J. K. chipman. *Biosciences, University of Birmingham, Birmingham, United Kingdom.* Sponsor: R. Roberts.

Nongenotoxic carcinogens are thought to act *via* disturbance of the balance between cell death and cell proliferation, leading to clonal expansion of pre-neoplastic cells. The precise mechanisms that can lead to this disruption are varied and this complicates the ability to use short term tests to predict nongenotoxic carcinogenic potential. Biomarkers indicative of a chemical's ability to influence cell survival and proliferative advantage are important in recognising this activity in rodent studies. This may be helpful in the interpretation of rodent carcinogenicity that may occur in the absence of genotoxicity, and to be able to extrapolate to human risk assessment. The dose response relationships and species differences in response are critical parameters in this process. Biomarkers can be relatively non-specific relating to apoptosis and proliferation in target organs but may be optimised and more refined by interrogation of the modulation of specific molecules involved in key signalling processes. Both the modulation of connexin-mediated gap junction intercellular communication and the status of DNA methylation have shown particular promise as relevant biomarkers in conjunction with functional studies as above. For example, the rodent non-genotoxic hepatocarcinogens Wy-14, 643, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, methapyrilene and hexachlorobenzene and the rat kidney carcinogens chloroform and p-dichlorobenzene all disrupt gap junction plaques containing connexin 32 in the target organs in the absence of toxicity and they all cause proliferation at the carcinogenic dose. Furthermore, compounds that deplete glutathione such as chloroform and carbon tetrachloride have the ability to induce a secondary genotoxicity through oxidative stress but only at high concentrations. Transcriptomic analyses are leading to the identification of further novel potential biomarkers associated with species- and tissue- specific nongenotoxic carcinogenesis such as the induction of hepatic fibrinogen / angiopoietin-related protein by monoethylhexylphthalate.

1547 BIOMARKERS OF ENVIRONMENTAL EXPOSURE TO GENOTOXICANTS.

D. E. Shuker. *Chemistry, The Open University, Milton Keynes, United Kingdom.* Sponsor: R. Roberts.

The latest technologies for detection and measurement of biomarkers of exposure to environmental genotoxicants can be seductive. Ever lower exposures can be detected using increasingly sophisticated analytical tools. 32P postlabelling has revealed many types of DNA damage. Mass spectrometry has been used to characterise a range of modified amino acids in proteins. Accelerator mass spectrometry can quantitate DNA damage with exquisite sensitivity. Does this information improve our ability to make better risk assessments or increase our knowledge about the mechanisms of cancer? The few exposures for which we have extensive data, in both animals and humans, such as aflatoxin B1, suggest that it does. However, there remain some significant challenges: What is the significance of background levels of DNA and protein adducts? Are measurements of average levels of DNA adducts informative or do we need to look for gene-specific DNA damage? These issues will be addressed using exposures to environmental genotoxicants that are of current concern, such as 1, 3-butadiene, acrylamide and benzene.

1548 BIOMARKERS FROM NEW TECHNOLOGIES.

R. Roberts[1], J. Leonard[1], M. Duchesne[2], P. Fabiene[2], M. Courcol[1], C. Saulnier[1] and J. Gautier[1]. *[1]Drug Safety Evaluation, Aventis, Vitry sur Seine, France and [2]Functional Genomics, Aventis, Vitry sur Seine, France.*

During the development of a new product, toxicity may not be seen until after repeat dose toxicity studies in rodents. However, much is being done to integrate toxicology as early as practically possible in order to provide early warning and even to guide chemistry away from potential toxicity. Biomarkers offer great potential to give early indication of potential problems. This is exemplified by the class of drugs known as kinase inhibitors. This class is relevant to several therapeutic areas but

some compounds have, in some cases, been associated with liver and kidney toxicity. The discovery and evaluation of potential biomarkers of this drug-induced toxicity would be invaluable in guiding drug development to avoid this potential issue. In order to detect potential biomarkers, we used a toxicoproteomics (TPX) approach to ask which proteins were altered in rat liver after 14 days of dosing at the toxic but not at the pharmacologically active dose. At the pharmacologically active dose, 52 proteins were up-regulated and none were down-regulated but at the toxic dose of 200mg/kg/day, 88 and 26 were up- and down-regulated, respectively. The bioanalysis of proteomics data showed that many biochemical pathways of lipid, fatty acid and carbohydrate metabolism were affected as well as proteins belonging to the oxidative stress pathways. Interestingly, toxicity was associated with the modulation of pI variants within several protein families. This suggests that these modifications could be used as a toxicity-specific signature of compounds within a chemical class; this approach can be evaluated by correlating toxicity with protein expression using specific antibodies and an isoelectric Western blot technique.

1549 HORMONE REPLACEMENT THERAPY: A CHALLENGE OF RISKS AND BENEFITS.

V. C. Moser[1] and A. R. Scialli[2]. *[1]Neurotoxicology Division, USEPA, Research Triangle Park, NC and [2]Department of Obstetrics and Gynecology, Georgetown University Medical Center, Washington, DC.*

A large epidemiological study of the risks and benefits of estrogen plus progestin hormone therapy was stopped early due to the unanticipated but significantly higher incidence of invasive breast cancer, coronary heart disease, and stroke in postmenopausal women receiving this therapy (JAMA 2002, 288:321-333). The medical community was faced with difficult questions, since estrogen has been so commonly prescribed for relieving menopausal symptoms and preventing bone loss. Questions remain, including whether the results are valid for other pharmaceutical formulations and whether clinicians could have predicted the toxicity of estrogen and progestin based on experimental studies and previous clinical trials. This workshop will present the basic and epidemiological studies of hormone therapy for women and men, as well as the challenge of managing the apparent and real risks and benefits of hormone therapy.

1550 HORMONE THERAPY IN MENOPAUSE: THE CLINICAL CONTEXT.

A. R. Scialli. *Ob-Gyn, Georgetown University Hospital, Washington, DC.*

Estrogen has been prescribed for menopausal women for more than fifty years. Some estrogen prescriptions have been for resolution of vasomotor symptoms; however, there has been substantial prescription of estrogen for prevention or treatment of diseases associated with aging such as heart attack, stroke, dementia, and osteoporosis. The concept that estrogen represented a fountain of youth was adopted by many consumers in response to popular books, advertising, and physician recommendation. The association of estrogen use and increased endometrial cancer risk in the mid 1970s caused a transient decrease in popularity of these preparations, but the addition of a progestin to the prescription was believed to reduce the excess risk to zero. Concerns about breast cancer arose from theoretical considerations, *in vitro* and experimental animal studies, and observational studies in women, but were dismissed as unproven. Randomized clinical trials of estrogen with or without progestin began to appear in the 1990s, demonstrating a lack of effectiveness of menopausal hormone therapy in the prevention or treatment of cardiovascular disease. The largest of these studies, the Women's Health Initiative (WHI) issued its first publication in July 2001. This and subsequent publications from the WHI demonstrate clearly that the estrogen-progestin product most commonly prescribed in the United States does not improve quality of life measures or decrease the incidence of cardiovascular disease or dementia; in fact there is an increase in these diseases associated with hormone therapy. The incidence of breast cancer is increased by this hormone combination and the associated breast cancers are more aggressive. Hip fracture and colon cancer are reduced by the hormone combination, but overall measures of total harm do not favor estrogen-progestin therapy. The WHI publications have not to date addressed estrogen-only regimens, nor did the WHI include formulations other than equine estrogen and medroxyprogesterone acetate; however, data from small studies do not suggest that use of estradiol alone, even without a progestin, provides cardiovascular benefits.

1551 ALTERNATIVES TO HORMONE THERAPY.

A. Fugh-Berman. *Health Care Sciences, George Washington University School of Medicine, Washington DC, DC.* Sponsor: V. Moser.

The use of herbs and dietary supplements has become very popular, especially among women. There are a variety of CAM treatments promoted for hot flashes, depression, prevention of osteoporosis, etc. Large, appropriately designed trials are lacking for many CAM therapies. For hot flashes, clinical trials have not supported a beneficial effect for red clover (Trifolium arvense), evening primrose oil, vitamin E, dong quai (Angelica sinensis) or ginseng. Trials of soy or other dietary phytoestrogens for menopausal symptoms are mixed, and trials on black cohosh are mixed. The herbs and dietary supplements that are most commonly used for hot flashes have not been linked to serious adverse effects. Possible long-term adverse effects will be discussed. So-called natural or bioidentical hormones, including estriol, Tri-Est (estrone, estradiol, and estriol, usually 1:1:8), and natural progesterone cream, are also popular. Although some alternative medicine practitioners have promoted estriol as safe for breast and uterus, recent studies have confirmed that estriol does stimulate endometrial proliferation, and that systemic use increases the risk of endometrial cancer. Additionally, there is no credible evidence for claims that estriol reduces the risk of breast cancer. Clinical trials have shown that topical progesterone cream does not improve bone density and does not provide adequate serum levels to protect the uterus from estrogen-induced stimulation.

1552 RESULTS AND PREDICTABILITY OF ANIMAL STUDIES FOR HUMAN RISK.

A. jordan. *fda, rockville, MD.* Sponsor: G. moser.

To determine if studies in animals were predictive of the outcomes of the Womens Health Initiative study, published literature and information submitted to the FDA were examined. The increased incidence of breast cancer in women was preceded by studies showing an icreased incidence of mammary gland cancer in rats. This carcinogenic response to sex steroids is highly variable between rodent species and strains. Because of the variable response and the availability of what was then considered a better model, the dog, the results from 2 year rodent carcinogenicity studies were largely discounted as being predictive of human risk. The beagle was considered the most predictive model for carcinogenicity due to its apparent ability to differentiate between progestins in their carcinogenic response. Results from studies in beagles led to the removal from the US market of medroxyprogesterone acetate. After a study by the World Health Organization showed no increase in breast cancer in women injected with depot MPA, the FDA eliminated the beagle testing requirement. Ten year studies in monkeys were essentially negative for tumors with some increases in breast cellular proliferation. Studies of coagulation were initiated in animals after the first reports of stroke and thromboembolism in women taking oral contraceptives. The early data were inconsistant but tended to show little if any clear effect of estrogens and progestins on various parameters of blood clotting. More recent studies with the progestin nomegestrol showed no effects on coagulation factors IX and X and antithrombin III in ovariectomized rats or on coronary vascular reactivity in intact and ovariectomized monkeys. It is clear the estrogens and progestins are carcinogenic in some animals at some dose. There is little evidence that safe doses in humans can be extrapolated from results in animals using current testing methods. The data for hormone induced blood coagulation are inconsistent. Newer research paradigms such as genomics and a better understanding of the underlying mechanisms of disease may help increase the relevance of preclinical testing of hormonal steroids.

1553 TESTOSTERONE AND AGING: CLINICAL RESEARCH DIRECTIONS; INSTITUTE OF MEDICINE REPORT ON TESTOSTERONE THERAPY IN OLDER MEN.

E. Vaughan[17], D. Blazer[3], E. Barrett-Connor[2], B. A. Brody[4], R. M. Califf[3], J. P. Costantino[5], D. D. Federman[6], L. P. Fried[7], D. G. Grady[8], W. R. Hazzard[9], S. B. Heymsfield[10], S. W. Lagakos[11], M. S. Litwin[12], C. T. Liverman[1], P. A. Lombardo[13], P. S. Nelson[14], E. S. Orwoll[15] and L. R. Schover[16]. *[1]Committee on Assessing the Need for Clinical Trials of Testosterone Therapy, Institute of Medicine, Washington, DC, [2]University of California, San Diego, San Diego, CA, [3]Duke University, Durham, NC, [4]Baylor College of Medicine, Houston, TX, [5]University of Pittsburgh, Pittsburgh, PA, [6]Harvard Medical School, Boston, MA, [7]Johns Hopkins University, Baltimore, MD, [8]University of California, San Francisco, San Francisco, CA, [9]University of Washington, Seattle, WA, [10]Columbia University College of Physicians and Surgeons, New York, NY, [11]Harvard School of Public Health, Boston, MA, [12]University of California, Los Angeles, Los Angeles, CA, [13]University of Virginia, Charlottesville, VA, [14]Fred Hutchinson Cancer Research Center, Seattle, WA, [15]Oregon Health Sciences University, Portland, OR, [16]M.D. Anderson Cancer Center, Houston, TX and [17]Cornell University, New York, NY.* Sponsor: V. Moser.

In 2002, the National Institute on Aging and the National Cancer Institute asked the Institute of Medicine to conduct a 12-month study to review and assess the current state of knowledge related to the potential beneficial and adverse health effects of testosterone therapy in older men, and to make recommendations regarding clinical trials of testosterone therapy, including the parameters that should be considered in study design and conduct. More specifically, the committee was asked to review and consider the epidemiologic data on normal levels of testosterone during

the lifespan and the associations with morbidity and mortality; the risks and benefits of testosterone therapy; the potential public health impact of testosterone therapy in the United States; and the ethical issues related to the conduct of clinical trials of testosterone therapy. This presentation will provide an overview of the committee's findings and recommendations regarding future clinical trials of testosterone therapy in older men.

1554 STRATEGIES TO IDENTIFY BIOACTIVE SUBSTANCES IN COMPLEX AIR POLLUTANT MIXTURES.

M. C. Madden[1] and J. R. Harkema[2]. *[1]NHEERL\Human Studies Division, USEPA, Chapel Hill, NC and [2]Department National Food Safety and Toxicology, Michigan St. University, East Lansing, MI.*

Both indoor and outdoor air contains a very complex mixture of gas and particulate matter (PM) pollutants. The assessment of the role of each pollutant in the complex atmosphere in the induction of an associated health effect or a response can be difficult due to many factors, including the vast number of pollutants that may potentially induce or modify the health effect. The aim of this session is to present different strategies that have been used by researchers in attempts to identify airborne toxic components in complex mixtures. The range of strategies to be presented spans a wide spectrum of possible approaches (from study of whole populations to predictive toxicology modeling). Findings will be presented on: appropriately designed field epidemiological studies and controlled exposure studies with humans subjects aimed at determining the components of airborne ambient PM that induce increased morbidity and mortality (e.g., premature deaths, hospitalizations, asthma symptomatology); determination of airway irritants in indoor air using rodent exposures; comparative potencies of components of diesel exhaust using controlled *in vitro* cell exposures; and prediction of toxicity using quantitative structure-activities relationships. In each case, individual components of a polluted atmosphere were isolated or considered, and then assessed for potential bioactivity using a variety of methods. In some cases where individual components appeared to be relatively biologically inactive, different individual components were added together to assess potency. This session is timely in that the topics provide insights that may be utilized by researchers to aid in risk assessment and management of complex atmospheres such as combustion emissions, outdoor PM, and indoor air quality, all currently subject to regulation at local to federal levels. [This abstract may not represent official EPA policy.]

1555 THE EPIDEMIOLOGICAL APPROACH TO INVESTIGATING AIR POLLUTION MIXTURES.

J. M. Samet. *Department of Epidemiology, Johns Hopkins University, Baltimore, MD.* Sponsor: M. Madden.

Air pollution comprises a mixture of diverse gaseous and particulate components that vary spatially and temporally. Epidemiologic research, while often focused on estimating the effect of particular pollutants, can also address the effects of mixtures. A means of estimating population exposure to the mixture is needed; for this purpose, multiple components may be measured to profile the mixture or, one or several components may be used as summary indicators. To carry out epidemiologic research on mixtures, populations are needed that experience measurable variation in exposures to the air pollution mixture, across spatial or temporal gradients. Multi-site studies may be most informative. This presentation will consider epidemiological approaches to investigating the health risks of air pollution mixtures in urban environments. Emphasis is given to approaches that may prove informative and to the data resources on exposures and health outcomes that will be needed. Air pollution mixtures have long been a vexing problem for epidemiologists and toxicologists, but new approaches and data resources could lead to further insights.

1556 LUNG RESPONSES IN HEALTHY HUMAN SUBJECTS INHALING COARSE FRACTION PARTICULATE MATTER (PMCF).

N. E. Alexis[1], W. Bennett[3], T. Huang[2] and S. Becker[2]. *[1]Pediatrics, UNC-Chapel Hill, Chapel Hill, NC, [2]Human Studies Division, USEPA, Chapel Hill, NC and [3]Pulmonary Medicine, UNC Chapel, Chapel Hill, NC.* Sponsor: M. Madden.

Based on results from our laboratory, *in vitro* exposures of human lung macrophages to coarse fraction particulate matter (PMcf)appears to induce phagocyte responses to a greater degree than smaller sized PM, and bacterial endotoxin was implicated as the most responsible agent generating this effect. To further assess the role of PMcf and endotoxin in inducing lung toxicity *in vivo*, ambient Chapel Hill (NC) PMcf was collected, sterilized (UV irradiation) and split into two for inhalation directly (naive PMcf) or following heat inactivation of endotoxins by baking at 120oC for 20 hours. Subjects underwent 3 inhalation challenges (filtered air (FA) first, then randomly PMcf-treated or PMcf-untreated)each challenge separated by at least 2 weeks. PMcf was delivered and deposited (0.5 mg)to each subject's airways *via* a closed inhalation system (mouthpiece/pneumotachograph attached to an aspirator). Controlled shallow breathing (500 ml tidal volume at 30 breaths/min) was used to achieve preferential deposition of PMcf to the central airways. Following the FA exposure, subjects also inhaled saline droplets with radiolabeled Tc99m-sulfur colloid (SC) particles (5um MMAD)and then received a 2h gamma camera scan to measure lung dosimetry and mucociliary clearance. Induced sputum was collected at the end of the gamma camera scan to examine particle uptake by airway macrophages. Two hours following each PMcf exposure, induced sputum was collected. Its contents were analyzed for various markers of inflammation, and its cells used for *in vitro* functional assays (phagocytosis, cell-surface phenotype expression, oxidative burst). Other endpoints that were measured 2 hours after PMcf exposure were symptoms and lung function. To date, 10 subjects (N=15) have completed all arms of the study. Data analysis is in progress.

1557 AIRWAY AND PULMONARY EFFECTS OF TERPENE/OZONE REACTION PRODUCTS IN MICE.

A. C. Rohr. *Electric Power Research Institute, Palo Alto, CA.* Sponsor: M. Madden.

Reactions between ozone and unsaturated hydrocarbons (e.g., terpenes) produce gas- and condensed-phase products that may be more irritating than their precursors. Indeed, while neither terpenes nor ozone alone induce airway irritation in mice, when combined the mixture has a marked effect, indicating the formation of irritating substances. Terpenes are emitted from vegetation, and are common volatile organic compounds (VOCs) in consumer/cleaning products. Ozone is present both indoors and outdoors. Some gas-phase products formed through terpene ozonlysis, including methacrolein and formaldehyde, are highly irritating. Ultrafine particles are also formed from these reactions. In mice, the oxidation products of *d*-limonene, α-pinene, and isoprene have been shown to cause airway irritation in mice during 30-minute acute exposures, as evidenced by a reduction in respiratory rate (*f*). Two subsequent studies have shown additional effects. Over a 60-minute exposure, with pre-reaction concentrations of 3.4 ppm (ozone), 47 ppm (pinene), 51 ppm (limonene), and 465 ppm (isoprene), airway irritation was a prominent effect. Airflow limitation also developed, persisting for at least 45 minutes. During repeated exposures (3 hours/day for 4 days) to isoprene oxidation products (OPs), marked enhancement of the irritation and airflow limitation effects was observed, suggesting a cumulative effect. Respiratory frequency decreased from 46.0 ± 2.3% of baseline (Day 1) to 34.2 ± 2.1% (Day 4)($p=0.00002$). Peak expiratory flow normalized for tidal volume (PEF/VT; a measure of airflow limitation) decreased from 75.6 ± 3.9% of baseline (Day 1) to 53.1 ± 3.7% (Day 4)($p<0.00001$). A significant reduction in airway responsiveness was observed, suggesting possible mucous accumulation. These findings have important implications for indoor environments, where terpenes are common and building occupants may be chronically exposed to terpene OPs. The findings are also relevant to outdoor environments, where the reaction products of ozone with biogenic terpene emissions may partition onto existing PM in the atmosphere to contribute to secondary organic aerosol.

1558 AIR-LIQUID INTERFACE CULTURE: TOWARDS MORE PHYSIOLOGICAL *IN VITRO* TOXICOLOGY OF AEROSOLS.

J. Seagrave, J. D. McDonald and J. L. Mauderly. *Lovelace Respiratory Research Institute, Albuquerque, NM.*

Exposure to combustion emissions has been convincingly shown to be associated with adverse health effects. However, the chemical/physical properties of the emissions and toxicological mechanisms responsible for health effects are still unclear. Understanding these processes is important in terms of modifying technology, regulating the emissions, or interventions to protect public health. This has created a need for rapid, inexpensive methods to screen for biological responses to these pollutants. Such methods must accurately model biologically relevant exposure pathways, doses, and responses, as well as the emissions' physical properties and chemical composition. We evaluated air-liquid interface exposure of cultured lung epithelial cells to diesel exhaust as a model of air pollution constituent-induced biological effects. We used two types of diesel engines, with or without modification of the emissions by removal of particles or stripping of semi-volatile organics. One engine was evaluated at two loads and several dilutions of the exhaust. In some cases, the cells were "primed" by pre-treatment with TNFa. We examined the effects of these alterations in exposure conditions on various toxicological endpoints including membrane damage, metabolic inhibition, oxidant stress, and pro-inflammatory potential. Effects were observed at exposure levels close to the physiologi-

cally relevant. However, there were differences in the types and magnitudes of the responses as a function of the exposure parameters. Most conditions tested caused little overt cytotoxicity, but metabolic inhibition and oxidant stress were reasonably sensitive to exposure conditions. Oxidant stress appeared to be a transient effect of exposure, but metabolic inhibition was more sustained. This method has potential applications for assessing the effects of alterations in engine technology as well as for mechanistic studies of the biological responses. Supported by FreedomCAR and Vehicle Technology Program of the US Department of Energy, Office of Energy Efficiency and Renewable Energy.

1559 THE RELATIVE TOXICITY OF SUBSTITUTED PHENOLS REPORTED IN CIGARETTE MAINSTREAM SMOKE.

C. J. Smith[1], T. A. Perfetti[4], M. J. Morton[4], A. Rodgman[4], R. Garg[2], C. D. Selassie[3] and C. Hansch[3]. *[1]Wake Forest University, Winston-Salem, NC, [2]Clarkson University, Potsdam, NY, [3]Pomona College, Claremont, CA and [4]RJRT, Winston-Salem, NC.*

Cigarette mainstream smoke (MS) is a very complex aerosol with more than 4800 identified chemical constituents. MS and its fractions display a number of biological and toxicological activities. Only a minority of MS biological activities are attributable to a single compound, e.g., relative hypoxia with carbon monoxide. Quantitative structure-activity relationship (QSAR) studies can be conducted on structurally similar families of MS constituents. A QSAR study on 253 different substituted phenols reported in MS is shown here as an example of the application of QSAR to a complex mixture. From a laterally validated equation based on published data on the toxic effects of phenols on cultured cells, the relative toxicity, on a molar basis, of the 253 MS phenols has been determined. Based on this scheme, the most toxic phenols in MS include in descending order of toxicity: 2-(dimethylamino)-phenol, 2-ethyl-6-methyl-1, 4-benzenediol, 2-methoxy-1, 4-benzenediol and 4-ethyl-2-methoxy-6-methylphenol. The least toxic phenols include in ascending order of toxicity: 4-hydroxybenzoic acid and 3-hydroxybenzenepropanoic acid. In the human exposure situation, the toxicity of MS phenols is a complex interaction with contributions made by the following factors: toxicity per mole; MS concentration; synergistic, additive or antagonistic interactions with other MS components; host susceptibility; metabolism; and individual smoking behavior and inhalation patterns. QSAR studies of this type can play an important role in identifying MS components for reduction or removal by rank ordering their potential toxicity. A QSAR rank ordering process can help guide future toxicology testing.

1560 NEUROMODULATION OF TELEOST CATECHOLAMINES BY PROPRANOLOL AND FLUOXETINE.

W. Smith[1], L. Blank[1], C. M. Foran[2], D. B. Huggett[3] and B. W. Brooks[4]. *[1]Chemistry and Biochemistry, University of Oklahoma, Norman, OK, [2]Biology, West Virginia University, Morgantown, WV, [3]Pharmacology, University of Mississippi, University, MS and [4]Environmental Studies, Baylor University, Waco, TX.* Sponsor: M. Kanz.

Information on mechanistic toxicity of pharmaceuticals to aquatic vertebrates is insufficient, particularly for neurologically active therapeutics. Recent research has identified fluoxetine and propranolol in municipal effluents and effluent-dominated streams, characterized fluoxetine and propranolol ecotoxicity to model organisms, and assessed fish neuro-endocrine responses after fluoxetine and propranolol exposures. To assess potential neuromodulatory effects of waterborne propranolol exposure on fish, Japanese medaka (Oryzias latipes) were exposed to 0, 0.5, 1, 50 and 100 ug/L for 28 days. A second study with a similar experimental design was performed with medaka exposed to 0, 0.1, 0.5, 1 and 5 ug/L fluoxetine for 28 days. Analysis of whole medaka brains for norepinephrine, epinephrine, dopamine, 3, 4-dihydroxyphenylacetic acid, 5-hydroxytryptamine, and 5-hydroxyindole-3-acetic acid content was performed by liquid chromatography with electrochemical detection. All propranolol treatment levels significantly increased norepinephrine levels in female fish and decreased male dopamine and norepinephrine levels. Similar to previous fluoxetine studies with rat models, an upregulation of dopamine in male fish was observed by 0.1, 1, and 5 ug/L fluoxetine treatment levels. We also detected a decrease of norepinephrine in male medaka at all fluoxetine treatment levels and an increase of 5-hydroxyindole-3-acetic acid at 5 ug/L. These studies provide baseline information for fish neurotransmitter responses to widely prescribed medications. In addition, these data indicate that neuromodulation following aqueous exposure to fluoxetine and propranolol may occur in fishes at concentrations similar to those reported in environmental matrixes.

1561 BEHAVIORAL AND NEUROLOGICAL BIOMARKERS OF STRESS EXPOSURE IN A FISH MODEL.

J. Salierno[1], A. Murphy[2], J. Shields[3] and A. Kane[1]. *[1]Vet. Med., University MD, College Park, MD, [2]Biology, Georgia State University, Atlanta, GA and [3]VIMS, College of William & Mary, Gloucester Pt, VA.* Sponsor: K. Squibb.

Chemical or physical stress exposure can alter behavior and have deleterious consequences for the survival of an organism in the wild. This work developed and tested the utility of behavioral and neurological assays as biomarkers of stress exposure in a killifish model, Fundulus heteroclitus. Alterations in startle behavior and brain activity were evaluated from fish exposed to brevetoxin, domoic acid, and swarming Pfiesteria shumwayae dinoflagellates (harmful algal bloom stressors); MS-222 (an anesthetic); and physical transport stress. Startle responses were quantified using a computer-controlled, video-based behavioral exposure system. Fos expression, visualized using immunocytochemistry, was investigated because it is a biomarker of neural activation in mammals. Fos is the protein product of c-Fos, an immediate-early gene, expressed in stimulated neurons. Startle response behaviors were significantly reduced when fish were exposed to brevetoxin and MS-222. This response was associated with longer reaction times and decreased reaction velocities. Exposure to domoic acid, dinoflagellates, and transport stress increased Fos labeling in the optic lobes of exposed fish brains. A dose response was observed in brains of fish exposed to dinoflagellates, where increases in dinoflagellate density were associated with increased Fos labeling. A six- and two-fold maximal increase in Fos expression was observed in brains of fish exposed to P. shumwayae and transport stress, respectively. Transport stress, dinoflagellates, and to a lesser extent domoic acid, increased neuronal Fos expression. Unlike mammals, Fos expression in fish brains appears to be more sensitive to physical than chemical stress. General alterations in brain activity, as well as knowledge of specific, stress-activated regions within the brain, may provide valuable insights into the neural control of fish behavior and sublethal effects of environmental stressors.

1562 IMMUNOTOXIC EFFECTS OF *IN VIVO* CHLORPYRIFOS EXPOSURE ON MURRAY COD (*MACCULLOCHELLA PEELI*).

P. F. Wright[1], A. J. Harford[1] and K. O'Halloran[2]. *[1]Key Centre for Toxicology, RMIT-University, Melbourne, VIC, Australia and [2]CENTOX, Landcare Research, Lincoln, Canterbury, New Zealand.* Sponsor: M. Karol.

Murray cod is the largest freshwater fish in Australia and an important economic species. Our group has adapted several functional assays to test the immunotoxicity of chemicals that either pollute the Australian Murray-Darling basin, or are relevant to the aquaculture of this species. Organophosphate (OP) chemicals are neurotoxic and may have immunotoxic activity through disruption of the hypothalamo-pituitary-interrenal axis. This study investigated the immunotoxic effects of the OP pesticide chlorpyrifos, 14 days after *in vivo* exposure (single i.p. dose of 0, 0.4, 2, 10 and 50 mg/kg). Immune parameters analysed were: immune organ somatic indices and cell yields, serum lysozyme concentration, phagocytosis (*via* flow cytometry) and lymphoproliferation. Results: there was a dose-related decrease in fish body weight, splenic somatic index, and cell yields from head kidney and spleen. Serum lysozyme concentration was increased by 20% at 50 mg/kg. Lymphoproliferation was initially increased by 15% at 0.4 mg/kg, before being reduced by 17% at 50 mg/kg. Flow cytometry revealed an increase in granulocyte counts, while lymphocytes were marginally decreased. Phagocytic activity (i.e. number of granulocytes with engulfed fluorescent beads) increased by 40% at 2 mg/kg. This study has demonstrated that chlorpyrifos causes a dose-dependant atrophy of the spleen and reduction in spleen and head kidney immune cell numbers. It can also modulate the immune system, causing stimulation of some parameters at lower doses (i.e. phagocytosis and lymphoproliferation) and then suppression of many immune parameters at very high doses (50 mg/kg), where neurotoxicity is also apparent.

1563 USING STRUCTURAL EFFECTS ON THE ORGANIZATION OF THE CYTOSKELETON OF RAINBOW TROUT HEPATOCYTES TO SORT PATHWAYS OF REACTIVE TOXICITY.

K. Flynn, P. K. Schmieder and R. D. Johnson. *USEPA, Duluth, MN.* Sponsor: J. Nichols.

Reactive toxicants have been shown to be more acutely toxic to aquatic organisms than chemicals neither capable of direct interaction with cellular nucleophiles nor potentially metabolized to free radicals. For the development of accurate QSAR models, *in vitro* toxicity assays are being used to distinguish the more toxic arylating chemicals from those that primarily redox cycle. The cytoskeleton has multiple components providing an array of potential targets for direct arylation or oxidative stress induced by redox cycling. Four quinones served as "model" chemicals used to determine if alterations in the organization of the cytoskeleton of rainbow trout

(RBT) hepatocytes could adequately distinguish toxic pathways. Primary cultures of RBT hepatocytes were exposed to 2-methyl-1, 4-naphthoquinone (MNQ), 1, 4-naphthoquinone (NQ), 1, 4-benzoquinone (BQ), or 2, 3-dimethoxynaphthoquinone (DMONQ). At several time points, exposed cells were collected, and labeled *via* immunocytochemistry for confocal imaging of three principle cytoskeletal components: microtubules (MT), intermediate filaments (IF) and microfilaments (MF). The organization of each component was quantified and a toxic "fingerprint" was determined for each quinone. BQ, which primarily arylates, altered MT and MF immediately, and IF after a longer exposure. NQ, considered to primarily arylate but also redox cycle, also affected MT and MF before IF. MNQ altered IF and MT most with little effect on the MF, presumably through mixed reactivity with redox cycling dominating over arylation. DMONQ redox cycles only, and like MNQ, altered IF structure first, followed by effects on MT. These results indicate that trout MT are the most sensitive to arylation, while MF are the least sensitive; with IF most susceptible to redox cycling, but only after much longer exposures. Therefore, a cytoskeletal endpoint assay shows promise in its ability to distinguish pathways of soft electrophile chemical reactivity.

1564 MECHANISMS OF PAH- AND PCB-MEDIATED IMPACTS ON EMBRYONIC DEVELOPMENT IN THE KILLIFISH, *FUNDULUS HETEROCLITUS*.

D. Wassenberg and R. T. Di Giulio. *Integrated Toxicology Program, Duke University, Durham, NC.*

Mechanisms by which polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) perturb development in vertebrates, including fish, are not completely understood. Previous studies have shown that some PAHs and PCBs are agonists for the aryl hydrocarbon receptor (AHR) and induce AHR regulated genes such as cytochrome P4501A (CYP1A), while some PAHs and PCBs inhibit CYP1A enzyme activity. Interactions between CYP1A inducing and inhibiting compounds in environmental mixtures may be important to consider when addressing mechanisms by which real world mixtures impact fish development. In this study, we explored the relationships among CYP1A induction, CYP1A inhibition, and developmental deformities in *Fundulus heteroclitus* embryos. We exposed embryos to aqueous doses of an environmental PAH mixture from a creosote-contaminated sediment, a model PAH-type CYP1A inducer, β-naphthoflavone (BNF), or a model PCB-type CYP1A inducer, PCB126 alone and in combination with the AHR antagonist and CYP1A inhibitor, α-naphthoflavone (ANF). The PAH mixture, BNF and PCB126 all induced CYP1A activity, and co-treatment with ANF dampened this induction. Embryonic deformities were observed in embryos treated with the all three CYP1A inducers. Embryos co-exposed to PCB126 with 100 ug/L ANF were less severely deformed than those exposed to PCB126 alone, suggesting an ANF-mediated rescue of PCB126-induced deformities. However, embryos co-exposed to the PAH mixture and ANF were deformed at concentrations of the mixture two orders of magnitude lower than those exposed to the mixture alone. Similar increases in deformities were observed in BNF and ANF co-treated embryos. The interaction between the PAH-type inducers and ANF on deformities cannot be explained by an additive model of toxicity. Ongoing research is exploring mechanisms behind the differences observed between the effects of co-treatment with ANF on PCB versus PAH-type CYP1A inducers and the impacts environmentally occurring CYP1A inhibitors may have on predicting the embryotoxicity of PAH and PCB mixtures.

1565 DEVELOPMENT OF A QUANTITATIVE VITELLOGENIN ELISA FOR DETECTION OF ENDOCRINE DISRUPTOR EFFECTS IN ZEBRAFISH (*DANIO RERIO*).

F. F. Mikkelsen[1], J. K. Eidem[1], B. M. Nilsen[1] and A. Goksoyr[1, 2]. [1]*Biosense Laboratories AS, Bergen, Norway and* [2]*Department of Molecular Biology, University of Bergen, Bergen, Norway.* Sponsor: E. Dybing.

The yolk protein precursor vitellogenin (Vtg) is normally produced in response to estradiol in mature female fish, but is also produced in juvenile and male fish after exposure to environmental estrogens. During the last decade, Vtg in plasma or whole body homogenate of fish has become an accepted measure of xenoestrogenic potency of chemicals, effluents and discharges. Within international bodies such as the Organization for Economic Cooperation and Development (OECD), work is ongoing to develop screening and testing programmes for endocrine disrupting effects of new chemicals, and in the focus of this development are the small fish test species zebrafish (*Danio rerio*), fathead minnow (*Pimephales promelas*) and Japanese medaka (*Oryzias latipes*). We have developed quantitative ELISAs for detection of vitellogenin in all these species, and here we present results from the development and validation of a zebrafish Vtg ELISA. The zebrafish ELISA was developed using a combination of a monoclonal antibody as a capture antibody and a polyclonal antibody as a detecting antibody in an indirect sandwich assay. Stabilized Vtg purified from zebrafish was used as calibration standard. The zebrafish Vtg ELISA has a working range of 0.5- 63 ng/mL and a minimal detection limit of 0.4 ng/mL. Various sample matrices (plasma, whole body homogenate) were tested and the practical detection limit in whole body homogenate samples was 250 ng/mL. The intra-and inter-assay coefficients of variations were below 20 percent. The assay was evaluated using a set of samples spanning the wide dynamic range of Vtg-levels found in fish exposed to environmental estrogens, and the results showed that the assay distinguished between samples with Vtg concentrations ranging from about 2 µg/mL up to several mg/mL. Work is currently ongoing to increase the sensitivity of the assay. An international inter-lab validation of the assay has been performed.

1566 ACUTE TOXICITY AND BIOACCUMULATION OF TRIBUTYLTIN IN TISSUES OF UROLOPHUS JAMAICENSIS (YELLOW STINGRAY). J DWIVEDI AND L D TROMBETTA. ST. JOHNS UNIVERSITY, NEW YORK, NY.

J. Dwivedi[1] and L. D. Trombetta[2]. [1]*Biological Sciences, St. John's University, New York, NY and* [2]*Pharmaceutical Sciences, St. John's University, New York, NY.*

Tributyltin (TBT) is a widely used biocide with many industrial applications including the manufacture of marine antifouling paint. TBT has been shown to cause imposex in several marine gastropods, immune suppression in marine mammals, and is a known endocrine disruptor. This study is concerned with the effects and bioaccumulation of TBT in tissues of the elasmobranch Urolophus jamaicensis (yellow stingray) following acute exposure to 4 ppb, 2 ppb and .2 ppb. Individuals of a captured population of University.jamaicensis were housed in isolated tanks, and after a minimum of 30 days of acclimation, were exposed to one of the three experimental doses. A fourth group was isolated as a control population. Three hours after exposure, individuals were sacrificed and blood, brain and gill tissue were collected from both control and exposed animals. Electron microscopy was performed on gill tissue to determine any morphological changes in gill epithelium. Graphite furnace AA spectrophotometry was performed on tissue samples to determine the concentration of Sn. Results indicate that University.jamaicensis is hypersensitive to TBT exposure. Elasmobranch gill showed a distorted, swollen epithelium with exfoliation following acute exposure to 4 ppb TBT for 3 hours. GFAAS results indicate that exposed gill tissue and blood contained significantly elevated concentrations of Sn compared to controls. Marine teleosts have been reported to have less than 40% necrosis in gill tissue following 30 day exposure to 4 ppb TBT. The threat of moderate exposure to TBT may be greater to apex predators such as elasmobranchs, due to the effects of bioconcentration of Sn in the food web. Stingrays such as University. jamaicensis may be at still greater risk since they inhabit the sediment where ambient TBT concentrations are reportedly higher.

1567 CORRELATION BETWEEN OBSERVED ACUTE AQUATIC TOXICITY OF A SERIES OF OLEFINIC MATERIALS USING SOLID PHASE MICROEXTRACTION - BIOAVAILABLE PETROLEUM HYDROCARBON.

D. J. Caldwell, E. J. Febbo, D. J. Letinski, C. L. Dzamba, R. F. Blattenberger, D. A. Winkelmann and T. F. Parkerton. *Toxicology and Environmental Sciences Division, ExxonMobil Biomedical Sciences, Inc., Annandale, NJ.*

Bioavailable petroleum hydrocarbon (BPH) refers to the measurement of dissolved, bioavailable hydrocarbons from aqueous samples using solid phase microextraction (SPME) as a lipid surrogate. In the present study, the existing data set on hydrocarbons has been expanded to include olefinic substances. In this study, extracted hydrocarbons/olefins from increasing loading rates of complex chemical substances in water accommodated fractions are analyzed by gas chromatography with flame ionization detection and quantified against a series of hydrocarbon standards. Measured BPH levels can be correlated with exposure concentrations assessed for toxicity potential in guideline studies. As such, BPH is considered a biomimetic extraction technique in which analytical methodologies are employed to estimate the exposure and effects of hydrocarbon mixtures on aquatic organisms. The correlation between BPH and aquatic toxicity is predicated on the fish acute toxicity syndrome of non-polar narcosis, where the sum of individual hydrocarbons exerts a cumulative narcotic effect on an aquatic organism, eventually reaching a critical body concentration and imparting a toxic effect. BPH has previously been demonstrated to be an accurate predictor of the acute aquatic toxicity of hydrocarbon solvents and petroleum mixtures. BPH results compared favorably with observed toxicity and further validates SPME - BPH as a cost effective tool for evaluating toxicity and developing testing strategies for use in the classification of substances for aquatic toxicity. Additionally, it provides a *via*ble alternative to animal testing.

1568 NOTOCHORD DISTORTION BY THIURAM AND OTHER DITHIOCARBAMATE IN ZEBRAFISH EMBRYO.

H. Teraoka[1], S. Urakawa[1], S. Nanba[1], W. Dong[1], T. Imagawa[2], H. Handley[3], J. J. Stegeman[3] and T. Hiraga[1]. *[1]School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan, [2]Faculty of Agriculture, University of Tottori, Tottori, Tottori, Japan and [3]Woods Hole Oceanographic Institution, Woods Hole, MA.*

Dithiocarbamates include major agricultural chemicals, used as fungicide or rejectant for several decades. It has been reported that dithiocarbamates including thiuram caused wavy distortion of notochord in rainbow trout embryos. In the present study, we studied the mechanism of toxicity by thiuram and other dithiocarbamates with zebrafish embryos. When newly hatched embryos were exposed to thiuram (5-20nM) in 3.5cm petri dish from 30% epiboly until 24 hours post fertilization (hpf), wavy notochord, disorganized somite and shortened yolk sac extension with almost the same concentration dependency. Mortality at 24hpf was not increased for up to 1, 000nM thiuram. Body length but not notochord is shortened upon thiuram exposure. Distortion of the notochord began from 18 somite stage, when rhythmic spontaneous trunk contraction occurred. Abolishment of spontaneous contraction by anesthesia with tricaine blocked notochord malformation. While mRNA expression of no tail (zebrafish brachyury) and chondromodulin was not affected, specific expression of collagen type 2a in notochord was not markedly decreased by thiuram treatment. Other dithiocarbamates such as ziram or zisulfiram caused similar malformation to thiuram. These results suggest that thiuram disrupts rigidity of the trunk in zebrafish embryos, followed by notochord distortion upon voluntary trunk contraction. As the concentration of thiuram we used was under the environmental quality standard for environmental water in Japan, urgent laboratory and field investigation is required.

1569 REPROGRAMMING OF THE GENETIC NETWORK FOR CELLULAR DEFENSE AGAINST OXIDATIVE STRESS.

C. N. Giroux, J. Fan and A. Weiss. *Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI.*

The cellular responses to oxidative stress are highly conserved and comprise a dose dependent, graded phenotypic series: adaptation, cell cycle checkpoint delay, apoptosis, and necrosis. We hypothesize that these distinct phenotypes are determined by the gene expression program (i.e. the cellular state) of an underlying and similarly conserved genetic response network. The discrete response states of this network have been visualized by microarray gene expression profiling of hydrogen peroxide stressed yeast cells. Similarity clustering of genes based on their co-responsive behavior over multiple states of stress identifies specific pathways and processes whose gene expression levels are coordinated during the phenotypically complex cellular responses to oxidant challenge. To further characterize these cellular stress responses, we have developed a high-throughput, cell growth based assay system that provides quantitative phenotypic data for modeling of gene-environment interactions in yeast. Using this assay system, we demonstrate that the discrete oxidative stress response states are stable and that they are separated by unstable transitions in which cell populations exhibit increased variability in their quantitative behavior. In the adaptation response to oxidative stress, exposure to a low concentration of hydrogen peroxide results in increased cellular resistance to subsequent challenge by a high concentration or oxidant. This protective response is acquired in less than 1 hour and gradually decays over 24 hours. Establishment of the adapted state requires protein synthesis and the action of the stress-activated YAP1 transcription factor. These observations support our working hypothesis that adaptation is accomplished by a reprogramming of the genetic response network to create a novel, meta-stable protective state. We conclude that the graded series of phenotypic responses to oxidative stress reflects the action of an underlying genetic network whose expression state can be modulated by the interaction of genetic and environmental factors.

1570 DISSECTING ANTIOXIDANT RESPONSE ELEMENT (ARE)-DRIVEN GENE EXPRESSION INDUCED BY TERT-BUTYLHYDROQUINONE: A COMPARATIVE STUDIES USING LONG AND SHORT OLIGONUCLEOTIDE MICROARRAYS.

J. A. Johnson, J. Li and M. L. Spletter. *School of Pharmacy, University of Wisconsin at Madison, Madison, WI.*

At present, there are three popular microarray systems: in situ synthesized short oligonucleotide (oligo) arrays, long oligo arrays, and long cDNA array. Data are lacking on the comparison of the three platforms in the same biological system. In this study, we investigate the gene expression profiles identified by short or long oligo arrays. We have already established a model for upregulation of a cluster of antioxidant responsive element (ARE)-driven genes in human neuroblastoma cell line (isogenic) and neural progenitors (nonisogenic) by treatment with tert-butylhydroquinone (tBHQ) for 24h. Total RNA was generated and accessed using an Agilent Bioanalyzer, and the high-quality RNA was used for cRNA synthesis. Widely used Affymetrix U95AV2 arrays (25 mer) and Agilent Human 1A arrays (60 mer) were chosen to do the comparative study. Based on our stringent screening process (fold change over 2.0, coefficient of variation (CV) below 1.0) on three pair-match comparisons, both platforms showed similar gene expression patterns for upregulation of ARE-driven phase II detoxification enzymes and antioxidant genes in neuroblastoma cells and neural progenitors. RT-PCR confirmed these changes. The Agilent system showed greater dynamic changes in gene expression and lower CV values for those changed genes compared to the Affymetrix system. In terms of the sample preparation and hybridization, however, success with the Agilent system relies more heavily on the experimenter's expertise due to the lack of automation. There was a definite set of overlapping genes in all functional categories. The data generated from both systems, however, also revealed unique gene expression profiles after tBHQ treatment based on the platform used for the analysis. In conclusion, both short- and long- oligo microarray technologies offer reproducible and relatively reliable data, and could become the major competing microarray platforms.

1571 AGE-RELATED IMPAIRMENT OF THE TRANSCRIPTIONAL RESPONSES TO OXIDATIVE STRESS IN THE MOUSE HEART AND SKELETAL MUSCLE.

T. Prolla. *University of wisconsin, Madison, WI.* Sponsor: J. Kramarik.

To investigate the transcriptional response to oxidative stress in the heart and how it changes with age, we examined the cardiac gene expression profiles of young (5-mo-old), middle-aged (15-mo-old), and old (25-mo-old) C57BL/6 mice treated with a single intraperitoneal injection of paraquat (50 mg/kg). Mice were killed at 0, 1, 3, 5, and 7 h after paraquat treatment, and the gene expression profile was obtained with high-density oligonucleotide microarrays. Of 9, 977 genes represented on the microarray, 249 transcripts in the young mice, 298 transcripts in the middle-aged mice, and 256 transcripts in the old mice displayed a significant change in mRNA levels (ANOVA, $P < 0.01$). Among these, a total of 55 transcripts were determined to be paraquat responsive for all age groups. Genes commonly induced in all age groups include those associated with stress, inflammatory, immune, and growth factor responses. Interestingly, only young mice displayed a significant increase in expression of all three isoforms of GADD45, a DNA damage-responsive gene. Additionally, the number of immediate early response genes (IEGs) found to be induced by paraquat was considerably higher in the younger animals. These results demonstrate that, at the transcriptional level, there is an age-related impairment of specific inducible pathways in the response to oxidative stress in the mouse heart. Similar experiments aimed at understanding the transcriptional response to oxidative stress in skeletal muscle are in progress.

1572 ENHANCED EXPRESSION OF MAMMALIAN PROTEASOME THROUGH THE KEAP1-NRF2 SIGNALING PATHWAY.

M. Kwak[1], N. Wakabayashi[1, 2], J. L. Greenlaw[1], M. Yamamoto[2] and T. W. Kensler[1]. *[1]Environmental Health Sciences, Johns Hopkins University, Baltimore, MD and [2]University of Tsukuba, Tsukuba, Japan.*

26S proteasomes can recognize and remove oxidatively damaged proteins, thereby promoting cell survival. Decreased function of the proteasome system in aged subjects is strongly associated with the development of tissue degeneration. Transcription factor Nrf2 is a critical factor in governing the regulation of xenobiotics detoxifying genes. Keap1 inhibits the action of Nrf2 by confining it in the cytoplasm of cells. In the present study, we suggest that the proteasome system is a novel gene category regulated by the Keap1-Nrf2 signaling pathway in mouse. Dithiolethione increased levels of transcripts for 24 genes out of 34 subunits of the 26S proteasome in gene chip analysis using mouse liver. Seventy nine percent of the proteasome subunits increased by dithiolethione were only elevated in wild-type mice but not in *nrf2*-disrupted mice. Protein levels of proteasome subunits PSMA1, PSMB5 and PSMC1 were elevated up to 3-fold and proteolytic activities toward fluorogenic peptides were enhanced in wild-type mice following dithiolethione treatment. No induction was seen in mice where Nrf2 was disrupted. The promoter of *PSMB5* encoding catalytic subunit of the 20S proteasome was activated by dithiolethione-treatment and overexpression of Nrf2. Basal activity of the *PSMB5* promoter was 10 times higher in *keap1*-deficient cells and stimulation of PSMB5 promoter by dithiolethiones was blunted in *nrf2*-deficient cells. Further studies using promoter truncation and mutation showed that tandem AREs located in the proximal promoter of *PSMB5* were necessary for the activation of this promoter in response to inducers and Nrf2. Results from chromatin-immunoprecipitation assays also indicated that these AREs can bind Nrf2 in intact cells. Taken together, we propose that the proteasome system can be enhanced through the Keap1-Nrf2 pathway, implicating possible contribute to the protection/retardation against human degenerative diseases associated with aging.

1573 THE TAK1-TAO1 COMPLEX MEDIATES STRESS-ACTIVATED SIGNALING.

W. HuangFu and J. Ninomiya-Tsuji. *Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.*

Environmental stress stimuli such as osmotic shock and UV irradiation trigger activation of cell signaling to adapt the cells to or resist the stress. Mitogen-activated protein kinases (MAPs) JNK and p38 are major mediators of stress signaling, which are activated through the MAP kinase cascade, a protein kinase cascade composed of MAP3K, MAP2K, and MAPK. A number of MAP3Ks that can activate JNK/p38 have been identified. However, which MAP3K is involved in each stress and how MAP3K is regulated are largely unidentified yet. Here we found that TAK1 MAP3K was activated by osmotic stress. Furthermore, we isolated a kinase TAO1 (thousand and one amino acid protein kinase 1) as a TAK1 binding protein from the yeast two-hybrid screen with TAK1 bait. TAO1 has been reported to have a MAP3K-like activity. TAO1 was also activated by osmotic stress, suggesting that the TAK1-TAO1 complex participated in osmotic stress signaling. It was demonstrated that TAK1 could activate JNK/p38 as well as NF-kB pathway. However, osmotic stress solely activates JNK/p38 but not NF-kB pathway. We found that TAO1 did not activate NF-kB, but rather inhibited TAK1-induced NF-kB activation. These results suggest that TAK1, when activated by osmotic stresses, forms a complex with TAO1 resulting in activation of JNK/p38 but not NF-κB pathway.

1574 USE OF AFFINITY CHROMATOGRAPHY TO IDENTIFY NOVEL PROTEINS THAT MEDIATE STRESS-INDUCED GENE ACTIVATION.

N. Macdonald, D. A. Clynes, J. G. Moggs, I. Kimber and G. Orphanides. *Syngenta Central Toxicology Laboratory, Alderley Park, United Kingdom.*

Exposure of cells to mitogenic or stress stimuli, including a broad range of toxicants, results in the induction of immediate-early (IE) protein kinase cascades and gene activation. The resulting changes in gene expression induce alterations in cell growth, division, differentiation, and metabolism. The mechanisms by which IE cascade activation promotes transcription are yet to be clearly defined, but involve the rapid modification of the histone components of chromatin. In particular, the N-terminal tail of histone H3 is phosphorylated at serine 10 (S10p) and acetylated at lysines 9 and 14 (K9ac and K14ac). However, the way in which these post-translational modifications promote the remodelling of chromatin required for gene activation is unclear. Using affinity chromatography, we have identified human proteins that recognise histone H3 tails containing stress- and mitogen-associated modifications. Synthetic peptides corresponding to unmodified (H3um), diacetylated (H3K9acK14ac), and phospho-diacetylated (H3K9acK14acS10p) histone H3 tails were immobilised onto agarose beads and incubated with HeLa cell nuclear extract. Six proteins bound in a modification-specific manner and were identified by peptide mass spectrometry. RbAp46/48, nucleophosmin, and NAP1, bound preferentially to H3K9acK14ac or H3um. Three proteins with molecular masses of 27.7, 28.3 and 29.1 kDa bound only to the H3K9acK14acS10p peptide, suggesting that they may play a role in mediating the stress and mitogen-induced transcriptional response. Peptide binding competition assays using surface plasmon resonance confirmed that these proteins bind to H3K9acK14acS10p with high affinity and revealed that the S10p modification was the major determinant of binding. Identification of these proteins will shed light on mechanisms by which toxicants induce changes in chromatin structure that facilitate gene activation and the pleotropic stress response.

1575 INHIBITION OF NUCLEAR FACTOR KAPPA B BY PHENOLIC ANTIOXIDANTS: INTERPLAY BETWEEN ANTIOXIDANT SIGNALING AND INFLAMMATORY CYTOKINE EXPRESSION.

Q. Ma and K. Kinneer. *HELD/TMBB, CDC/NIOSH, Morgantown, WV.*

Phenolic antioxidants inhibit the induction of inflammatory cytokines by inflammatory stimuli. Here, we analyzed the mechanism by which the antioxidants inhibit LPS-induced expression of TNFα in macrophages. Hydroquinone (HQ) and t-butyl hydroquinone (tBHQ), prototypes of phenolic antioxidants, block LPS-induced transcription of TNFα and a NF-κB-mediated reporter gene expression, suggesting NF-κB as a target in the inhibition. Analyses of the NF-κB activation pathway revealed that the antioxidants do not inhibit LPS-induced activation of the IκB kinase activity, degradation of IκBα, or translocation of activated NF-κB into the nucleus, but block the formation of NF-κB/DNA binding complexes. *In vitro* experiments showed that the antioxidants do not directly interfere with DNA binding of NF-κB. Structure-activity analyses suggest that inhibition of NF-κB function involves the redox cycling property of the antioxidants. These findings implicate a redox-sensitive factor important for the binding of NF-κB to its DNA recognition sequence as a target molecule in the inhibition of NF-κB function and inflammatory cytokine expression by phenolic antioxidants.

1576 RESTRAINT STRESS ACTIVATES STAT3 VIA ADRENOCEPTOR STIMULATION AND IL-6 PRODUCTION IN MOUSE LIVER.

E. A. Johnson[1], J. P. O'Callaghan[2] and D. B. Miller[1]. *[1]Chronic Stress and Neurotoxicology Laboratory, Toxicology and Molecular Biology Branch, Centers for Disease Control-NIOSH, Morgantown, WV and [2]Molecular Neurotoxicology Laboratory, Toxicology and Molecular Biology Branch, Centers for Disease Control-NIOSH, Morgantown, WV.*

In vivo stress causes an activation of the JAK-STAT signaling pathway as evidenced by the large magnitude increases in phosphorylated STAT3 in the livers of mice subjected to restraint. Restraint can elevate IL-6 levels in the liver and this cytokine is known to instigate signaling through the JAK-STAT pathway but the role of the stress mediators, epinephrine, norepinephrine and corticosterone in this cascade is not well understood. Using a combination of focused microwave irradiation to preserve protein phosphorylation state, and phospho-specific immunoblotting, we found that the greater than 10 fold increase in liver PSTAT3tyr705 caused by 2 hrs of restraint is blocked by both alpha and beta adrenergic antagonists. Adrenergic agonists (phenylephrine 10 mg/kg or isoproterenol 10 mg/kg s.c.) which mimic the stress response *in vivo* also activate liver STAT3. Liver responses to both restraint stress and the adrenergic agonists were reduced in IL-6 deficient animals, suggesting that IL-6 is required for signal transduction initiated by activation of adrenoceptors. Pretreatment of IL-6 deficient mice with exogenous IL-6 (1 mg/kg i.p. 60 min before restraint) restored liver STAT3 activation in response to restraint stress. Hepatocyte growth factor and epidermal growth factor were ruled out as potential mediators of restraint stress-induced activation of STAT3 using real time PCR as only message for IL-6 was elevated. Altogether, these data suggest that restraint stress and adrenergic agonist-induced activation of liver STAT3 are dependent on IL-6 synthesis mediated by activation of adrenoceptors in liver. Stress is known to modulate the liver toxicity of numerous compounds. Stress-induced changes in JAK-STAT signaling and subsequent protein transcription will help to clarify the molecular basis of stress-induced modulation of liver toxicity. This work was supported by intramural funds of CDC-NIOSH

1577 EVALUATION OF THE DERMAL BIOAVAILABILITY OF AQUEOUS XYLENE IN F344 RATS AND HUMAN VOLUNTEERS.

K. D. Thrall and A. D. Woodstock. *Battelle, Pacific Northwest Laboratories, Richland, WA.*

Xylene is a clear, colorless liquid used as a solvent in the printing, rubber, and leather industries and is commonly found in paint thinners, paints, varnishes, and adhesives. Although humans are most likely to be exposed to xylene *via* inhalation, xylene is also found in well and surface water. Therefore, an assessment of the dermal contribution to total xylene uptake is useful for understanding human exposures. To evaluate the significance of these exposures, the dermal absorption of o-xylene was evaluated in F344 male rats and human volunteers using a combination of real-time exhaled breath analysis and physiologically based pharmacokinetic (PBPK) modeling. Animals were exposed to o-xylene at a 0.2 mg/ml aqueous concentration using a 2.5-cm diameter occluded glass patch attached to a clipper-shaved area on the back of the rat. Immediately following the initiation of exposure, individual animals were placed in a glass off-gassing chamber and exhaled breath was monitored. Human volunteers participating in the study placed both legs into a stainless steel hydrotherapy tub containing an initial concentration of approximately 0.5 mg/L o-xylene. Exhaled breath was continually analyzed from each volunteer before, during, and after exposure to track absorption and subsequent elimination of the compound in real time. In both animal and human studies, a PBPK model was used to estimate the dermal permeability coefficient (Kp) to describe each set of exhaled breath data. Rat skin was found to be approximately 12 times more permeable to aqueous o-xylene than human skin. The estimated human and rat aqueous o-xylene Kp values were 0.005 +/- 0.001 cm/hr and 0.058 +/- 0.009 cm/hr, respectively. (Supported by NIEHS grant 1-P42-ES10338-01).

1578 A NEW QSAR MODEL FOR HUMAN SKIN ABSORPTION.

W. Luo[1], H. Nguyen[1], Q. Telesford[2] and W. Fung[1]. *[1]Toxicology, L'Oreal USA, Clark, NJ and [2]Bioengineering, Rutgers University, New Brunswick, NJ.*

The objective of this study was to create a model for the quantitative prediction of percutaneous absorption based on physicochemical properties of chemicals. Such a model is valuable as a tool for screening and prioritization purposes for safety evaluation and risk assessment. It is very beneficial to the industry to be able to predict the skin permeability of compounds before they are made or on occasions when no experimental values exist. A five-parameter QSAR model to predict the permeability coefficient Kp, was completed using a training set of 270 chemicals and their

physico-chemical descriptors. All Kp values were experimentally determined and obtained from literature sources, while the values for the selected molecular descriptors were experimentally measurable and can be calculated readily. The analyzed descriptors included calculated octanol-water partition coefficient (Kow), octanol-water partition coefficient of all species (unionized and ionized) at pH 5 and 7.4 (D), molecular weight (MW), molecular volume, polar surface area, water solubility (Sw), topological polar surface area, melting point (m.p.) and hydrogen donors/acceptors. Using the CAChe program's (Fujitsu America Inc., Portland, OR) stepwise regression analysis, five out of the ten molecular descriptors in the data set were determined to have significant statistical correlation: log P, log DpH 7.4, molecular weight (MW), solubility (SW), and Melting Point (m.p.). LogKp = -0.005530X(MW) + 0.6205X(log Kow) + 3.030e-07X(Sw) + 0.08239X(log DpH7.4) - 0.001483*(m.p.) - 2.759 In conclusion, an algorithm to estimate human skin absorption is derived based on physical properties of over three hundred compounds. Compared to other skin absorption QSAR models in the literature, our model has incorporated more physical properties like Sw, log DpH7.4 and m.p. in addition to the commonly used MW and log Kow with good correlation coefficient. An independent test data set will be generated to test the reliability of this model.

1579 DERMAL TOXICITY OF SUPER VASMOL 33 WITH AYURPRASH, SUPER VASMOL 33, COMBINED EXTRACT AND TWO SOLUTION DYE SAMPLES.

S. L. Bodhankar, S. Sankaran, K. R. Khandelwal, S. N. Shah and N. A. Mhetre. *Pharmacology, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, India.* Sponsor: H. Mehendale.

Hair dye containing hydrogen peroxide and para-phenylenediamine is popularly used for cosmetic purpose. Ayurprash, a combination of Embilica officinalis, Trigonella foenum graecum, Eclipta alba, Hibiscus rosasinensis in definite proportion imparts glow and smoothens hair. Toxicity reports of Ayurprash in combination with hair dye/hydrogen peroxide are not available. The objective was to compare and investigate acute dermal toxicity of the following formulations in rabbits 1) Super Vasmol 33 with Ayurprash (SVA), 2) Super Vasmol 33 without Ayurprash (SVWA), 3) Combined extract (CE) of the plant material used as Ayurprash. Two marketed hair dye samples containing hydrogen peroxide viz., 4) Sample A(SA) and 5)Sample B(SB) were also used for the study.New Zealand white rabbits of either sex weighing 1.54 to 2.0 kg with its back shaved in 4 round patches of equal diameter, 2 on each side were used. After application of 1ml/kg of formulations to the shaved patches on the dorsal side of rabbits hair growth and color, skin color, its texture and toxicity (inflammation) were noted weekly for 10 weeks. SA group showed slight rough texture of skin till 10th week. SVA, CE groups showed slow hair growth on days 7 and 14 and rapid hair growth subsequently. SVWA, Control, SA, SB groups showed no hair growth upto day 21. SA showed roughness of skin and SB showed deposition of black dye leading to darkening of skin. SVA, SVWA, SA and SB showed brownish black color and CE and control groups showed white hair colour. Hydrogen peroxide formulations showed more dermal toxicity than SVA. These studies indicate that SVA is safer than hair dyes containing hydrogen peroxide. Ayurprash was beneficial for rapid hair growth and is non toxic to skin.

1580 THE PIG AS AN EXPERIMENTAL MODEL IN WOUND HEALING RESEARCH.

P. Glerup, M. Skydsgaard, J. T. Jensen and S. Klastrup. *General toxicology and pharmacology, Scantox A/S, Lille Skendsved, Denmark.* Sponsor: R. Harling.

The use of pigs in studies of wound repair is known to provide results of high reproducibility and reliability for the efficacy and safety evaluation of new pharmaceuticals or medical device products. The skin of pigs has been shown to be anatomically, biochemically and immonulogically similar to human skin. The poster gives an overview of some methods and practical procedures, in the context of the investigations currently performed at Scantox. They are based on extensive experience with pigs and minipigs as animal models. Studies are performed using Gottingen minipigs or Landrace pigs. The wounds, excisional split-thickness or full-thickness wounds, are surgically prepared under general anaesthesia. A number of variations in the nature of wound models are possible, for example wound shape and size, eschar induction (for evaluation of debriding agents) or determination of infectious burden. Dressings can be changed on a daily basis until complete healing. During the recovery phase wounds are evaluated macroscopically in detail, including planimetric measurement of wound areas. In addition, bacteria in the wound site can be determined, or blood samples may be taken in order to assess systemic exposure to test article(s). After harvesting, histopathological analysis of the wound tissues is performed, including special stainings to estimate angiogenesis and collagen formation. The generation of collagen may also be evaluated indirectly by HPLC assay of hydroxyproline concentration. A full necropsy evaluating possible adverse systemic effects of test compound may be performed. Data from wound observations and planimetric measurements will be presented in the poster as well as histopathological results.

1581 SIMULATED SOLAR UV LIGHT (SSL) INDUCES INFLAMMATION AND OXIDATIVE STRESS IN THE SKIN OF SKH-1 HAIRLESS MICE.

A. R. Murray[1], E. Kisin[2], V. Castranova[1, 2], B. J. Miller[3], P. C. Howard[3] and A. A. Shvedova[1, 2]. *[1]Physiology and Pharmacology, West Virginia University, Morgantown, WV, [2]PPRB, NIOSH, Morgantown, WV and [3]NCTR, USFDA, Jefferson, AR.*

The skin is continuously exposed to a variety of hazardous environmental insults, such as ultraviolet light, ozone, and ionizing radiation. These exposures result in the production of free radicals and reactive oxygen species, which may play a role in the development of inflammatory skin disorders, skin cancer, cutaneous autoimmune disorders, phototoxicity, and premature ageing. A variety of antioxidant defense mechanisms are present to prevent oxidative stress; however, UV irradiation has been shown to overwhelm these mechanisms. We hypothesized that UV-light exposure results in the formation of free radicals, which causes antioxidant depletion, lipid peroxidation and DNA damage. To experimentally evaluate the effects of simulated solar light (SSL) on SKH-1 hairless mice, animals were exposed to SSL (13.7 mJ (CIE)/cm^2) for 1 hour, 5 days a week for 3 weeks. Twenty-four hours following the last exposure, mice were sacrificed. The skin was evaluated for changes in several parameters of oxidative stress. A significant amount of GSH oxidation as well as decreases in the levels of protein thiols, total antioxidant reserves, and vitamin E were seen as a result of exposure to SSL. A significant increase in lipid peroxidation and myeloperoxidase activity (MPO), indicating infiltration of neutrophils into the skin, was observed following SSL irradiation. The production of pyrimidine dimers seen after exposure is indicative of DNA damage occurring as a result of the direct action of SSL on DNA. Histological evaluation of the skin following exposure to SSL irradiation showed increased skin thickness and inflammatory cell infiltration. These data indicate that SSL exposure induces the production of pyrimidine dimers and free radicals in the skin, which result in the development of oxidative stress and inflammatory cell infiltration.

1582 MOLECULAR CHANGES IN RAT SKIN RELATED TO IRRITATION BY JP-8 JET FUEL.

J. N. McDougal, C. M. Garrett and C. M. Amato. *Pharmacology/Toxicology, Wright State University, Dayton, OH.*

Dermal irritation due to occupational exposure of Air Force personnel to jet fuels can be an important occupational health issue. Irritant contact dermatitis from a wide variety of chemicals is the most common occupational skin disease. It costs the US government millions of dollars each year. Having a mechanistic understanding of the early process of skin irritation provides the potential for prophylactic and therapeutic intervention. We have previously studied the changes in gene and protein expression in rat skin for up to four hours after one-hour cutaneous exposures to JP-8. These studies in male, Fisher 344 rats address molecular changes in the skin induced by longer *in vivo* exposures; 4, 8 and 24 hours. Five hundred microliters of JP-8 was placed in a modified Hill Top Chamber and held in place on the clipped back with a modified Lomir rodent jacket. Sham-treated controls with an empty chamber to control for the effect of hydration were used for comparisons at each time point. At the end of the exposures, skin was excised, flash frozen and pulverized for the isolation of total RNA and proteins. Affymetrix gene array (RG-U34A) was used to determine changes in gene expression. Real time PCR was used to quantify changes in mRNA levels and sandwich enzyme linked immunosorbent assay (ELISA) was used to quantify changes in specific protein levels. Gene array studies showed that the JP-8 treatment caused up regulation of genes involved in the process of inflammation and protein synthesis. Genes down regulated were related to metabolism and support functions of the skin. Real time PCR confirmed up and down regulation in some but not all genes investigated. ELISA measurements of some of the inflammatory and regulatory proteins related to the gene changes showed that protein levels in whole skin are affected by the JP-8 treatment especially at later time points. The results of this study increase our understanding of the JP-8-induced inflammatory process and may allow us to build quantitative mathematical models of the irritant process. (supported by the Air Force Office of Scientific research)

1583 ASSESSMENT OF SKIN BARRIER CREAMS TO LOWER PENETRATION OF JP-8 JET FUEL.

J. J. Schlager[1], D. L. Pollard[2] and A. J. Guilfoil[1]. *[1]AFRL/HEST, Wright-Patterson AFB, OH and [2]Mantech Enviromental Technology, Inc., Wright-Patterson AFB, OH.*

Skin irritation as well as skin and systemic toxicity from accidental JP-8 jet fuel exposure is a concern for the USAF and their fuel handling personnel. Personal protection equipment and clothing (PPEC) is a necessity under normal operations; but

requirements to meet workload goals can lengthen individual exposure causing PPEC failure under non-life threatening JP-8 discharge or accident. To insure work conditions are amended to produce the lowest JP-8 exposure, we proposed to add a skin-enhancement barrier cream to the PPEC armament. Initial work was to select an over-the-counter product that claims to have skin enhancement and/or barrier properties, which could be quickly fielded. For studies to assess penetration lowering performance of 13 products to JP-8, we applied either 0.1 mm (neat) or 0.22 mm (cotton gauze supported) barrier formulation on a 10 cm^2 silastic membrane, allowed a 1 hr drying time, and placed the coated membrane into a static penetration cell containing 2 ml of JP-8 in the upper chamber over the barrier. JP-8 component membrane penetration was isolated with 20 ml VOLPO-saline in the lower receiving chamber over a 4 hr period. Samples (20 µl) were withdrawn from the lower chamber sidearm and capped into 20 ml headspace sample *via*ls for gas chromatography analysis. Samples were heated (140°C) to stable vapor phase using a headspace sampler and components separated on a non-polar SPB-1 column with FID detection. Total area of eluted hydrocarbon vapor from the sample was compared between the coated and non-coated membrane penetration. Generally, products tested had either 1) no to little barrier properties, 2) initial barrier properties but latent breakthrough rates often similar to the non-coated surface or 3) a constant lower penetration rate through the 4 hr test. The most successful barrier to JP-8 was SERPACWA cream having breakthrough rates of 3.4 to 8% of control depending on coating thickness. The results show that not all creams promoting non-polar barrier qualities would create a sufficient barrier for JP-8 penetration.

1584 A NEW TECHNIQUE TO ASSESS DERMAL ABSORPTION OF CHEMICAL VAPORS *IN VITRO* BY THERMAL GRAVIMETRIC ANALYSIS.

T. S. Isaksson and G. Johanson. *Work Environment Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.*

There is a lack of quantitative dermal absorption data for industrial chemicals. When available, absorption rates can vary up to 5 orders of magnitude for the same chemical. This indicates a need for a standardized approach. The aim of this study was to explore the possibility to use a gravimetric *in vitro* method, to measure dermal absorption of solvent vapors. Method: Epidermal membranes from stillborn porcine were placed in a Thermal Gravimetric Analysis (TGA) balance (TA Instruments, USA) under constant air flow (90 ml min^{-1}), temperature (35°C) and humidity (RH 45%). After equilibration, a constant level of chemical vapor (corresponding to 45% saturation) was added to the air flow. Results: The weight changes during exposure of vapors (water, methanol, toluene, ethanol, dimethylformamide, trimethyl benzene, isopropyl alcohol, gamma-butyrolactone and n-hexane) were readily recorded. Upon addition of vapor, as expected, the skin weight initially increased rapidly and approached steady-state. A reverse pattern was seen following removal of chemical vapor. The gravimetric method seems to be a promising approach for dermal absorption studies. The method can easily be automated and only small amount of skin is required. However, the method cannot be used with chemicals with low vapor pressure. Future directions: Studies of additional chemicals, development of a model that translates the weight curves to absorption rates, and validation against a standardized method for dermal absorption, using diffusion cells. This study was supported by the Swedish Council for Working Life and Social Research (FAS).

1585 INFLUENCE OF CUTTING FLUID CONTAMINANTS ON THE DERMAL DISPOSITION OF THE BIOCIDE, TRIAZINE.

R. E. Baynes, J. D. Brooks, B. Beth, R. Wilkes and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Cutting fluids can become contaminated with metals (e.g., nickel, Ni) and nitrosamines (e.g., N-nitrosodiethanolamine, NDELA), and there is concern that these classes of contaminants will modulate dermal disposition and ultimately the toxicity of cutting fluid additives such as biocides (e.g., triazine, TRI). Biocides are added to these formulations to prevent bacterial degradation of commercial cutting fluids. The purpose of this study was to assess the dermal absorption and deposition of C14-TRI when topically applied to porcine skin in an *in vitro* flow-through diffusion cell system as mineral oil (MO) or polyethylene glycol (PEG) mixtures. C14-TRI mixtures were formulated with NDELA and/or Ni or with 3 other cutting fluid additives (5% linear alkylbenzene sulfonate, 5% triethanolamine, and 5% sulfurized ricinoleic acid) and one or both of these contaminants. C14-TRI absorption ranged from 2.72 - 3.29% dose in MO and 2.29 - 2.88% dose in PEG with significantly greater TRI absorption in MO than PEG when all additives and contaminants were present. TRI permeability was consistently and significantly greater in MO than in PEG when NDELA was present. Ni appears to have little or no effect on TRI absorption, although the trends suggest a possible negative effect on triazine permeability and deposition in skin. These observations suggest that metal-machining workers should not only be concerned about dermal toxicity of these contaminants, but they may also enhance dermal disposition of cutting fluid additives. (Supported by NIOSH Grant R01-OH-03669)

1586 A COMPARATIVE INVESTIGATION OF THE EFFECTS OF WATER, ETHANOL AND WATER/ETHANOL MIXTURES ON CHEMICAL PARTITIONING INTO PORCINE STRATUM CORNEUM AND PERMEABILITY IN PORCINE SKIN.

D. van der Merwe and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

The effects of vehicles on transdermal skin absorption is a critical factor in determining risk from skin exposure to toxins and for assessing the transdermal route for drug delivery. Water and ethanol are commonly used solvents in the chemical and pharmaceutical industries. 0%, 50% and 100% aqueous ethanol solutions were used as solvents for radio labeled phenol, 4-nitrophenol, pentachlorophenol, nonylphenol, methyl parathion, ethyl parathion, chlorpyrifos, fenthion, triazine, atrazine, simazine and propazine. The porcine stratum corneum/solvent partitioning coefficients of these compounds were estimated. Permeability in porcine skin of these compounds in the same solutions was estimated using flow-through diffusion cells. Partitioning into the stratum corneum was highest when water was used as a solvent across all compounds. Partitioning from 50% ethanol was higher than from 100% ethanol, except for ethyl parathion, atrazine and propazine. Stratum corneum partitioning was significantly correlated with octanol/water partitioning. Divergence between octanol/water partitioning and stratum corneum/solvent partitioning became wider in 50% and 100% ethanol as the octanol/water partitioning coefficient increased. This divergence was more marked in 100% ethanol than in 50% ethanol. Correlation also existed between molecular weight and stratum corneum partitioning, but with a lower significance. Stratum corneum partitioning was not correlated with porcine skin permeability. It was concluded that chemical partitioning into the stratum corneum from water and aqueous ethanol solutions is dependent on differences in thermodynamic activity between the solvent and stratum corneum. Among the compounds tested, stratum corneum partitioning can be predicted using the compound's physico-chemical properties, but stratum corneum partitioning does not predict skin permeability.

1587 INVESTIGATION OF THE SENSITIZATION POTENTIAL OF VARIOUS ESSENTIAL OILS IN THE LOCAL LYMPH NODE ASSAY (LLNA).

J. Lalko and A. Api. *Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ.*

Essential oils are commonly used fragrance ingredients. The oils themselves are complex mixtures, which may contain varying amounts of naturally occurring contact sensitizers. As part of a dermal sensitization risk assessment, the local lymph node assay (LLNA) was used to evaluate the sensitization potential of 7 essential oils and 3 of their major components. The essential oils tested were clove leaf oil, citronella oil, geranium oil, basil oil, lemon grass oil, litsea cubeba and palmarosa oil. The components of each essential oil were characterized by GC/MS. The individual components tested were eugenol, citral and geraniol. The LLNAs were conducted according to OECD guideline 429. Each fragrance material was tested at five dose levels ranging from 2.5 to 50% w/v in 1:3 ethanol:diethyl phthalate. The Stimulation Index (SI) values were calculated for each dose level, an SI of 3 or more was considered to give a positive response. Linear interpolation of the dose response data from each LLNA was then used to derive an estimated concentration (EC3) required to elicit an SI value of 3. The EC3 value was then taken as a measure of relative potency. Eugenol, geraniol and citral were observed to have SI values greater than 3 and, as such, were considered to be potential sensitizers under the conditions of the test. The EC3 values were calculated to be 5.4%, 6.3% and 11.4%, respectively. Based on EC3 values, the potency of each of these three materials can be classified as weakly sensitizing. Positive responses were observed with lemon grass, litsea cubeba, palmarosa and basil oil. The respective EC3 values and subsequent potency classification for each material are 6.5% (weak), 8.4% (weak), 9.6% (weak), 7.1% (weak) and <2.5% (<=moderate). Citronella and geranium oil were not observed to elicit a positive response. However, a mild dose response and SI values nearing three at the highest dose indicate that a positive response and subsequent EC3 value could be expected at doses > 50% classifying each material as extremely weak to non-sensitizing.

1588 TOPICAL HYDROCARBON ABSORPTION IN PORCINE SKIN PREVIOUSLY EXPOSED TO JP-8 JET FUEL.

F. Muhammad, N. A. Monteiro-Riviere and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

The percutaneous absorption of topically applied jet fuel hydrocarbons (HC) through skin previously exposed to JP-8 has not been investigated, although this exposure scenario is the occupational norm. Pigs were exposed to JP-8 soaked cotton fabrics for 1 and 4 days with repeated daily exposures. Pre-treated and untreated skin was then dermatomed and placed in in-vitro diffusion cells. Five cells with exposed skin and four cells with unexposed skin were dosed with a mixture of 14 different HC consisting of nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, ethyl benzene, o-xylene, trimethyl benzene (TMB), cyclohexyl benzene (CHB), naphthalene, and dimethyl naphthalene (DMN) in water + ethanol (50:50) as diluent. Another five cells containing JP-8 exposed skin were dosed with diluent only in order to determine the residue potential of jet fuel HC in skin. The data indicated that there was 2-3-fold and 3-4-fold increase in absorption of nonane, undecane, dodecane and tridecane through 1 and 4 days JP-8 pre-exposed skin respectively. Similarly, ethyl benzene, o-xylene and TMB were absorbed 2-3 times more than in controls in 1 day while > 4 times in 4 days JP-8 pre-exposed skin experiments. The absorption of naphthalene and DMN was 1.5 times higher than controls in both 1 and 4 days pre-exposures. The CHB, naphthalene and DMN were found to have significant persistent skin residues capable of considerable further absorption in 4 days pre-exposures as compared to 1-day exposures. The absorption parameters of flux, diffusivity and permeability were calculated for the studied HC. The possible mechanism of increased HC absorption in fuel pre-exposed skin may be *via* lipid extraction from stratum corneum as indicated by electron microscopy. This study suggests that single dose application data for jet fuel HC cannot be used to predict the toxic potential for repeated exposures and that for certain compounds, persistent absorption may occur days post-exposure. Supported by USAFOSR F49620-01-1-0080.

1589 A MURINE MODEL FOR CUTANEOUS PHOTOAGING: OBSERVATIONAL, HISTOPATHOLOGIC AND MOLECULAR ENDPOINTS.

D. B. Learn[1], C. P. Sambuco[1], P. D. Forbes[1], M. J. Mayo[2], C. S. Johnson[2] and A. M. Hoberman[1]. *[1]Charles River Discovery and Development Services, Argus Division, Horsham, PA and [2]Pathology Associates, West Chester, OH.*

Photoaging connotes changes in aging skin chronically exposed to sunlight. Clinically, the changes involve skin texture and appearance (wrinkling, sagging, discoloration), microarchitecture (e.g., epidermal thickness, elastin and collagen fiber content and arrangement, cell type and number,) and functional capacity (e.g., repair). Identifying compounds that affect (e.g., increase, halt or reduce) these deleterious responses to solar exposure begins with employing a reliable experimental model to define those responses. The (HRS/Skh-1) hairless albino mouse undergoes photoaging changes when chronically exposed to UVR. Groups of female hairless mice were exposed to 0, 3, 3.75, 4.5, 5.25 or 6 minimal erythema doses (MED) of solar-simulated, environmentally relevant UVR per week, for 4 or 8 weeks. Clinical observations were performed weekly and skinfold thickness (SFT) measured at weeks 0, 2, 4, 6 and 8 weeks. Skin samples harvested at 4 or 8 weeks were frozen or formalin-fixed, sectioned, stained and evaluated histopathologically using visual and image analysis techniques. Mean SFT increased in a UVR dose-dependent manner up to 5.25 MED/week. Incidence and severity of erythema, edema, flaking, thickening (assessed visually) and wrinkling increased in a UVR dose-dependent manner up to 4.5 MED/week. Mast cell density (MSD) and average epidermal thickness (AET) was greatest at 4 weeks at UVR exposures of 5.25 MED and 6.0 MED, respectively. Elastin content (EC) was increased at 4 weeks but the increase was not UVR dose-dependent. MSD, AET and EC was reduced at 8 weeks for all groups, as compared with 4 weeks. The hairless mouse along with the use of environmentally relevant UVR is a predictable and repeatable photoaging model for the evaluation of both safety (i.e., enhancement) and efficacy (i.e., prevention and/or amelioration) by novel compounds using these observational and histopathological endpoints.

1590 DIFFERENTIAL REGULATION OF COX-2 EXPRESSION BY ULTRAVIOLET LIGHT IN KERATINOCYTES AND MACROPHAGES.

A. T. Black[1], A. M. Vetrano[1], R. Sur[1], D. E. Heck[1] and J. D. Laskin[2]. *[1]Rutgers University, Piscataway, NJ and [2]Environmental and Community Medicine, UMDNJ-Robert W Johnson Medical School, Piscataway, NJ.*

Chronic exposure to sunlight is a significant causative factor in the development of skin cancer. Modifications of DNA and other critical cellular macromolecules by the higher energy shorter solar wavelengths comprising the UVB spectra (290 to 320 nm) are the most damaging to the skin. Recent studies have demonstrated a link between expression of cyclooxygenase-2 (COX-2), formation of prostaglandins and the development of skin cancer. UVB is also known to induce COX-2 in human skin cells. In the present studies, we compared the effects of UVB on COX-2 expression in JB-6 and PAM 212 cells, two murine keratinocyte cell lines, and RAW264.7, a murine macrophage cell line. In macrophages, bacterially derived lipopolysaccharide (LPS, 1 μg/ml) was found to rapidly induce COX-2 protein expression as determined by western blotting. The keratinocyte cell lines constitutively expressed COX-2. LPS caused a 3-4 fold increase in expression of this protein. Whereas in PAM 212 cells, UVB light (10-25 mJ/cm^2) caused a 3-4 fold increase in COX-2 expression, it had no effect on expression of the protein in JB6 keratinocytes or RAW264.7 macrophages. In PAM 212 cells, the combination of UVB light and LPS did not further increase COX-2 expression while in JB6 cells, UVB light had no effect on LPS-induced COX-2 expression. In contrast, in macrophages, UVB light was found to suppress LPS-induced expression of COX-2. In macrophages, UVB light-induced the JNK and p38 MAP kinases. Inhibition of these kinases with JNK inhibitor II (1, 9-pyrazoloanthrone) or the p38 kinase inhibitor SB-203580 was found to reverse the inhibitory effects of UVB light on LPS-induced expression of COX-2. Taken together these data indicate that the effects of UVB light on COX-2 expression is dependent on cell type. In macrophages, MAP kinases appear to mediate the inhibitory effects of UVB light on LPS-induced COX-2 expression. Supported by NIH grants CA100994, ES005022 and ES006897.

1591 EPIDERMAL CYTOKINE SECRETION INDUCED BY CHEMICAL CONTACT AND RESPIRATORY ALLERGENS.

M. Cumberbatch, R. J. Dearman and I. Kimber. *Syngenta CTL, Macclesfield, United Kingdom.*

Repeated topical exposure of BALB/c strain mice to chemical contact and respiratory allergens results in preferential T helper (Th)1- and Th2-cell activation, respectively. In addition, it has been shown that respiratory allergens, such as trimellitic anhydride (TMA), stimulate epidermal Langerhans cell (LC) migration with delayed kinetics compared with contact allergens, such as 2, 4-dinitrochlorobenzene (DNCB). Experiments using anti-interleukin (IL)-10 antibodies *in vivo* suggest that cutaneous IL-10 may contribute to the differential regulation of LC migration by these chemicals. To investigate further the mechanistic basis for the development of polarised immune responses, we have examined the production of epidermal cytokines provoked following a single topical application to BALB/c strain mice of DNCB (1%), TMA (25%) or vehicle (acetone:olive oil, 4:1; AOO). Skin explants were excised from mice exposed on the dorsum of both ears for various periods (30min-6hr) to chemical and were cultured on medium prior to analysis of supernatants for the presence of tumour necrosis factor α (TNF-α), IL-1β, IL-1α, IL-6, IL-10, IL-12p40, IL-12p70 and IL-17 using the Bio-PlexTM cytokine array system. Enhanced production of IL-1β, a cytokine involved in the initiation of LC migration, was detected only following exposure to DNCB, with 15-fold increases induced by 6hr of exposure. In addition, only exposure to DNCB was associated with early (2hr) secretion of IL-17. In contrast, up-regulation of IL-10, a cytokine that inhibits LC mobilization, was evident only for TMA during the first 3hr of exposure, with 2 to 3-fold increases in IL-10 release being induced. Small increases in IL-1α levels were apparent for both chemicals. No alterations in either IL-6, IL-12p40 or IL-12p70 secretion were recorded and TNF-α remained undetectable throughout. These data suggest that discrete epidermal cytokine secretion profiles induced following exposure to chemical contact and respiratory allergens might contribute to the polarisation of immune responses, possibly through effects on LC function.

1592 *IN VIVO* AND *IN VITRO* DERMATOTOXICITY OF CUTTING FLUID MIXTURES.

N. A. Monteiro-Riviere, A. Inman, B. Barlow and R. E. Baynes. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Cutting fluids are widely used in the metal-machining industry to lubricate and reduce heat generation when metals are cut by a metal-cutting tool. Unfortunately, these cutting fluids have caused occupational contact irritant dermatitis (OCID), and many of the additives used in these cutting fluid mixtures are thought to be responsible for OCID in workers. The purpose of this study was to assess single or various combinations of these additives in initiating the OCID response following an acute 8 hr exposure in porcine skin *in vivo* and *in vitro* using the isolated perfused porcine skin flap (IPPSF). Pigs (n=4) were exposed to 5% mineral oil (MO) or 5% polyethylene glycol (PEG) aqueous mixtures containing various combinations of 2% triazine (TRI), 5% triethanolamine (TEA), 5% linear alkylbenzene sulfonate (LAS), or 5% sulfurized ricinoleic acid (SRA). Erythema and edema were evaluated and skin biopsies for histology were obtained at 4 and 8 hrs. IPPSF's

(n=4) were exposed to control MO or PEG mixtures and complete MO or PEG mixtures, and perfusate samples were collected hourly to determine IL-8 release. The only significant mixture effects were SRA+MO+LAS+TRI+TEA causing an increase in IL-8 release. Exposure to TRI alone appeared to increase erythema, edema, and dermal inflammation compared to the other additives, while SRA alone was least likely to initiate a dermal inflammatory response. In 2-component mixture exposures, the presence of TRI appeared to increase the dermal inflammatory response at 4 and 8 hrs especially with the PEG mixtures. In the 3- and 4-component mixtures, MO mixtures are more likely to incite an inflammatory response than PEG mixtures. In summary, these preliminary studies suggest that the biocide, TRI, is the more potent of the 4 performance additives in causing dermal irritation, and this may vary depending on whether the worker is exposed to a synthetic- or MO-based fluid.(Supported by NIOSH Grant R01-OH-03669)

1593 INVESTIGATION OF THE SENSITIZATION POTENTIAL OF FRAGRANCE INGREDIENT 3 AND 4-(4-HYDROXY-4-METHYLPENTYL)-3-CYCLOHEXENE-1-CARBOXALDEHYDE (HMPCC).

A. Api, J. Cocchiara and C. Letizia. *Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ.*

3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC, CAS Nos. 31906-04-4 and 51414-25-6) is a fragrance ingredient with a sweet, light and floral odor used in all types of cosmetic and household products. Three guinea pig studies (a modified Draize and two Magnusson and Kligman maximization tests) and a murine local lymph node assay indicate that the material has a weak potential to induce skin sensitization. The results from human predictive tests (numerous repeated insult patch tests and one human maximization test) conducted at various concentrations, also predict that the material is a weak skin sensitizer. The diagnostic patch test data indicate that the material is a moderate to strong skin sensitizer. The skin sensitization potential of this material is summarized and a dermal sensitization risk assessment is presented.

1594 ASSESSMENT OF SKIN ABSORPTION AND METABOLISM OF ARACHIDONIC ACID & GLYCERYL ARACHIDONATE USING *IN VITRO* DIFFUSION CELL TECHNIQUES.

A. R. Eppler[1, 2], M. E. Kraeling[1], R. R. Wickett[2] and R. L. Bronaugh[1]. [1]*Office of Cosmetics and Colors/ Cosmetic Toxicology Branch, US Food & Drug Administration, Laurel, MD and* [2]*College of Pharmacy, University of Cincinnati, Cincinnati, OH.*

Arachidonic acid (AA), has been used in cosmetics as a surfactant-cleansing and emulsifying agent. Glyceryl arachidonate (GA), a skin conditioning agent and emollient, may be partially metabolized by ester hydrolysis in skin to AA. Based on the Cosmetic Ingredient Review (CIR) Panel's concern that there was a lack of dermal absorption data for AA, *in vitro* percutaneous absorption and metabolism studies were initiated. To simulate normal consumer use, AA and GA were applied in an oil in water emulsion (2mg/cm^2) to skin samples 200um thick in flow-through diffusion cells perfused with a physiological buffer. To assay for permeation, *via*ble fuzzy rat and human skin were dosed with [^{14}C]AA containing ~0.5uCi (0.01mg) AA /cell while [^{3}H]GA was applied to *via*ble and cadaver human skin at ~0.5uCi (0.003mg) GA/cell. For metabolism analysis, the skin equivalent Epiderm was dosed with [^{3}H]GA under similar conditions. Receptor fluid fractions were collected at 6h intervals over a 24h dosing period. Skin penetration was determined by liquid scintillation counting and expressed as a percent of the applied dose. High performance liquid chromatography was used to assess metabolism to AA. Absorption of AA through rat skin was 19.8 ± 5.3 % (mean ± SEM) compared to only 1.4 ± 0.3 % through human skin. Total AA penetration (receptor fluid plus skin levels) in rat and human skin was 52.3 ± 7.3 and 20.1 ± 5.4 %, respectively. Total GA penetration of *via*ble skin was found to be 11.3 ± 2.1% with only 3.2 ± 0.5% absorbed through the skin. In cadaver skin, 4.8 ± 0.8% GA was absorbed through skin with a total penetration of 6.7 ± 1.2%. Assay of the Epiderm receptor fluid found ~50% absorption of radioactivity and 3.0 ± 2.1% GA conversion to AA. In conclusion, percutaneous absorption of AA was less through human than rat skin, while GA absorbed through Epiderm was metabolized to AA.

1595 MIXTURE EFFECTS OF JET FUEL ALIPHATIC AND AROMATIC HYDROCARBONS ON HUMAN EPIDERMAL KERATINOCYTES.

C. Chou[1], J. Yang[2], C. Lee[1] and N. A. Monteiro-Riviere[3]. [1]*Veterinary Medicine, National Chung-Hsing University, Taichung, Taiwan,* [2]*Chung-Shan Medical University, Taichung, Taiwan and* [3]*Center for Cutaneous Toxicology and Research Pharmacokinetics, NC State University, Raleigh, NC.*

Jet fuels are complex mixtures of aliphatic (ALI) and aromatic (ARO) hydrocarbons that vary significantly in individual cytotoxicity and proinflammatory activities in human epidermal keratinocytes (HEK). In order to elucidate the dermatotoxicity of a complex mixture like jet fuels, structural differences, exposure time and dosage were investigated on HEK toxicity assessed by mortality and IL-8 release. ALI and ARO hydrocarbons were grouped into 4 categories: highly cytotoxic (octane, nonane, decane for aliphatics and cyclohexalbenzene, trimethylbenzene, xylene for aromatics), low cytotoxic (tetradecane, pentadecane, hexadecane for aliphatics and benzene for aromatics), high IL-8 release (decane, undecane, dodecane for aliphatics and dimethylnapthalene, cyclohexylbenzene, ethylbenzene for aromatics) and low IL-8 release (tetradecane, pentadecane, hexadecane for aliphatics and benzene, toluene, xylene for aromatics). The 4 categories of ALI hydrocarbons were mixed with each other, or cross-mixed with each of the 4 categories of ARO hydrocarbons. The resulting cytotoxicity and IL-8 production from HEK were evaluated at 24 hrs. The results showed an antagonistic cytotoxic effect between ALI and ARO hydrocarbons in which ALI attenuated the degree of HEK mortality caused by the ARO hydrocarbons. On the other hand, the ARO hydrocarbons reduced the significant increase of IL-8 induced by ALI hydrocarbons. Synergistic effects between low IL-8 inductive and low cytotoxic hydrocarbons were found and the highest cytotoxic and IL-8 inductive responses did not completely correspond to the mixture of highly cytotoxic and highly IL-8 inductive hydrocarbons. This study supports the concept that the ARO dictate the degree of HEK mortality, while the ALI are the major contributor to inciting the proinflammatory response. Mixture effects must be considered when evaluating cytotoxicity to HEK. Support: NSC Taiwan 92-2313-B-005-117

1596 DIPHOTERINE:SKIN SENSITIZATION STUDY IN THE GUINEA PIG.

L. MATHIEU[1], F. BURGHER[1] and A. H. HALL[2]. [1]*Researches and Communication, PREVOR, TALENCE, France and* [2]*TCMTS, Inc., Elk Mountain, WY.*

Diphoterine is an eye/skin decontamination solution for chemical splashes, produced by the PREVOR laboratory, France. Its chemical and physical properties allow a quasi polyvalent rinsing of chemical splashes with a quick return to a physiological state. Diphoterine has been used since several years by workers in industries on European Market. No sensitization cases have been reported. Here we present the results obtained on a possible delayed sensitizing capacity of Diphoterine. It was evaluated in the Guinea pig, in accordance with the general requirements of OECD Guideline and Directive 67/548/EEC. The experimental technique is based on those of Magnusson-Kligman and Guillot and coll. The sensitivity and the reliability of the experimental method are verified at least six months, by use of a positive control group in which animals are treated with DNCB used as a 1% alcoholic solution. The application of the test substance Diphoterine did not induce coloration of the application site. Grading of any skin lesions was therefore possible. Primary induction was performed at day 1 by intradermal injection, the sensitization phase by topical application at day 9 and the challenge at day 22 by topical application with 100% v/v concentration of the substance. Determination of the degree of allergenicity at times 24 and 48 hours was based upon the percentage of animals in the group showing a reaction, rather than on the severity on the latter. Under the experimental conditions adopted, results obtained were as follows:no irritation reaction was noted at times 24 and 48 hours in animals of the negative control group and in animals treated during the challenge phase with Diphoterine at the Maximum Non-Irritant Concentration. The test substance showed no allergenicity at 24 and 48 hours. The test substance showed no allergenicity of Class at 24 hours and 48 hours. According to the terminology employed, it is considered that Diphoterine is free of sensitizing capacity in the Guinea pig. Keywords:Diphoterine, Skin, Allergy

1597 ROLE OF SP-1 IN SDS-INDUCED ADIPOSE DIFFERENTIATION RELATED PROTEIN SYNTHESIS IN HUMAN KERATINOCYTES.

C. L. Galli, O. Zancanella, L. Lucchi, B. Vi*via*ni, M. Marinovich and E. Corsini. *Department Pharmacological Sciences, University of Milan, Milan, Italy.*

Skin irritation is a complex phenomenon, and keratinocytes, owing of their anatomical location and production of inflammatory mediators, play an important role in it. We have recently characterized the expression and protective role of adipose differentiation related protein (ADRP) in skin irritation (J. Invest. Dermatol. 121: 337, 2003). In particular, ADRP expression is induced to recover functional cell membrane following the cell damage induced by skin irritants. The purpose of this study was to characterize in a human keratinocyte cells line (NCTC 2544) the biochemical events that lead to ADRP expression following SDS treatment, and, in particular, to investigate the role of transcription factor SP-1. Analysis of ADRP promoter region revealed indeed a potential binding site for the transcription factor SP-1 together with AP-2, NF-1, c-myc and Pax-1. We found that SDS induces a dose and time related SP-1 activation as evaluated by measuring the DNA binding activity, which is correlated with SDS-induced ADRP mRNA expression. Furthermore, SDS-induced SP-1 activation, ADRP mRNA expression and lipid droplets accumulation could be modulated by mithramycin A, an antibiotic that selectively binds to the GC box preventing SP-1 binding and gene expression,

demonstrating that SDS-induced ADRP expression is mediated in part through the transcription factor SP-1. SDS-induced SP-1 activation as well as ADRP expression could be modulated by the calcium chelator BAPTA, indicating a role of calcium in ADRP-induction. The following scenario can be proposed: every time an irritant perturbs membrane barrier and renders the membrane leaky, extracellular calcium can enter the cells triggering SP-1 activation and ADRP expression to help restore normal homeostasis and to repair membrane. Acknowledgement. This work was partially funded by FIRST, UNIPRO and by the Center for Risk Assessment of the University of Milan.

1598 INFLUENCE OF SKIN THICKNESS ON PERCUTANEOUS PENETRATION OF CAFFEINE, BUTOXYETHANOL AND TESTOSTERONE *IN VITRO*.

S. C. Wilkinson[1], L. Greaves[2] and F. M. Williams[1]. *[1]Clinical and Lab. Sciences, University of Newcastle, Newcastle upon Tyne, Tyne and Wear, United Kingdom and [2]Neurology, University of Newcastle, Newcastle upon Tyne, Tyne and Wear, United Kingdom.*

In vitro percutaneous penetration studies have historically used full or split thickness skin. As part of a multicentre study of *in vitro* dermal absorption measurement using diffusion cells (part of the EDETOX project), the influence of skin thickness on the percutaneous penetration of test chemicals with different physicochemical properties (caffeine, testosterone and butoxyethanol) was investigated. Test compounds and their ^{14}C-labelled analogues were applied to full thickness (less than 1 mm thick) or dermatomed (330 μm) human skin from the same donors (2 per study, 3 cells each) mounted in flow through diffusion cells (skin temperature approximately 32°C, 0.64 cm^2. The receptor fluid was saline (pH 7.4, 1.5 ml/h) with 5% (w/v) BSA added for studies with testosterone. Caffeine and testosterone were applied in ethanol:water (1:1 v/v) at 4 mg/ml (100 μg/cm^2) and receptor fluid samples collected. After 24 h, the skin surface was swabbed with vehicle, the diffusion cell washed with ethanol, and the skin digested in 1.5 M KOH in ethanol:water (4:1 v/v). Butoxyethanol was applied in water (50% v/v) at 200 μl/cm^2 and the skin surface swabbed as above after 4 h. Receptor fluid samples were collected for 8 h and the diffusion cell washed and skin digested as above. All studies were carried out with occlusion. The maximum flux of testosterone increased from 0.18 ± 0.04 μg/cm^2/h with full thickness skin to 1.82 ± 0.37 μg/cm^2/h with dermatomed skin, whilst the maximum flux of butoxyethanol increased from 674.6 ± 92.8 μg/cm^2/h to 1694.8 ± 178.9 μg/cm^2/h. Significant effects on distribution were limited to the receptor fluid. Caffeine penetration was not significantly affected. Use of split skin clearly contributed to an increase in flux but did not greatly increase the coefficient of variation when compared with full thickness skin. This increase in flux was not limited to the lipophilic test compound.

1599 AN *IN VITRO* MODEL FOR CUTANEOUS PHOTOAGING USING A LUCIFERASE REPORTER GENE TO MEASURE HUMAN ELASTIN PROMOTER ACTIVITY.

D. B. Brown, M. D. Schwartz, S. M. Ksenzenko, E. F. Bernstein, P. D. Forbes, C. P. Sambuco and A. M. Hoberman. *Charles River Discovery and Development Services, Argus Division, Horsham, PA.*

Using transgenic mice, we have previously reported on the correlation between elevated levels of human elastin promoter (HEP) activity after exposure to UVR, and increased expression of steady-state mRNA and elastotic deposition, considered hallmarks of *solar-aged* skin. In order to develop a rapid and sensitive *in vitro* screening model for evaluating the *anti-aging* potential of compounds, immortalized human skin fibroblasts containing the HEP/luciferase reporter gene construct and selected ultraviolet radiation sources were used. Exposure to unfiltered FS lamps produced a 5.5-fold increase over baseline promoter levels with 2 mJ/cm^2 (using an erthyma weighted detector, corresponding to about 10% of a human erythema dose (p<0.0001). Using a Xenon Arc solar simulator with cut-off filters of different thickness revealed increases in relative promoter activity from 2.5- to 1.5-fold over baseline activity (p<0.001). Initial results indicate that this sensitive and quantitative, solar simulator-based model is readily applicable to evaluating potential photo-screening and photo-protection compounds for reducing the development of photodamaged and *photoaged* skin.

1600 RETINOIC ACID INDUCED EXPRESSION OF THE HELIX-LOOP-HELIX INHIBITORY PROTEIN ID-1 IN NORMAL HUMAN KERATINOCYTES.

C. Villano[2], L. A. White[1] and E. Myers[1]. *[1]Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ and [2]Joint Graduate Program in Toxicology, Rutgers University, New Brunswick, NJ.*

Retinoids, vitamin A analogues, are powerful regulators of cell growth and differentiation and is necessary for the maintenance of epithelial differentiation. We have found that exposure of normal human keratinocytes to all-trans retinoic acid, the main signaling retinoid in the body, results in a significant increase in Id-1 mRNA and protein. The Id proteins, which lack a DNA binding domain, function by forming inactive heterodimers with other helix-loop-helix (HLH) proteins and reducing their transcriptional activity. Importantly, ectopic overexpression of Id-1 results in immortalization or delayed senescence of normal keratinocytes, demonstrating that Id-1is an important regulator of keratinocyte differentiation. Our hypothesis is that retinoic acid induced expression of Id-1 is a critical step in retinoic acid mediated transcription in normal human keratinocytes. Our data show that retinoic acid stimulation of Id-1 is specific; no change was observed in Id-2 and Id-3 expression following retinoic acid exposure. Retinoic acid stimulation of Id-1 does not involve mRNA stability, suggesting that it is regulated at the level of transcription. To investigate this possibility, we have isolated 2.2 kb of the Id-1 promoter and linked it to a reporter gene (luciferase; pGL3) for transfection into normal human keratinocytes. The goals of these experiments are to determine the mechanism of retinoic acid induced Id-1 expression, as well as elucidate the role of Id-1 in mediating retinoic acid effects in skin. These data will provide a better understanding of the mechanism of action of retinoic acid in skin, as well as to define the role of Id-1 in this pathway.

1601 *IN VITRO* PERCUTANEOUS ABSORPTION OF SALICYLIC ACID IN HAIRLESS MOUSE AND HUMAN SKIN.

M. E. Kraeling and R. L. Bronaugh. *Office of Cosmetics and Colors, USFDA, Laurel, MD.*

Salicylic acid (SA) is one of the most commonly used beta hydroxy acids used in skin care products. This study was conducted in support of a SA photocarcinogenicity study conducted at the National Center for Toxicological Research (NCTR). The purpose of this study was to determine the rate of skin absorption of SA from cosmetic creams and to determine the amount of SA residing in hairless mouse (HM) skin at the time of UV irradiation. SA penetration was determined from 2% and 4% [14C]SA creams applied to excised *via*ble HM skin (full thickness) and human skin (200 um thick) mounted in flow-through diffusion cells perfused with a physiological buffer (HEPES-buffered Hanks balanced salt solution, pH 7.4). In HM skin, the formulations remained on the skin for 4 and 24 h. Four hours after application of SA cream, half of the skin was exposed to UVB radiation (16 mJ/cm2) while the unexposed half was used as the control. At the end of the studies, the amount of SA penetrated into skin layers and receptor fluid was determined and expressed as the applied dose penetrated for each formulation. At both concentration levels, SA was rapidly and extensively absorbed into HM skin in 24 h. Total penetration of SA was 90-94%, with 87-91% absorbed into receptor fluid and 2-3% remaining in skin. UVB treated and control skin showed similar absorption profiles with no difference in SA penetration. In the 4 h studies, absorption of SA into receptor fluid at both SA concentration levels was rapid at 37-39%. At the time of UV exposure, 3-5% of the SA applied resided in HM skin. Human skin absorption studies were conducted with only a 24 h application of 2% and 4% [14C]SA creams. SA absorption was less extensive with 34-44% of the applied SA absorbed into receptor fluid in 24 h and 6.5-10.5% of the SA remaining in skin. Variability in SA skin penetration through human skin was observed. SA penetration increased as the permeability of the human skin to [3H]water increased. In conclusion, SA rapidly penetrated HM skin and was found in skin at the time of irradiation.

1602 RESPONSE OF SKH-1 MOUSE SKIN FOLLOWING THE ACUTE INJURY OF TATTOOING.

N. V. Gopee[1, 2], Y. Cui[1, 2], G. Olson[3], A. Warbritton[3], B. J. Miller[1, 2], L. H. Couch[1, 2], W. G. Wamer[4] and P. C. Howard[1, 2]. *[1]Division Biochemical Toxicology, NCTR, USFDA, Jefferson, AR, [2]NTP Center for Phototoxicology, NCTR, USFDA, Jefferson, AR, [3]Charles River Companies, Jefferson, AR and [4]CFSAN, USFDA, College Park, MD.*

Tattooing is a rapidly increasing art form in the US. The toxicity of the pigments used in tattoo inks have not been reported. As an initial step to assess their phototoxicologic properties we have studied the cutaneous impact of tattooing using SKH-1 hairless mice and commercial tattoo inks or inks made with CdS or Fe_3O_4. The dorsal tattoos were ~2x25 mm, and the mice were sacrificed at 0.5, 1, 3, 4, 7 or 13 days (d) following tattooing. Histological evaluation of the skin revealed dermal hemorrhaging at 0.5 and 1 d, and necrosis at 3 d decreasing to 17% by 13 d. Dermal inflammation was present from 3-13 d. Epidermal necrosis and inflammation were immediately apparent and decreased in occurrence and frequency to 0% by 13 d. External inguinal and axillary lymph nodes contained tattoo pigments, the inguinal being the most reactive evidenced by hyperplasia at all timepoints. Orinithine decarboxylase (ODC), cyclooxygenase-1 (COX1) and COX2 protein levels increased significantly at 0.5-4 d and decreased to control levels by 13 d. The increase was greatest in the tattooed skin compared to the intra-tattooed skin.

ODC and COX2 protein were elevated in the inguinal lymph nodes. Interleukin-1β (IL-1B) and IL-10 proteins were dramatically increased at all timepoints in the inguinal lymph nodes but suppressed in the tattoo and intra-tattoo skin, with maximal suppression between 0.5-4 d. Concentrations of cutaneous nuclear factor-kappa B (NF-κB) protein, the transcription factor for several inflammatory mediators, were elevated between 0.5-4 d. Inducible nitric oxide synthetase mRNA was increased in tattooed (1 d) and intra-tattoed (0.5 d) skin and lymph nodes (1 d). These data demonstrate that tattooing skin induces an inflammatory response similar to acute injury, inflammatory cells respond to the presence of the pigment removing the pigment to the regional lymph node, and the skin in general recovers from the insult within 13 d.

1603 MECHANISM OF PARAQUAT-INDUCED TOXICITY IN MOUSE KERATINOCYTES.

A. Vetrano[1], D. E. Heck[1], A. T. Black[1], M. Thiruchelvam[2], E. Richfield[3], D. A. Cory-Slechta[2] and J. D. Laskin[2]. *[1]Pharmacology and Toxicology, Rutgers University, Piscataway, NJ, [2]Environmental and Community Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ and [3]Pathology and Lab. Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

An important herbicide known to cause significant dermatotoxicity in exposed field workers including both allergic and contact dermatitis is paraquat (1, 1'-dimethyl-4, 4'-bipyridinium). In cells, paraquat undergoes redox cycling and, during intracellular metabolism, it can generate active radical species which cause modifications in cellular macromolecules and promote lipid peroxidation. In the present studies we examined the cytotoxicity of paraquat in PAM 212 cells, a skin-derived mouse keratinocyte cell line. We found that paraquat caused a time- and concentration-dependent inhibition of keratinocyte cell growth. Cells exposed to paraquat for prolonged periods (96 hr) were significantly more sensitive to growth inhibition that cells exposed to the herbicide for 3 or 6 hr (IC50 = 50 μM and 100-300 μM, respectively). In keratinocytes, redox sensitive agents and oxidants readily deplete intracellular glutathione. At concentrations up to 1 mM and for periods up to 6 hr, paraquat had no effect on this thiol tripeptide in PAM 212 cells. Previous studies have demonstrated that redox active agents and oxidants readily glutathionylate cellular proteins and are potent activators of NF-κB. We found that paraquat did not induce protein glutathionylation in PAM 212 cells. Moreover, it inhibited NFκB activation in the cells, as measured by electrophoretic mobility shift assays. Taken together, these data demonstrate that paraquat is highly cytotoxic to keratinocytes. However, its mechanism of action in these cells may not be due to altered intracellular redox balance. Supported by NIH grants ES05022, ES10791, ES03647 and CA100994.

1604 MECHANISMS OF UVB LIGHT-INDUCED OXIDANT FORMATION IN THE SKIN.

D. E. Heck. *Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

It is recognized that modifications of critical cellular macromolecules by the higher-energy shorter solar wavelengths comprising the UVB spectra (290-320 nm) are the most damaging to the skin. This process is mediated, in part, by the intracellular generation of reactive oxygen species. In previous studies we identified catalase as an important protein responsible for UVB-induced reactive oxidant-generating activity in keratinocytes. To investigate the mechanism by which this protein converts damaging solar radiation into less energetic oxidant species, we analyzed the effects of inhibitors of the peroxide degrading catalatic activity of catalase on UVB-mediated oxidant generation. The inhibitor 3-amino-1, 2, 4-triazole, which blocks the formation of the enzyme-hydrogen peroxide transition state C1, and sodium azide, an inhibitor of the dissociation of C1, stimulated hydrogen peroxide production. Interestingly, cyanide, which interacts directly with the heme iron to inhibit electron transfer, was ineffective in inhibiting the UVB-stimulated peroxidatic reaction. This profile of inhibition is strikingly similar to that of the peroxidase activity of some bacterial bifunctional catalase-peroxidases, and prompted us to analyze the heme binding pockets of human red blood cell catalase, a representative monofunctional catalase, the heme binding pocket derived from the crystal structure of pea cytosolic ascorbate peroxidase, a mono-functional Class I intracellular peroxidase, and the heme environs of Burkholderia pseudomallei catalase-peroxidase Katg, a bifunctional catalase-peroxidase. Structural analysis identified several critical residues in the active site likely to be important in the activities. Taken together, these data imply that there are distinct reaction pathways for the catalatic and UVB catalyzed reactions. We hypothesize that UVB alters the overall electronic environs at the catalase heme to render the distal histidine less imidazole-like in character, and similar to the highly polar distal histidine in the active site of bifunctional catalases undergoing peroxidase activity. Support: NIH CA093798 and ES03647.

1605 SUNSCREENS WITH PHYSICAL UV BLOCKERS CAN INCREASE TRANSDERMAL ABSORPTION OF THE HERBICIDE 2, 4 D.

R. M. Brand, J. M. Pike and A. R. Charron. *Internal Medicine, Evanston Northwestern Healthcare and Feinberg School of Medicine, Evanston, IL.* Sponsor: P. Iversen.

Farmers are encouraged to wear sunscreen to reduce the risk of skin cancer. We have demonstrated that the dermal penetration of agrochemicals can be enhanced by chemical UV absorbers and the insect repellent DEET. We have extended these studies to determine whether commercially available sunscreens containing either/or titanium dioxide or zinc oxide enhance the transdermal absorption of the herbicide 2, 4 D. Hairless mouse skin was placed in an *in vitro* Bronough Style flow through diffusion chamber. The epidermal side of the skin was pre-treated for 30 minutes with one of nine commercially available sunscreens purchased through the internet. One hundred microliters of 2, 4 D in the amine form spiked with ^{14}C labeled 2, 4 D was placed on top of the epidermis for 24 hours. The radiolabeled pesticide diffused through the skin, into the receiver compartment where it was collected in 90 minute fractions and placed in a liquid scintillation counter. Data were compared by ANOVA followed by a Dunnett's Multiple Comparison Test and are reported as mean ± sem. The total percentage of 2, 4 D penetrating through the skin in 24 hours ranged from 36.8 ± 2.4 for the no sunscreen control to 57.6 ± 6.3 for the TiSilc product. Five of the nine sunscreen tested led to a significant enhancement of total 2, 4 D penetration as compared to the control ($p<0.01$). This demonstrates that physical UV absorbers can also enhance transdermal absorption similar to the chemical absorbers. Inert ingredients appear to be the major factor in determining the enhancement produced by the active chemicals. Careful selection of sunscreen during pesticide application could reduce potential exposure.

1606 DERMAL ABSORPTION AND CHEMISTRY OF ARSENIC IN RESIDUES FROM WOOD PRESERVED WITH CHROMATED COPPER ARSENATE (CCA).

Y. W. Lowney[1], R. Wester[2], P. Nico[3] and M. Ruby[1]. *[1]Health Risk Group, Exponent, Boulder, CO, [2]Department of Dermatology, University of California, San Francisco, CA and [3]Chemistry Department, California State University, Stanislaus, Turlock, CA.* Sponsor: J. Tsuji.

In assessing possible risks associated with dermal exposure to arsenic, regulatory agencies have relied on information from the early 1990s that assessed dermal absorption of arsenic from radiolabeled soluble arsenic, either in solution or freshly mixed with soil. Because the naturally high background intake of arsenic in the diet precludes detection of low-level absorption of arsenic, studies on environmental matrices (i.e., natural soils or CCA-treated wood, which cannot be radiolabeled) have not been undertaken previously. However, recent efforts using specific low-arsenic diets for lab animals has lowered the limit of detection for absorbed arsenic. Based on this new animal research model, this study was undertaken to evaluate whether the arsenic in residues from CCA-treated wood demonstrates a different dermal absorption profile than soluble arsenic. The dermal absorption research was conducted in Rhesus monkeys, and was designed to replicate, to the extent possible, the earlier research using radiolabeled arsenic. In tandem with the dermal absorption research, state-of-the-art spectroscopic techniques were used to understand the nature of arsenic in the CCA residues. Specifically, x-ray absorption spectroscopy was used to evaluate the chemical structure of arsenic in the residue on the surface of new and weathered wood. These two lines of research provide evidence both from a biological system standpoint and from chemical analyses to demonstrate that the arsenic in CCA residues is present in a stable, complexed form that is not well absorbed compared to soluble arsenic.

1607 *IN VITRO* ASSESSMENT OF STYRENE ABSORPTION THROUGH HUMAN SKIN.

S. Madden, C. Roper and R. Greenough. *In Vitro Sciences, Inveresk Research, Edinburgh, United Kingdom.*

Skin absorption studies provide data that can be an alternative to the use of worst case default values in risk assessments of dermal exposure. Historically, these studies have been performed in animal models *in vivo*, however, in recent years there has been growing acceptance by regulatory authorities for alternative *in vitro* models. In this present study we have assessed the absorption of styrene in human skin *in vitro*. The study examined skin absorption of pure styrene and also styrene as formulated into an unsaturated polyester resin. The resin was typical of the worst case that might be found in the glass reinforced composite industry (high styrene content and long cure time). Human skin was obtained with full informed consent from elective plastic surgery patients. Split thickness membranes were prepared from the sample at a thickness of *ca* 350 μm using a Zimmer electric dermatome. Sections of skin membranes were then mounted in flow through diffusion cells and a tritiated

water barrier integrity assessment performed. [^{14}C]styrene or [^{14}C]styrene in resin was applied to the stratum corneum of intact sections. The cells were covered with non-occlusive carbon filters to allow collection of volatile materials. Receptor fluid (tissue culture medium) was collected until 24 h post dose application. At 24 h, the majority of the [^{14}C]styrene (*ca* 70-80%) from both formulations had been collected in the charcoal filters. Recovery of radioactivity in the receptor fluid (absorbed dose) was 1.25% for the [^{14}C]styrene and 1.15% for the [^{14}C]styrene in resin. Dermal delivery was 1.68 and 1.23% in each formulation, respectively. Total recovery was *ca* 88% for [^{14}C]styrene and 95% for [^{14}C]styrene in resin. In conclusion, following topical application of [^{14}C]styrene or [^{14}C]styrene in resin to human skin the extent of absorption, and, therefore, predicted human systemic exposure, was similar. The degree of absorption was much lower than would be considered from default risk assessment assumptions.

1608 STUDIES ON PERCUTANEOUS ABSORPTION, BIOPHYSICAL AND BIOCHEMICAL CHANGES IN THE SKIN BY NONANE AND DODECANE IN HAIRLESS RATS.

J. B. RAMAPURAM[1], A. Chatterjee[1], B. Locke[2], S. Gibbs[2] and M. Singh[1]. *[1]COLLEGE OF PHARMACY, FLORIDA A&M UNIVERSITY, TALLAHASSEE, FL and [2]Department of Chemical Engineering, FAMU-FSU College of Engineering, Tallahassee, FL.*

Occupational skin diseases are one of the most significant health concerns in the US and cost the government millions of dollars each year. Skin exposures can occur from occupational chemicals such as organic solvents and fuels leading to irritant contact dermatitis. Understanding the mechanisms of chemical irritation will assist in assessing the risks to exposures. Nonane and dodecane were investigated for (a) their *in vitro* skin permeation and retention using Franz diffusion cells, (b) skin barrier changes due to acute chemical exposures by measuring transepidermal water loss (TEWL) and magnetic resonance imaging (MRI) of skin and (c) expression of molecular markers (IL-1α, TNFα, and MCP-1 by enzyme immunometric assay and NF-κB and IkB by western blot analysis) in the skin due to acute chemical exposures. The flux and deposition in the skin layers (stratum corneum, epidermis and dermis) of nonane was significantly lower than dodecane ($P < 0.001$) and nonane showed higher TEWL than dodecane ($P < 0.05$). The MRI of nonane treated skin showed significantly higher signal intensities in the epidermis, dermis, hair follicles and lipid regions of skin than control ($P < 0.05$). Further, nonane produced higher cytokine and chemokine expressions in the skin ($P < 0.01$) mediated through NF-κB activation and its corresponding IkB deactivation. Dodecane, even though demonstrated higher retention in the skin layers, did not show significant increase in the TEWL as compared with control ($P > 0.05$). Further, cytokine and chemokine expression in the skin was not significantly increased by dodecane as compared with control ($P > 0.05$) and this data was in agreement with the results of NF-κB and IkB levels in the skin, which did not alter due to dodecane treatment. The results indicate that MRI and TEWL showed strong correlations with cytokine and chemokine expressions in the skin and can serve as powerful tools for dermal risk assessment of irritant chemicals.

1609 PHOTODECOMPOSITION OF PIGMENT YELLOW 74, A PRIMARY COLOR CHEMICAL IN TATTOO INKS.

L. H. Couch[1,3], Y. Cui[1,3], A. Spann[1], N. V. Gopee[1,3], F. E. Evans[2], M. I. Churchwell[1], L. Williams[1], D. R. Doerge[1] and P. C. Howard[1,3]. *[1]Division of Biochemical Toxicology, NCTR, USFDA, Jefferson, AR, [2]Division of Chemistry, NCTR, USFDA, Jefferson, AR and [3]NTP Center for Phototoxicology, NCTR, USFDA, Jefferson, AR.*

Tattooing has become a popular recreational practice in the past decade. Very little is known concerning the pigments used in the inks or the toxicity, phototoxicity, or photochemistry of the pigments. Yellow tattoo inks were obtained from commercial sources and the organic chemical responsible for the color was extracted and quantitatively determined by HPLC with UV-VIS and mass spectral (MS) detection. The monoazo pigment yellow 74 (PY74; CI 11741) was the major chemical pigment used in seven yellow tattoo inks. PY74 was dissolved in tetrahydrofuran, deoxygenated using argon gas, and exposed in 2 mm cuvettes to simulated solar light equivalent to terrestrial irradiance using a 6.5 kWatt xenon arc solar simulator. Spectrophotometric and HPLC analysis indicated PY74 photodecomposed in the simulated solar light. Multiple products were isolated using a combination of silica chromatography and HPLC. Two of the major products were identified by NMR and MS as N-(2-methoxyphenyl)-3-oxobutanamide and its 2-hydrazine derivative. These products are indicative of photochemical reduction at the hydrazone of PY74. The rate of formation of these products is solvent dependent and decreased in the presence of oxygen. These results suggest that the photochemical decomposition of PY74 results in the release of aromatic amine products.

1610 PHOTOCHEMICAL DECOMPOSITION OF THE DICHLOROBENZIDINE-BASED TATTOO PIGMENT ORANGE 13 IN SIMULATED SOLAR LIGHT.

P. C. Howard[1,3], N. V. Gopee[1,3], Y. Cui[1,3], F. E. Evans[2], L. H. Couch[1,3], M. I. Churchwell[1] and D. R. Doerge[1]. *[1]Division of Biochemical Toxicology, NCTR, USFDA, Jefferson, AR, [2]Division of Chemistry, NCTR, USFDA, Jefferson, AR and [3]NTP Center for Phototoxicology, NCTR, USFDA, Jefferson, AR.*

The art of tattooing has experienced a renaissance over the past several decades, with an increase in popularity especially amoung youth, and migrating from the use of inorganic metals (e.g. HgS) as the color agent to organic pigments. Many of the yellow, orange or red pigments used in tattoo inks are derivatives of 3, 3'-dichlorobenzidine. We used Pigment Orange 13 (PO13; CI 21110), a bisazo pigment of 3, 3'-dichlorobenzidine and 3-methyl-1-phenyl-2-pyrolozin-5-one (MPPone), as a model bisazo pigment for examining photochemical stability of this class of compounds. PO13 was dissolved in tetrahydrofuran, deoxygenated using argon, and exposed in 2 mm cuvettes to simulated solar light using a 6.5 kW xenon arc solar simulator, with irradiance equivalent to terrestrial summer sunlight. The PO13 solution decolorized indicating photochemical decomposition. The products were analyzed by HPLC and isolated using a combination of column and thin layer silica chromatography and HPLC, and analyzed using NMR and mass spectroscopic (MS) methods. The initial photochemical reduction of PO13 involves cleavage at one of the hydrazone groups to yield the mono-MPPone derivative of 3, 3'-dichlorobenzidine and the mono-MPPone derivative of 3, 3'-dichloro-4-aminobiphenyl. 3, 3'-Dichlorobenzidine and 3, 3'-dichloro-4-aminobiphenyl were also identified, presumably through sequential photochemical reduction. The identification of other photochemical products is in progress to elucidate further the pathways for photodecomposition of this pigment. These data demonstrate that photochemical decomposition of a representative 3, 3'-dichlorobenzidine-based tattoo pigment using simulated solar light in an aprotic solvent results in the release of potentially carcinogenic 3, 3'-dichlorobenzidine and 3, 3'-dichloro-4-aminobiphenyl.

1611 CUTANEOUS UVR AND CIS-UROCANIC ACID EXPOSURE ENHANCES BURULI ULCER DISEASE IN THE CRL:IAF(HA)-HRBR HAIRLESS GUINEA PIG.

R. Cope[1], N. Stang[1], B. Valentine[1], P. L. Small[2] and L. Bermudez[1]. *[1]College of Veterinary Medicine, Oregon State University, Corvallis, OR and [2]Department of Microbiology, University of Tennessee, Knoxville, TN.*

Buruli ulcer disease (BUD) is a significant, high morbidity, emerging human cutaneous disease caused by Mycobacterium ulcerans (MU) infection. In endemic areas, it is now the second most common mycobacterial infection of humans. MU ulcers are primarily located on the appendages and a single lesion can cover up to 15% of body surface area. Antibiotic therapy is ineffective in human cases and current treatment regimes are based on local excision/skin grafting or amputation. In rodent models, ultraviolet radiation (UVR) exposure increases the severity of tuberculosis and leprosy; however the effects of UVR on BUD have not been previously examined. Thus, the aim of this study was to test the hypotheses that exposure to ultraviolet radiation (UVR) or cis-urocanic acid (cUCA), an initiator of photoimmunosuppression, would result in an increased severity of MU infection in a hairless guinea pig model of BUD. Pre-infection exposure to UVR or cUCA results in substantial and significant ($p < 0.001$) increases in size of MU lesions. UVR exposure was associated with significant ($p < 0.001$) delays in the healing of MU infected skin sites. Both UVR and cUCA exposure were associated with significant ($p < 0.001$) suppression of delayed-type hypersensitivity responses to MU cell wall antigens in infected animals. These results suggest that UVR exposure is a significant factor in the development of BUD and that UVR/cUCA susceptible immune pathways are important in the pathogenesis of BUD.

1612 HELICOBACTER PYLORI AND ETHANOL INDUCED TOXICITY AND APOPTOSIS IN GASTRIC EPITHELIAL CELLS.

A. N. Nunley[1], R. L. Copeland[1] and D. T. Smoot[2]. *[1]Pharmacology, Howard University College of Medicine, Washington, DC and [2]Medicine, Howard University Hospital, Washington, DC.*

Infection with Helicobacter pylori (HP) induces chronic gastritis in virtually all infected persons, which is associated with increased risk of developing gastric cancer. In the gastric mucosa, ethanol increases histamine, gastrin and acid secretion, and stimulates ulcer formation. This study examined the effects of HP, ethanol, and acetaldehyde on gastric epithelial cells. AGS cells were infected with either a cag+ HP strain or cag- HP strain for 24 hrs (37oC). AGS were then exposed to EtOH (100, 500, 1000, 1500mM) or Acet (10, 50, 100, 200uM) for 1, 3 and 6 hours. EtOH caused a concentration dependent increase in toxicity (500-1500mM) which was

enhanced by its combination with HP. EtOH (1500mM) alone and HP/EtOH (500-1500mM) decreased AGS cell *via*bility (trypan blue and proliferation assays). Acetaldehyde did not significantly increase AGS toxicity. However, the combination of acetaldehyde and HP was toxic and lead to decreased *via*bility. To elucidate underlying mechanisms behind the observed damage, apoptosis and p53 and bcl-x expression studies were performed. Both HP and EtOH alone induced apoptosis. AGS cells exposed to the cag+ HP strain or HP/EtOH did not induce apoptosis. AGS cells exposed to cag- HP strain induced apoptosis at a higher rate compared to control AGS cells. p53 and bcl-x expression (western blot analysis) found increased expression of p53 following EtOH exposure, cag- HP strain infection, and HP/EtOH. Bcl-x expression was increased following exposure to cag+ HP strain and HP/EtOH. Acetaldehyde treatment induced the expression of both p53 and bcl-x in all treatment groups. This study demonstrated the toxic effects of EtOH and its combination with HP on AGS cells. The effect of acetaldehyde on AGS cells may be due to its rapid metabolism or evaporative properties. Furthermore, the EtOH and cag- HP strain damage may be irreparable as seen by the induction of apoptosis and p53 expression in those cells. This damage may somehow bypass the signals to terminate and elude apoptosis.

1613 INVOLVEMENT OF APOPTOSIS IN THE HMG-COA REDUCTASE INHIBITOR-INDUCED MYONECROSIS IN HUMAN SKELETAL MUSCLE CELLS.

A. Wolf[1], L. Ndountse-Tchapda[1], S. Ursula[1] and W. E. Trommer[2]. [1]*Biomarker Development, Novartis Pharmacology AG, Basel, Switzerland and* [2]*Department of Chemistry, University of Kaiserslautern, Kaiserslautern, Germany.*

3-Hydroxymethylglutaryl-CoA reductase inhibitors (HMGCRI) are potent cholesterol lowering agents, causing rhabdomyolysis as a clinically relevant side effect in man. Previous studies in human skeletal muscle cells (hSkMC) suggested that caspase-3 activation might be an important early event in the development of HMGCRI-induced rhabdomyolysis and that the intracellular ATP pool might serve as a checkpoint, which could drive cells into necrosis. A direct link between necrosis and apoptosis however has not been shown so far. In the present study, we investigated caspase-3 activation (apoptosis) and LDH-leakage (necrotic membrane damage) in hSkMCs after incubation with the HMGCRIs simva-, lova-, atorva-, prava-, and fluva-statin and co-incubation with the caspase-3 inhibitor Ac-DEVD-CHO or the major down stream HMGCR metabolites mevalonate, geranylgeranyl- pyrophosphate, farnesyl-pyrophosphate, geranylgeraniol, farnesol and mevalonic acid. The results showed that the caspase-3 inhibitor completely inhibited caspase-3 activity and reduced, but did not completely suppress, the HMGCRI-induced LDH-leakage. All metabolites reduced the HMGCRI-induced capase-3 activation, with farnesol showing the strongest effect. Similar potencies were found concerning the reduction of the HMGCRI-increased LDH-leakages. Among the metabolites farnesol was most effective. The present results confirm that apoptosis is an important event, which at least partially contributed to the generation of the HMGCRI-induced myonecrosis in human skeletal muscle cells.

1614 PULMONARY TOXICANTS AMIODARONE AND PARAQUAT CAUSE INJURY BY APOPTOSIS IN RAT PLEURAL MESOTHELIAL CELLS.

S. Seth and J. Cerreta. *Pharmaceutical Sciences, St. John's University, New York, NY.* Sponsor: L. Trombetta.

The antiarrhythmic drug amiodarone and the herbicide paraquat are known to cause pulmonary fibrosis, but their mechanisms remain unclear. We hypothesized that such dissimilar agents may cause injury in part through induction of apoptosis in rat pleural mesothelial cell line. Cell *via*bility was determined using the MTT assay. Apoptosis was measured by DNA Fragmentation assay, Caspase-3 enzyme activity and *In Situ* terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay (TUNEL). MTT cytotoxicity assay determined that cells treated with amiodarone at concentrations of 0.93nM to 15.6µM and with paraquat at concentrations of 9.3nM to 2.4µM for 24 hrs. had no significant loss of *via*bility. Cultures incubated with 50µM amiodarone and 100µM paraquat for 24 hrs. had a significant decline in cell *via*bility as compared to controls (approx.50%). and these concentrations were selected as the test concentrations for the study. For DNA fragmentation assay, paraquat treatment resulted in the formation of fragments that could be visualized by gel electrophoresis as a characteristic ladder pattern. In the caspase assay, after 24hr. amiodarone and paraquat exposure the concentration of DEVD-pNA cleavage product of the treated was found to have increased significantly above that of the control. The numbers of TUNEL positive cells were greater in treated groups as compared to controls. Amiodarone and paraquat appear to activate specific intracellular death-related pathways indicated by the caspase-3 enzyme activation. Such data support the hypothesis that dissimilar toxicants may cause lung injury by acting in part through an apoptotic pathway.

1615 SUPPRESSION OF TGF-β_1 INDUCED APOPTOSIS *IN VITRO* BY THE ALDOSE REDUCTASE INHIBITOR, ZOPOLRESTAT.

L. Fasulo and D. Amacher. *Safety Sciences - Groton, Pfizer Global Research and Development, Groton, CT.*

The aldose reductase inhibitor (ARI) zopolrestat was developed for the treatment of late-stage diabetic complications such as neuropathy, nephropathy, cataractogenesis, and macrovascular disease. Recently, some ARIs were shown to prevent apoptosis in lens epithelial cells (Murata et al., Chemico-Biol. Interact. 130:617, 2001) and retinal pericytes (Naruse et al., Exp. Eye Res. 71: 309, 2000). Because apoptosis is readily studied in primary hepatocyte cultures, it was of interest to determine if zopolrestat had properties similar to these other ARIs. The present study was conducted in primary rat hepatocyte cultures to investigate the ability of zopolrestat to suppress apoptosis induced by transforming growth factor-β_1 (TGF-β_1). We have employed a working model for the experimental study of the induction or suppression of apoptosis *in vitro* using primary rat hepatocytes. Cells were cultured in collagen-coated flasks using serum-free hormone defined medium for 48 hours. The cultures were then treated with zopolrestat in the presence or absence of 1ng/ml TGF-β_1. Twenty-four hours later cell *via*bility was determined by trypan blue exclusion and apoptosis assessed using Hoechst 33258 staining method for nuclear morphology. In the presence of TGF-β_1, apoptosis increased 4-fold while the co-addition of 150µM zopolrestat significantly inhibited ($p \leq 0.001$) TGF-β_1 induced apoptosis. Further, zopolrestat alone suppressed intrinsic apoptosis as evident by its decrease in apoptosis compared with control cultures. In comparative studies, we have shown that TGF-β_1 caused up to a 7-fold increase in apoptosis in primary rat hepatocytes and that the hypolipidemic drug nafenopin (100µM) suppressed TGF-β_1 induced apoptosis. These *in vitro* data confirm that inhibition of apoptosis may be a common property of ARIs that is not confined to ocular tissues.

1616 THE ROLE OF DIMINISHED JNK SIGNAL TRANSDUCTION IN THE ACQUISITION OF APOPTOTIC RESISTANCE IN CADMIUM-TRANSFORMED HUMAN PROSTATE EPITHELIAL CELLS.

W. Qu[1], D. Broderick[1], W. E. Achanzar[1], M. M. Webber[2] and M. P. Waalkes[1]. [1]*Inorganic Carcinogenesis Section, NCI at NIEHS, Research Triangle Park, NC and* [2]*Departments of Zoology and Medicine, Michigan State University, East Lansing, MI.*

Cadmium (Cd) is a known human carcinogen that induces malignant transformation of human prostate epithelial cells upon chronic exposure. Our prior work shows these transformed prostatic cells (termed CTPE cells) acquire tolerance to the Cd cytotoxicity and become resistant to apoptosis. The present work sought to link Cd-induced apoptotic resistance to altered JNK signaling. After continuous exposure to 10 mM Cd for 8 weeks, CTPE cells became malignantly transformed, as confirmed by production of tumors upon inoculation into nude mice. Assessment of the cytotoxicity of Cd (as CdCl2) showed that the 24 hr LC50 in CTPE cells was 2.2-fold more than control cells, indicative of Cd tolerance. There was also a significant cross-tolerance to zinc in CTPE cells. Flow cytometry using annexin/FITC was used to evaluate apoptosis. Cd-induced apoptosis in transformed CTPE cells was markedly suppressed when compared to control cells. Apoptosis induced by the chemotherapeutics etoposide and cisplatin was also suppressed in CTPE cells. Immunocytochemical and densitometric protein analysis showed a dose-dependent decrease in phosphorylated JNK1/2 protein in CTPE cells. In addition, JNK kinase activity was suppressed in CTPE cells. RT-PCR analysis of RNA showed expression of the JNK1 and JNK2 genes in CTPE cells was reduced by 70% and 22%, respectively. A decrease in the expression of the apoptosis-related genes c-Jun and p-53 was also observed in CTPE cells, suggesting the involvement of these as factors in acquired resistance to apoptosis. These results indicate that Cd-transformed cells acquire a generalized resistance to apoptosis, and that the down-regulation of the JNK pathway plays an important role in tolerance. Generalized resistance to apoptosis may allow damaged cells to survive and promote tumor formation as a result of Cd exposure.

1617 CYTOPROTECTIVE EFFECTS OF GLYCYRRHIZAE RADIX EXTRACT AND ITS ACTIVE COMPONENT LIQUIRITIGENIN AGAINST CADMIUM-INDUCED TOXICITY.

K. Sangchan[1, 2], B. Sung Hui[1], Y. Chae Ha[1], K. Chul Young[2], K. Jin Woong[2] and K. Sang Geon[2]. [1]*College of Oriental Medicine, Daegu Hanny University, Daegu, South Korea and* [2]*College of Pharmacy, Seoul National University, Seoul, South Korea.*

Cadmium (Cd)-induced cell death mechanism, in which alterations in cellular sulfhydryls participate, involves Bad translocation, decrease in mitochondrial BclxL and cytochrome c, and PARP cleavage. Glycyrrhizae radix has been popularly used

as a frequently employed botanical in herbal medicine, and occupies an important place in food products. In the present study, we determined the effects of Glycyrrhizae radix extract (GRE) and its representative components on apoptotic and non-apoptotic cell death induced by Cd and explored the mechanistic basis of GRE protection against Cd-induced cell injury. Incubation of H4IIE cells with GRE (0.3-1 mg/ml) inhibited cell death induced by Cd. Also, GRE effectively blocked Cd-induced cell death potentiated by buthionine sulfoximine (BSO) without restoration of cellular GSH. GRE prevented both apoptotic and non-apoptotic cell injury induced by Cd or BSO+Cd. Inhibition of Cd-induced cell injury by pretreatment of cells with GRE suggested that the cytoprotective effect result from altered expression of the protein(s) responsible for cell *via*bility. GRE inhibited mitochondrial Bad translocation by Cd or BSO+Cd and caused restoration of mitochondrial BclxL and cytochrome c levels. Cd-induced poly(ADP-ribose)polymerase (PARP) cleavage in control cells or cells with sulfhydryl deficiency was prevented by GRE treatment. Among the major components present in GRE, liquiritigenin, but not glycyrrhizin, isoliquiritigenin or liquiritin, was active for cytoprotection against Cd. These results showed that GRE blocked Cd-induced cell death by inhibiting the apoptotic processes involving translocation of Bad into mitochondria, decreases in mitochondrial BcLxL and cytochrome c, and PARP cleavage.

1618 INTERACTION OF ARSENITE WITH B-CELL RECEPTOR- AND CD40- MEDIATED SIGNALING AND EFFECT ON APOPTOSIS IN B-LYMPHOMA CELLS.

D. Muscarella. *Cornell University, Ithaca, NY.*

Cell lines derived from human Burkitts lymphoma (BL) are an important *in vitro* model for receptor-mediated negative/positive selection of germinal center B-lymphocytes since they undergo apoptosis upon cross-linking of the surface IgM/B-cell receptor (sIgM) and are rescued from sIgM-induced apoptosis by ligation of CD40. These cell lines are also highly sensitive to the induction of apoptosis by many chemicals, including sodium arsenite, a significant environmental contaminant with immunotoxic activity. The purpose of this study was to identify the interactions of arsenite exposure with sIgM- and CD40- mediated signaling and subsequent effects on apoptosis induction. I found that cross-linking of sIgM initially provided protection against the early induction of apoptosis by arsenite. Specifically, sIgM-activation protected against arsenite-induced mitochondrial depolarization and the cleavage of caspase 9 and poly (ADP) ribose polymerase. The sIgM-mediated protection against apoptosis required the activation of two signaling pathways, the extracellular-signal regulated kinase (ERK) and the phosphoinositide-3 kinase (PI3-K) pathways. Inhibition of either pathway partially blocked the ability of sIgM-activation to protect against apoptosis induction. However, sIgM-mediated protection was transient, and decayed over a period of several hours, consistent with a model in which initial engagement of sIgM induces an immediate and potent anti-apoptotic response, but in the absence of appropriate costimulation (i.e. by CD40 engagement) the protective signals decay and the cells undergo apoptosis. Importantly, I also found that arsenite blocked the ability of CD40 to rescue BL cells from sIgM-mediated apoptosis by interfering with an important pro-survival pathway activated by CD40 ligation, the nuclear factor kappa-B pathway. Moreover, I have identified several key regulatory points within this pathway that are differentially sensitive to higher (≥ 200 μM) compared to lower (≤ 20 μM) concentrations of arsenite. (Supported by NIEHS grant ES-10815)

1619 MECHANISMS OF OCHRATOXIN A-INDUCED APOPTOSIS IN HUMAN LYMPHOCYTES.

H. Assaf[1, 2], H. Azouri[2] and M. Pallardy[1]. [1]*INSERM U461, Chatenay-Malabry, France and* [2]*Toxicology laboratory, Faculty of Pharmacy, Beirut, Lebanon.*

Mycotoxins are a group of fungal secondary metabolites that elicit a wide spectrum of toxicological effects. Of particular interest is ochratoxin A (OTA), a potent mycotoxin contaminating feed and food, which has been implicated in nephropathy in animals and humans in the Balkan states. In addition to being a nephrotoxin, animal studies indicate that OTA is a liver toxin, an immune suppressant, a potent teratogen and a carcinogen. The hazard that OTA poses to human health arises from both its widespread occurrence and its long plasma half-life of 35.5 days. All these toxicities coupled with its ability to be carried through the food chain, merit some concern. The aim of this study was to evaluate the apoptotic effects of OTA on human lymphocytes. We used two models: human peripheral blood lymphocytes (PBL) and the lymphoid T cell line, Kit 225. PBL, activated or not by phytohemagglutinin, as well as Kit 225 cells, underwent apoptosis in a time- (7, 24 and 48 h) and OTA dose- (10-6, 5x10-6 and 10-5 M) dependent manner. Moreover, OTA triggered mitochondrial membrane permeabilization, as shown by 3, 3' dihexyloxacarbocyanine iodide (DiOC6)/propidium iodide staining. Apoptosis was inhibited by z-VAD.fmk, a pan-caspase inhibitor, suggesting that caspases are responsible for the induction of apoptosis. Caspase-8 was not activated which suggest that the death receptor (Fas, TNF-R) pathway was not implicated. Conversely, caspase-9 and caspase-3 were activated. Interestingly, the Bcl-xL protein expression was decreased by OTA treatment as shown by western blot. On the contrary, the Bcl-2 protein was not affected. On the other hand, overexpression of Bcl-xL in Kit 225 cells protected them against mitochondrial perturbation and blocked OTA-induced apoptosis. These data suggested that mitochondria are a central component in OTA-induced apoptosis, and the loss of Bcl-xL due either to the degradation of the protein or to transcriptional downregulation of the bcl-xL mRNA was responsible for the OTA-induced cell death.

1620 THE ROLE OF APOPTOSIS IN THE OTA/OTB INDUCED NEPHROPATHY.

K. Kobras, E. O'Brien, A. H. Heussner and D. R. Dietrich. *Environmental Toxicology, University of Konstanz, Konstanz, Germany.*

The mycotoxins ochratoxin A (OTA) and ochratoxin B (OTB) are regularly found in human and animal food. Long-term exposure to OTA causes nephropathy and renal tumors in animals and is also associated with Balkan Endemic Nephropathy (BEN) and urothelial tumors in humans. Previous *in vitro* studies indicated a role for apoptosis in these processes. In the present study, the LLC-PK1 and NRK-52E cell lines were exposed to 0-50 μM OTA and OTB for up to 48 hours in the presence of serum. Standard assays revealed significant cytotoxicity after exposure to 50 μM OTA or OTB. Hoechst staining yielded no evidence of apoptosis with the same exposure regimen. In contrast, DNA-laddering was apparent after exposure of the LLC-PK1 cells to 25-50 μM OTA for 16 to 24 h. Interestingly, laddering could only be observed in cells which detached during OTA incubation. No DNA-fragmentation could be seen in adherent cells. OTB exposure did not result in apoptotic alterations in LLC-PK1 cells and neither method yielded evidence of apoptosis in NRK-52E cells following exposure to OTA or OTB. These results suggest OTA and OTB to have different mechanisms of action and furthermore, that the pro-apoptotic action of OTA is cell-type- or species-specific.

1621 DIELDRIN EXPOSURE IMPAIRS THE UBIQUITIN-PROTEOSOME FUNCTION AND PROMOTES α-SYNUCLEIN AGGREGATION IN MESENCEPHALIC DOPAMINERGIC NEURONAL CELLS: RELEVANCE OF ENVIRONMENTAL FACTORS IN THE ETIOPATHOGENESIS OF PARKINSON'S DISEASE.

F. Sun, V. Anantharam and A. Kanthasamy. *Biomedical Science, Iowa State University, Ames, IA.*

Exposure to pesticides has been linked to the etiopathogenesis of Parkinson's disease. Recently, we showed that dieldrin induces apoptosis in dopaminergic cells *via* caspase-3-dependent proteolytic activation of proapoptotic protein kinase PKCδ. Abnormal accumulation and aggregation of α-synuclein and impairment of the ubiquitin-proteosome system have been suggested to contribute to the dysfunction, degeneration and ultimate death of dopaminergic neurons in PD. To further understand the cellular mechanisms underlying dieldrin-induced dopaminergic neurodegeneration, we examined the effects of dieldrin on ubiquitin-proteosomal function in rat mesencephalic dopaminergic cells overexpressing human α-synuclein (α-SYN cells). Low dose (30 μM) dieldrin induced time-dependent cytotoxicity over a 24 hr period in α-SYN cells compared to vector expressing cells. α-SYN cells were slightly resistant to dieldrin toxicity up to 12 hr post exposure but were more sensitive at 24 hr. Dieldrin treatment for 24 hr also resulted in a significant increase in caspase-3 enzyme activity in α-SYN cells. Dieldrin treatment significantly increased the proteosomal activity at 3 hr in vector cells but not in α-SYN cells. At a later time point (24 hr), a significant reduction in proteosomal activity was observed in both vector and α-SYN cells, however, the impairment of proteosomal function was more pronounced in α-SYN cells than in vector cells. Immunocytochemical analysis of dieldrin-treated α-SYN cells showed aggregation of α-synuclein in a time-dependent manner, with small aggregates appearing as early as 6 hr and progressively increasing over a 24 hr. The proteosomal inhibitor lactacystin also induced a profound aggregation in α-SYN cells. Overall, these results suggest that α-SYN cells are more sensitive to dieldrin-induced apoptosis and that impairment of the ubiquitin-proteosome function may contribute to dieldrin-induced α-synuclein aggregation [NIH grant NS 045133].

1622 EFFECTS OF ENDOSULFAN AND PERMETHRIN EXPOSURE ON APOPTOTIC AND NECROTIC CELL DEATH OF MURINE SPLENOCYTES, *IN VITRO*.

V. Vemireddi[1] and H. P. Misra[1, 2]. [1]*Biomedical Sciences and Pathobiology, Virginia Maryland Regional College of Veterinary Medicine, Virginia Tech, blacksburg, VA and* [2]*Biomedical Science, Edward Via Virginia College of Osteopathic Medicine, Blacksburg, VA.*

The pesticides endosulfan and permethrin negatively affect the immune system in non-target organisms. We hypothesized that these pesticides cause immune suppression, at least in part, by enhanced apoptotic cell death. Splenocytes from adult

C57Bl/6 male mice were exposed to endosulfan, or permethrin or mixtures of these chemicals, *in vitro*. Cytotoxicity was evaluated using flow cytometer in combination with 7-amino-actinomycin D (7-AAD) staining. Endosulfan decreased the *via*bility (47-22%) in a dose-dependent manner (25-150 μM) when exposed for 12 hours. A dose-dependent increase in early apoptotic (28-32%) and late apoptotic/necrotic (25-46%) cell populations was also obsreved with endosulfan exposures. Endosulfan at 100 μM also caused a time-dependent (4-12 hours) decrease in live cells (71-30%), and an increase in early apoptotic (15-33%) and late apoptotic/necrotic (14-36%) populations. Permethrin exposure resulted in neither a time-dependent/dose-dependent loss of splenocyte *via*bility nor an induction of apoptosis in splenocytes. With mixtures of permethrin and endosulfan, depressed *via*bility and enhanced early apoptosis and late apoptosis/necrosis were also observed. Exposure to mixtures of 50 μM endosulfan with 50 or 100 μM permethrin increased the late apoptosis/necrosis compared to the results of exposure to either chemical alone. Visual evaluation of cell death using DNA ladder assay confirmed the contribution of both apoptotic and necrotic processes. These findings suggest that the immunotoxicity of endosulfan both individually and in mixtures with permethrin is associated with the occurrence of early and late apoptotic/necrotic processes.

1623 GENE EXPRESSION ANALYSIS DURING DIELDRIN EXPOSURE IN RAT MESENCEPHALIC DOPAMINERGIC NEURONAL CELLS: POTENTIAL MECHANISMS INVOLVED IN ENVIRONMENTAL CHEMICAL-INDUCED DOPAMINERGIC DEGENERATION.

Y. yang, K. D. Petry, A. Vellareddy, A. Kanthasamy and A. Kanthasamy.
Biomedical Sciences, Iowa State University, Ames, IA.

Environmental chemicals have been linked to the etiology of Parkinson's disease. Previously we demonstrated that dieldrin, a lipophilic organochlorine pesticide, can induce apoptosis in dopaminergic neuronal cells through proteolytic activation of protein kinase C delta (PKCδ). To fully understand the potential mechanisms of dieldrin-induced apoptotic death in dopaminergic neuronal cells, genomic gene expression analysis was performed using Affymetrix oligonucleotide microarray chips. Rat mesencephalic dopaminergic neuronal cells were exposed to either vehicle or 100 μM dieldrin for 6 hr and total RNA was isolated and labeled for hybridization with oligonucleotide gene chips. The hybridization signals were analyzed and compared in the vehicle and dieldrin-treated groups. A total of 40 genes were up-regulated and 33 genes were down-regulated. The following genes were increased by at least 4 fold: heme oxygenase (16 fold), an enzyme involved in oxidative stress; GRP78 (10 fold), an endoplasmic reticulum (ER) chaperone usually binding to unfolded protein to reduce ER stress; GADD153 (10 fold), a transcription factor C/EBP homologous protein-10; p38 MAP kinase (4 fold), a MAP kinase associated with cell death; MAP kinase phosphatase (10 fold), regulator of MAP kinase activation and Fra-1 (64 fold), a stress-induced immediate early gene. The genes that were down-regulated include: Mx3 (16 fold), DNA polymerase α(16 fold), Agrin (8 fold), etc. We also verified the transcription and translation of some of the above genes by using RT-PCR and Western blotting, respectively. Together, dieldrin treatment increases some key molecules that are associated with ER stress and apoptotic cell death. Greater understanding of the interaction of these signaling proteins in the orchestration of apoptotic cell death may provide new insights into mechanisms of environmental factor-induced dopaminergic degeneration (NS 45133 and ES 10586)

1624 DETERMINATION OF APOPTOSIS IN PLATEABLE CRYOPRESERVED HUMAN HEPATOCYTES.

T. A. Moeller, P. M. Silber and N. S. Jensen. *In Vitro Technologies, Baltimore, MD.*

Cryopreservation of human hepatocytes has provided the opportunity for study of toxicity and drug metabolism of compounds without the need to wait for fresh tissue. Some cryopreserved hepatocytes have the ability to form an adherent monolayer when thawed and plated on collagen-coated tissue culture plates. Previous work with these plateable cryopreserved human hepatocytes (PCHH) has demonstrated their usefulness for long-term (4 day) toxicity studies for necrosis. In this work we present data on the use of PCHH for evaluating chemically-induced apoptosis. PCHH were plated on collagen coated 48-well flat bottom plates and incubated for 24 hours at 37°C, 5% CO_2 in a humidified incubator. After monolayers were established, they were treated with camptothecin (100 nM, 1 μM, 10 μM and 100 μM) in serum–free medium for 4, 24 and 48 hours. Three different endpoints were determined; MTT assay for necrosis, and caspase 3/7 and DNA fragmentation for apoptosis. After 4 hours, no necrosis or apoptosis was identified by any of the assays. However, a significant increase in apoptotic markers was identified at 24 hours in both caspase 3/7 and DNA fragmentation assays. After 24 hours, at camptothecin levels of 10 and 100 μM, caspase 3/7 levels increased 4 fold, and DNA fragmentation levels increased 3–4 fold. At 48 hours, caspase 3/7 levels increased 7–8 fold, while DNA fragmentation results increased 2.5 fold. Small increases in caspase 3/7 and DNA fragmentation were seen at 1 μM camptothecin, with little or no increase at 100 nM. Viability, as measured by MTT, demonstrated little or no decrease after 48 hours at camptothecin levels of 100 nM and 1 μM, but decreased with 10 and 100 μM concentrations after 24 hours (50% *via*ble) and 48 hours (<25 % *via*ble). Camptothecin induced apoptosis in a time-dependent and concentration-dependent manner as indicated by both caspase 3/7 and DNA fragmentation assays. These results indicate that PCHH is a useful system for evaluating the ability of unknown compounds to initiate apoptosis in human hepatocytes.

1625 CELL-CELL CONTACT IS REQUIRED FOR PAH-INDUCED, STROMAL CELL-DEPENDENT, BONE MARROW B CELL APOPTOSIS.

D. H. Sherr, H. Ryu, J. K. Emberley, J. J. Schlezinger and L. L. Allan.
Environmental Health, Boston University School of Public Health, Boston, MA.

Polycyclic aromatic hydrocarbons (PAHs) and related environmental aryl hydrocarbon receptor (AhR) agonists are immunotoxic in a variety of *in vitro* and animal model systems. In a model system of B lymphopoiesis, PAH exposure rapidly induces CD43- pre-B and CD43+ pro/pre-B cell apoptosis. Apoptosis induction by DMBA is dependent upon AhR+ bone marrow stromal cells and likely involves DMBA metabolism. However, it was not known if PAH-treated stromal cells elaborate metabolites or soluble factors that may directly induce B cell death. In the present study it was demonstrated that supernatants from 7, 12-dimethylbenz(a)anthracene (DMBA)-treated stromal cells contained an activity capable of inducing apoptosis in pro/pre-B cells co-cultured with cloned stromal cells. This activity: 1) was produced at concentrations > 10-7 M DMBA, 2) was not produced when stromal cells were co-treated with DMBA and a-naphthoflavone, an AhR and cytochrome P450 inhibitor, 3) was retained by filtration through 50 kDa exclusion filters, 4) was ablated by trypsin or heat treatment, 5) did not directly interact with pro/pre-B cells to induce their death, 6) was partially dependent on the presence of a functional AhR in stromal cells supporting pro/pre-B cell growth, and 7) was able to induce AhR-regulated, CYP1A1 mRNA. The results: 1) argue against the likelihood of a stromal cell-derived metabolite or death factor which acts directly on developing B lymphocytes, 2) suggest the production of a relatively stable DMBA metabolite-protein complex capable of initiating a change in AhR+ stromal cell function at some distance, and 3) support the hypothesis that a DMBA-dependent death signal is delivered by stromal cells to neighboring B cells by cell-cell contact.

1626 DMBA-INDUCED PRO/PRE-B CELL APOPTOSIS: CASPASE 8-MEDIATED, TNF RECEPTOR-INDEPENDENT SIGNALING PATHWAYS.

J. K. emberley[1], H. Ryu[2], L. L. Allan[1], J. J. Schlezinger[2] and D. H. Sherr[2, 1].
[1]Microbiology, Boston University Sch. of Medicine, Boston, MA and [2]Environmental Health, Boston University Sch. of Medicine, Boston, MA.

PAH are common environmental pollutants which suppress the immune system in part by inducing bone marrow B cell apoptosis. Previously, we demonstrated that prototypic PAHs, including DMBA, induce pro/pre-B cell apoptosis when these cells are co-cultured with cloned bone marrow stromal cells (BMS2). The purpose of this study was to determine if DMBA-induced B cell apoptosis involves the initiator caspase 8 and/or TNF receptors which can activate caspase 8. Kinetics studies indicated that caspase 8 was induced within 4 hours of DMBA treatment while caspase 3 was not activated until 8-12 hours after DMBA treatment. Inhibitors of caspase 8 and effector caspase 3 almost completely protected pro/pre-B cells from DMBA-induced death. Since DMBA-induced pro/pre-B cell death required B cell-stromal cell contact, and since caspase 8 activation has been linked to TNFR signaling, the possibility that stromal cells deliver death signals through TNF-TNFR interactions was investigated. Vehicle- or DMBA-treated BMS2 cells did not make detectable levels of TNF-α, -β, or LT-β mRNA and/or protein. Furthermore, DMBA-treated stromal cells from TNF-α knock out mice were as effective at killing pro/pre-B cells as wildtype stromal cells. To assess whether caspase 8 activation was mediated by ligand-independent TNFR activation, populations of primary pro/pre-B cells from $TNFR1^{-/-}/2^{-/-}$ double mutant mice were expanded in rIL-7 and then co-cultured with BMS2 cells. Addition of DMBA induced the same percentage of $TNFR1^{-/-}/2^{-/-}$ B cells to undergo apoptosis as with wildtype B cells. Collectively, these results indicate that: 1) the PAH-induced apoptosis pathway in pro/pre-B cells involves caspases 8 and 3 and 2) ligand-dependent or ligand-independent TNFR activation appears not to be responsible for caspase 8 activation. The possible role of other death receptors is under investigation.

1627 THE ROLE OF P53 IN DEATH RECEPTOR EXPRESSION AND ACTIVITY IN MOUSE TESTIS AFTER MONO-2-(ETHYLHEXYL) PHTHALATE (MEHP) EXPOSURE.

Y. Chandrasekaran and J. H. Richburg. *College of Pharmacy, The University of Texas at Austin, Austin, TX.*

Testicular germ cell apoptosis is preceded in different injury models by the up regulation of various death receptor (DR)/ligand-signaling pathways, most notably Fas/FasL and DR5/TRAIL. The expression of both Fas and DR5 can be transcriptionally up regulated by the tumor suppressor protein p53. This protein may also be capable of influencing the expression of the DRs on the cell membrane in a transcription-independent manner. The aim of our current work is to clarify the role of p53 in death receptor expression and its influence on germ cell apoptosis induced after exposure to the Sertoli cell toxicant MEHP (1 g/kg). We examined death receptor expression in p53 knock-out (p53-/-) mice and their wild type littermates (p53 / ; C57BL/6J-129S/v mice) after toxicant exposure. After MEHP exposure p53-/- mice did not show increases in Fas or DR5 protein by western blot analysis of membrane fractions of testis homogenates. On the other hand, early DR5 increases (1.5-6h) and later increases in Fas membrane levels (12h) can be observed in p53 / mice. Similarly, the processing of pro-caspase 8, an indicator of DR activity, was unchanged in testes of p53-/- mice. Interestingly after toxicant exposure, the lack of processing of pro-caspase 8 in the p53-/- mice coincided with an increased expression of the caspase 8 inhibitor, c-FLIP, as early as an hour after exposure. A robust expression of c-FLIP protein (by western blots) and mRNA expression (*via* RT-PCR) in p53-/- mice indicates an alternate mechanism by which germ cell apoptosis is modulated after MEHP exposure. Future studies are directed at further examining the influence of p53 on receptor activity that occur independent of its transcriptional activity. (Supported, in part by grants from NIH/NIEHS ES09145 & ES07784).

1628 THE PROTEASOME INHIBITOR, BORTEZOMIB, STIMULATES APOPTOSIS BY INDUCING ENDOPLASMIC RETICULAR STRESS AND PROTEOTOXICITY.

S. Nawrocki, J. S. Carew and D. J. McConkey. *Cancer Biology, UT M.D. Anderson Cancer Center, Houston, TX.*

The role of the proteasome in regulating cell growth, survival, and metastasis of cancer cells make it an attractive therapeutic target. Bortezomib (PS-341) is the first proteasome inhibitor for use in cancer therapy, whose antineoplastic effects have been attributed to inhibition of nuclear factor kB (NF-κB) activity. However, this only accounts for a small fraction of the anticancer activity of the drug. Inhibition of proteasomal activity also prevents the clearance of misfolded proteins by the endoplasmic reticulum associated degradation (ERAD) pathway, resulting in endoplasmic reticular (ER) stress. ER stress activates signaling pathways that are collectively termed the unfolded protein response (UPR), which are required if the cell is to survive the stress. The UPR upregulates ER chaperone proteins (BiP) to increase protein-folding activity, attenuates translation by activation of PKR-like endoplasmic reticulum kinase (PERK) to reduce ER load, and increases protein degradation *via* ERAD. We hypothesize that proteasome inhibition causes ER stress-mediated apoptosis, which contributes to its anti-tumor activity. The pancreatic cancer cell line, L3.6pl, was selected to test this hypothesis. Consistent with known ER stress agents, bortezomib treatment resulted in an increase in BiP expression, indicating that proteasome inhibition results in ER stress. However, bortezomib treatment prevented phosphorylation of the PERK substrate, eukaryotic initiation factor 2a (eif2a), which is necessary for translational attenuation. Bortezomib treatment inhibits protein degradation and translational control resulting in an accumulation of proteins, which induces proteotoxicity. This toxicity induces apoptosis by activation of the cJUN NH2-terminal kinase (JNK) pathway and cleavage of caspase-3. Inhibition of translation by cyclohexamide treatment completely abrogates bortezomib-induced apoptosis. Therefore, the induction of proteotoxicity by bortezomib contributes significantly to the cytotoxic activity of the drug.

1629 THE ASSOCIATION OF APOPTOSIS WITH THE INDUCTION OF NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (NGAL).

J. P. Kehrer, Z. Tong and X. Wu. *Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.*

We previously identified a potential link between the induction of the murine lipocalin 24p3 by the 5-lipoxygenase activating protein inhibitor MK886 and apoptosis [Biochem. J. **372**: 203-210 (2003)]. The human homolog of 24p3 is NGAL, and the current study was designed to determine if a similar association exists in human cells. Human adenocarcinoma A549 cells treated with MK886 exhibited a dose- and time-dependent increase of NGAL expression that correlated with the extent of apoptosis. A similar induction of NGAL was observed in human breast cancer MCF7 cells. Treatment of MCF7 cells with 4 μg/ml of vitamin E succinate, another apoptosis inducing xenobiotic, also increased NGAL expression. The induction of NGAL in A549 cells was not *via* the alteration of the DNA binding activity of NF-κB. Treatment of A549 cells with purified NGAL had no effect on apoptosis. These data indicate that the induction of NGAL correlates with apoptosis, but that extracellular NGAL is not toxic. It remains possible that NGAL acts intracellularly or that NGAL/24p3 does not directly activate apoptosis pathways. It is also possible that 24p3/NGAL functions as a survival factor and the induction is a compensatory response to inhibition by these xenobiotics. (Supported by CA83701 and Center Grant ES07784.)

1630 AKT PLAYS A ROLE IN THE APOPTOSIS INDUCED BY THE 5-LIPOXYGENASE ACTIVATING PROTEIN (FLAP) INHIBITOR, MK886.

Z. Tong, X. Wu and J. P. Kehrer. *Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.*

MK886, a FLAP-inhibitor, induces apoptosis independently of both FLAP and lipoxygenase activities [Biochem. J. **340**: 371-375 (1999)]. The apoptosis-inducing mechanism of MK886 remains unclear. The serine/threonine kinase Akt plays a crucial role in cell proliferation and survival, particularly by preventing cells from undergoing apoptosis. In the present study, we examined the possible role of the Akt pathway in MK886-induced apoptosis. Exposure of cells to an apoptosis-inducing dose of MK886 inhibited IL3-induced and serum-induced Akt phosphorylation at Ser473 in murine pro-B FL5.12 and human Jurkat T cells, respectively. Treatment of FL5.12 cells with 0.5 μM of wortmannin, a PI3K inhibitor that will block upstream activation signals to Akt, significantly enhanced MK886-induced apoptosis. Furthermore, FL5.12 cells stably expressing constitutively active Akt were resistant to MK886-induced apoptosis and caspase-3 activation. In contrast, these cells were not resistant to etoposide-induced apoptosis. These results suggest that MK886 may inhibit the Akt pathway, at least partially explaining its ability to induce apoptosis. Similar mechanisms appear to apply with cyclooxygenase-2 inhibitor and related compounds that induce apoptosis independently of COX-2 activity. (Supported by CA83701 and Center Grant ES07784. Thanks are extended to Dr. David Plas, University Pennsylvania, for providing the Akt-FL5.12 cells.)

1631 α-SYNUCLEIN PREVENTS MITOCHONDRIAL AND NUCLEAR LOCALIZATION OF PRO-APOPTOTIC KINASE PKCδ DURING 1-METHYL-4-PHENYLPYRIDINIUM (MPP^+)-INDUCED CELLULAR APOPTOSIS IN DOPAMINERGIC NEURONAL CELLS. NOVEL ROLE OF α-SYNUCLEIN IN DOPAMINERGIC DEGENERATION.

S. Kaul, V. Anantharam and A. Kanthasamy. *Iowa State University, Ames, IA.*

We previously demonstrated that MPP^+ induced cellular apoptosis in rat mesencephalic dopaminergic neuronal cells (N27) *via* the proteolytic cleavage of PKCδ(Kaul *et al.*, 2003) and that the apoptotic cell death was completely attenuated by the expression of human wild-type α-synuclein (α-SYN) in these cells (Kaul *et al.*, 2002). In the present study, we investigated the cellular mechanisms involved in the protective effect of α-SYN against MPP^+-induced neuronal apoptosis. Expression of α-SYN in N27 cells significantly attenuated MPP^+(300 μM)-induced proteolytic activation of pro-apoptotic kinase PKCδ. Sub-cellular fractionation studies showed that PKCδ was proteolytically cleaved in VEC cells and the cleaved fragments translocated to the mitochondrial and nuclear compartments in a time-dependant manner. Interestingly this MPP^+-induced sub-cellular translocation of PKCδ was completely absent in α-SYN cells at all exposure periods. Co-immunoprecipitation analysis in the α-SYN cells revealed a physical association between α-SYN and PKCδ in untreated cells which was further increased following MPP^+ exposure. Additionally, MPP^+ treatment induced parallel increases in the association of α-SYN and the pro-apoptotic protein BAD, but none with the anti-apoptotic protein Bcl-2. These results suggest that α-SYN preferentially associates with the proapoptotic proteins PKCδand BAD. MPP^+ treatment also led to a increased translocation of cytosolic α-SYN to the nucleus over a 24 hr period, declining thereafter. Together, our results suggest that human wild-type α-SYN rescues dopaminergic neuronal cells from MPP^+-induced apoptotic cell death by acting as a molecular chaperone to regulate the proapoptotic functions of PKCδand BAD. Furthermore, translocation of α-SYN to the nucleus during MPP^+ treatment suggests that α-SYN may also function as a nuclear modulator protein during environmental neurotoxin exposures.[Supported by NIH grants ES10586 and NS38644].

1632 ROLE OF PROTEIN KINASE Cδ IN SILICA-INDUCED APOPTOSIS AND AUTOIMMUNITY.

J. M. Brown and A. Holian. *CEHS, University of Montana, Missoula, MT.*

Epidemiological studies have shown associations between silica exposure and several autoimmune diseases, including scleroderma and systemic lupus erythematosus. We have previously reported that lupus-prone New Zealand mixed mice develop an exacerbated autoimmune disease following exposure to crystalline silica. Fourteen weeks following exposure to silica, NZM mice develop high levels of autoantibodies to nuclear antigen. These mice also develop high levels of proteinuria due to increased immune complex and complement deposition within the glomerulus of the kidney. Silica exposure increases the level of pulmonary fibrosis and alters the numbers of T helper cells, B1a B cells and regulatory T cells. We have also reported that autoantibodies from silica-exposed mice specifically recognize apoptotic alveolar macrophages. Protein Kinase Cδ has been reported to induce apoptosis in many cell types and by several different stimuli. Recently, many reports have implicated apoptosis as playing a significant role in the development of several autoimmune diseases, therefore an increase in apoptotic proteins following silica exposure may provide a possible mechanism for the exacerbation of autoimmunity in NZM mice. In the present study, we are examining the activation of PKCδ in alveolar macrophages following silica exposure. PKCδ RNA and protein is increased 2-fold within the AMs of silica treated NZM mice. Lung sections obtained 14 weeks following silica exposure in NZM mice show increased staining of PKCδ and the staining appears to occur within the AM population as demonstrated by colocalized staining of PKCδ and F4/80. *In vitro*, using bone marrow derived macrophages, silica exposure increases the level of PKCδ within 2 hours following exposure. This increase in PKCδ at 2 hours also correlates with an increase in caspase-9 within 2 hours following silica exposure. Therefore, silica exposure appears to induce apoptosis by the activation of PKCδ which leads to activation of caspase-9 and the subsequent apoptosis may expose excess antigen to the immune system allowing the exacerbation of systemic autoimmune disease.

1633 MECHANISMS OF NORDIHYDROGUAIARETIC ACID (NDGA)-MEDIATED APOPTOSIS IN FL5.12 CELLS.

V. S. Deshpande and J. P. Kehrer. *Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.*

NDGA is a general lipoxygenase (LOX) inhibitor that induces apoptosis in a variety of cells, including human Jurkat T lymphocytes and FL5.12 cells (murine pro-B lymphocytes), independent to its role as a LOX inhibitor. Effects on various kinases may be involved in this pro-apoptotic effect. Western immunoblotting experiments showed that treatment of FL5.12 cells with 10 μM NDGA leads to phosphorylation of extracellular signal-regulated kinases (ERK1/2), peaking at 1 h post-treatment. Although ERK1/2 is mainly known as a pro-survival protein there is evidence that activation of ERK is involved in apoptosis [Cancer Res. **61**: 6569-6576 (2001)]. Consistent with this report, pre-treatment of FL5.12 cells with an ERK1/2 inhibitor, PD98059 (50 μM), followed by 10 μM NDGA, attenuated the apoptosis induced by NDGA. Another kinase signaling system involved in cell survival is the PI-3K/Akt pathway. Akt phosphorylation and activation is protective to cells as this kinase then phosphorylates and inactivates various pro-apoptotic substrates including ASK1 and members of the Bcl-2 family. Inhibition of the PI-3K/Akt pathway usually drives cells to apoptosis. NDGA treatments that induced apoptosis inhibited phosphorylation of Akt. In addition, pre-treatment of FL5.12 cells with the PI-3K inhibitor wortmannin (100 nM) enhanced NDGA-mediated apoptosis. These data support the hypothesis that NDGA-mediated apoptosis involves both activation of ERK1/2 as well as inhibition of the Akt survival pathway. These data further suggest a possible crosstalk between the MAP kinase and the PI-3K pathways. (Supported by CA83701 and Center Grant ES07784).

1634 ENVIRONMENTAL STRESS-MEDIATED SENSITIZATION OF B-LYMPHOID CELLS TO PESTICIDE-INDUCED APOPTOSIS AND INDUCTION OF MAP KINASE PATHWAYS.

S. E. Bloom and D. E. Muscarella. *Microbiology and Immunology, Cornell University, Ithaca, NY.*

Lymphocytes, at different stages of differentiation, can differ in resistance to drugs and environmental chemicals depending on expression level of Bcl-2 and other apoptotic proteins. Furthermore, cell resistance may be altered as a result of exposure to multiple environmental stresses. We have shown previously that a stress resistant B-lymphoid cell line, overexpressing Bcl-2, can be sensitized to arsenic-induced apoptosis following exposure to sub-lethal heat stress. However, it is unknown whether resistant B-cell lines can be sensitized to other classes of environmental chemicals. Therefore, we investigated whether the EW36 resistant B-cell line can be sensitized by heat stress to pesticide-induced apoptosis. The involvement of the c-jun N-terminal kinase (JNK) pathway in regulating enhanced apoptosis induction by the combination treatments was also investigated. EW36 cells were exposed to sub-lethal heat stress for 1 hr and then treated with 0, 5, 10, 25, 50 micromolar antimycin A (piscicide) or pyridaben (miticide) and incubated at 37 C. Cells were harvested at 2 hr post-treatment for studies of JNK activation and at 24 and 48 hours for c-jun expression and apoptosis induction. Exposure of EW36 cells to heat or antimycin individually failed to activate JNK or induce apoptosis. However, the heat plus antimycin combination treatment activated JNK, induced c-jun expression, and induced substantial apoptosis. An AP-1 inhibitor blocked apoptosis induction by the combination of heat plus antimyicn, and c-jun expression was not induced. The heat plus pyridaben combination treatment also induced substantial apoptosis compared to controls, but this effect was independent of activation of the JNK/c-jun pathway. These findings show that heat stress can sensitize resistant EW36 B-cells to pesticide-induced apoptosis, but the particular pesticide exposure determines whether the JNK/c-jun pathway will be activated. This signaling pathway, along with Bcl-2, may play an important role in regulating apoptotic cell death induced by pesticides.

1635 THIOREDOXIN AND TGHQ-INDUCED APOPTOSIS IN HL-60 CELLS.

M. Yang, S. S. Lau and T. J. Monks. *Pharmacology and Toxicology, University of Arizona Health Sciences Center, Tucson, AZ.*

2, 3, 5-*tris*-(Glutathion-S-yl)hydroquinone (TGHQ) is present in the bone marrow of rats and mice co-treated with hydroquinone and phenol, hematotoxic metabolites of benzene. TGHQ is also hematotoxic in rats, and induces DNA damage, growth arrest, and apoptosis in human promyelocytic leukemia (HL-60) cells. During TGHQ-induced apoptosis of HL-60 cells reactive oxygen species (ROS) generation occurs, with concomitant deceases in cellular glutathione (GSH). However, although co-treatment of HL-60 cells with catalase protects against TGHQ-induced apoptosis, it does not prevent depletions in cellular GSH. We have now investigated whether other cellular thiol pools, specifically thioredoxin (TRX), modulate TGHQ-induced apoptosis. Western blot analysis and flow cytometry revealed that TGHQ (200 μM) treatment of HL-60 cells decreased TRX levels. Consistent with this finding, c-Jun N-terminal kinase (JNK) and p38 MAPK were activated in response to TGHQ. Interestingly, although catalase treatment did not block TGHQ-induced depletions of cellular GSH, it did prevent decreases in TRX levels. Although cytochrome *c* is released from mitochondria, concomitant with the dephosphorylation of pS70 Bcl-2 and the translocation of Bax to mitochondria 2h after TGHQ treatment, $\Delta\Psi_m$ remained intact for at least 4h after treatment. The data indicate that TGHQ facilitates ROS production, which causes subsequent decreases in cellular TRX, which may be subsequently coupled to the apoptosis signal-regulating kinase 1 (ASK1) - JNK/p38 apoptosis signaling pathway, culminating in the release of cytochrome *c* without interruptions in $\Delta\Psi_m$ (ES09224).

1636 THE FATE OF INSTILLED APOPTOTIC MACROPHAGES IN THE LUNGS.

R. R. Mercer, J. Scabilloni, J. Antonini, V. Castranova and L. Wang. *HELD, NIOSH, Morgantown, WV.*

Our previous studies of particle exposures have implicated abnormal accumulations of apoptotic macrophages and apoptotic bodies in lung injury from high burdens of low toxicity particles and low burdens of highly toxic particles. To determine how the lungs process abnormal accumulations of apoptotic cells, we have used intratracheal instillation of apoptotic or normal macrophages from autologous donor rats. We characterized the fate of instilled apoptotic and normal macrophages by prelabeling the cells with UV fluorescent beads (0.5 micron, bright blue, polystyrene). One million labeled cells were instilled and the animals sacrificed at 4 hrs, 1 day, 1 week or 4 weeks after instillation. The right lung of each animal was lavaged with PBS and the left lung was inflation fixed with cryopreservative. Cytospins of lung lavage and cryosections of lung were stained for apoptotic cells with yellow-green fluorescence labeled nucleotides by terminal deoxytransferase (TdT). At 4 hours, 50% of the microsphere labeled, apoptotic cells which had been instilled were phagocytized. By 1 day, greater than 90% of the instilled apoptotic macrophages were phagocytized. There were no TdT positive nuclei in microsphere labeled cells at one week. No microsphere labeled, normal macrophages were found to be phagocytized at any time point. TdT positive cells in the lungs receiving normal

macrophages was too low to quantify. The results demonstrate that instilled apoptotic macrophages are rapidly ingested and processed by resident alveolar macrophages while otherwise normal macrophages are not.

1637 APOPTOTIC CELL INSTILLATION RESULTS IN ELEVATED TGF-β AND APOPTOSIS-INDUCED-APOPTOSIS IN RAT LUNG.

L. Wang[1], J. Scabilloni[1], J. Antonini[1], Y. Rojanasakul[2], V. Castranova[1] and R. R. Mercer[1]. *[1]PPRB, NIOSH, Morgantown, WV and [2]School of Pharmacy, West Virginia University, Morgantown, WV.*

Results from animal inhalation with either high burden or highly toxic particles showed increased apoptosis in the lungs. Previous studies by our group indicated that pulmonary administration of apoptotic lung cells induced inflammatory cell influx and lung fibrosis, indicating a direct linkage between apoptosis and fibrotic lung disorders (Ref.1). In this study, we further characterized the kinetics of pulmonary response to apoptotic cell instillation in rats and investigated the possible role of TGF-β in this process. Rats instilled with apoptotic cells showed a decrease in lung cell apoptosis at 1 week post-treatment, consistent with our previous finding showing the clearance of labeled apoptotic cells in rat lungs after 1 week (Ref.2). However, the level of lung cell apoptosis was higher at 4 and 12 weeks post-treatment compared to the control groups instilled with normal non-apoptotic cells. These results suggest that the apoptotic cells observed at latter time points were not those originally instilled but were subsequently induced after the treatment. Analysis of BAL fluids from treated rats showed an increase in TGF-β expression at 1 and 4 week post-treatment compared to the control groups. TGF-β is a known inducer of apoptosis and a key mediator of lung fibrosis, our results therefore suggest that increased TGF-β by apoptotic cell instillation may be responsible for the increased lung cell death and subsequent development of pulmonary fibrosis. References: 1. Wang L, Antonini J, Rojanasakul Y, Castranova V, Scabilloni JF, Mercer RR. Potential role of apoptotic macrophages in pulmonary inflammation and fibrosis. Am. J. Cell. Physiol. 194:215, 2002 2. Robert R. Mercer James Scabilloni, James Antonini, Vincent Castranova and Liying Wang. The fate of apoptotic macrophages instilled into the lung. American Thoracic Society Annual Meeting, Baltimore, MD, March 2004.

1638 PERTURBATION OF TESTICULAR CELL PROLIFERATION USING SODIUM ARSENITE.

N. L. Harmon and J. W. DuMond. *Biology, Texas Southern University, Houston, TX.*

In this study we report the effects of sodium arsenite exposure on the cell proliferation of the normalized mouse Leydig cell line, TM3. The Leydig cells were grown in culture medium containing 5% horse serum, 2.5% fetal bovine serum. Cell cultures were switched to serum-free medium prior to treatment with test compound. The treatments consisted of 1pg, 10pg, 100pg, 1ng, 10ng, and 100ng per ml sodium arsenite, along with a control. Results indicated a significant ($p \leq 0.05$) increase in cell proliferation in all treatments with growth ranging from 119.79 to 191.80%, with the exception of 100ng/ml when compared to the control. Peak proliferation for sodium arsenite was noted at 10pg/ml (191.80%). The other growth was recorded as follows: 166.88% at 1pg/ml, 163.03% at 100pg/ml, 155.32% at 1ng/ml, 126.80% at 10ng/ml, and 119.79% at 100ng/ml. These results are of concern given that sodium arsenite has previously been reportrd to decrease DNA repair. Hence, when coupled with our findings here, a mechanism for the induction of genomic instability can be postulated. This then may explain the carcinogenic activity of sodium arsenite.

1639 ARSENIC ACTIVATES NADPH OXIDASE THROUGH CDC42 AND THEIR INVOLVEMENT IN ACTIN FILAMENT REMODELING AND CELL MOTILITY IN ENDOTHELIAL CELLS.

Y. Qian[1], D. C. Flynn[2], V. Castranova[1] and X. Shi[1]. *[1]The pathology and Physiology Research Branch, National Institute for Occupaptional Safety and Health, Morgantown, WV and [2]Microbology, Immunology and cell biology, West virginia University, Morgantown, WV.*

Although arsenic is a human cancinogen, the molecular mechanisms of its action remain to be understood. By stimulating mouse endothelial cell lines (SVEC4-10) with 10 μM arsenic, we found that arsenic induced activation of NADPH oxidase as determined by the nitroblue tetrazolium test. Using confocal microscopy, the generation of $O^{\cdot}_2$ and H_2O_2 was observed to be increased in SVEC4-10 cells after exposure to arsenic. We also found that arsenic activated Small Rho GTPase CDC42. Disruption of CDC42 with a dominant negative CDC42 abolished arsenic-induced activation of NADPH oxidase and subsequent generation of $O^{\cdot}_2$ and H_2O_2, suggesting that CDC42 may mediate the effects of arsenic on NADPH oxidase. Furthermore, it was found that arsenic stimulation induced actin filament remodeling and increased cell motility in SVEC4-10 cells. These changes were abrogated by either disruption of CDC42 or inhibition of NADPH oxidase, indicating that the cell signaling changes induced by arsenic are relevant to these cellular functions. Taken together, the data imply that arsenic may promote motility-dependent metastasis of cancer cells through CDC42, NADPH oxidase and reactive oxygen species.

1640 TRANSGENERATIONAL EFFECTS OF CHROMIUM(III) ON OFFSPRING WEIGHT, SERUM TIIODOTHYRONINE, AND HEPATIC GENE EXPRESSION.

R. Y. Cheng and L. M. Anderson. *Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick, MD.*

Exposing male mice to chromium(III) chloride before mating results in increased tumors in offspring. An epigenetic mechanism has been postulated, involving alterations in hormones and in gene expression in the offspring. In pursuit of this hypothesis, male Swiss mice were injected i.p. with 1 mmol/kg chromium(III) chloride, 2 wks before mating. Offspring (10 wks old) were weighed and assessed for serum triiodothyronine (T3)and hepatic gene expression by cDNA microarray analysis. Both male and female offspring of Cr(III)-treated fathers were significantly heavier than those of control fathers: 31.4 +/- 0.4 g (S.E.) (N = 62) vs 30.6 +/- 0.2 (N = 90) (P = 0.035) for males and 24.6 +/- 0.3 (N = 54) vs 23.0 +/- 0.3 (N = 54) (P = 0.0003) for females. Serum T3 levels were also greater in the offspring of Cr(III)-treated fathers: 107.7 +/- 2.0 ng/dl vs 95.6 +/- 3.4 for males ($P<0.0001$) and 96.4 +/- 2.2 vs 89.3 +/- 2.8 for females (P = 0.054). Microarray analysis was carried out on eight pairs of livers from female offspring of Cr(III)-treated and control fathers, chosen to represent serum T3 ratios over the range 1.04 to 2.68. Linear regression analysis selected for hepatic genes showing microarray ratios correlating with serum T3 ratios with a significance of $P <= 0.001$. Fifty-eight genes met this criterion, including 25 named genes. Several are known to be regulated by T3, e.g. beta 2 adrenergic receptor and the calcium-regulator phospholamban. Others may pertain to growth: protein tyrosine phosphatase 4a2, pancreatic colipase, and complement component 3a receptor 1. Expressions of several genes associated with tumor suppression were negatively associated with serum T3 ratios: the transcription factors Ikaros, zinc finger protein 161, and Kruppel-like factor 5, as well as single-strand DNA binding protein and natural killer tumor recognition sequence. These results are consistent with hormone-mediated effects of paternal Cr(III)-treatment on offspring physiology, and with an epigenetic mechanism of transgenerational carcinogenesis.

1641 INDUCTION OF THE HYPOXIA MARKERS, CARBONIC ANHYDRASE IX AND Cap43, BY NICKEL OR CELL DENSITY IS RELATED TO ASCORBATE DEPLETION.

A. A. Karaczyn[1], K. S. Kasprzak[1], S. Ivanov[2] and K. Salnikow[1]. *[1]Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, Frederick, MD and [2]SAIC, NCI-Frederick, Frederick, MD.*

Nickel compounds are known carcinogens and represent potential hazard for environmentally and occupationally exposed people. Mechanisms of nickel-induced carcinogenesis are currently unknown. Recently, we have shown that carcinogenic nickel compounds induced HIF-1 transcription factor and all known hypoxia-inducible genes. HIF-1 activation likely plays important an role in malignant transformation caused by nickel since stimulation of soft agar growth by nickel can be observed in HIF-1 alpha normal but not in knock-out cells. In this study we further investigated mechanisms of nickel-induced activation of hypoxia-inducible genes. We have found that both carbonic anhydrase IX (CAIX) and Cap43, known as intrinsic marker of hypoxia and a tumor marker, are also induced by exposure to soluble nickel compounds in human lung epithelial (HAE) cells. In cells exposed to 0.5 mM nickel(II) sulfate, the induction of both proteins was observed as early as after 2 hrs. The involvement of HIF-1 transcription factor in the induction of these proteins was also confirmed in cells exposed to cobalt(II), desferoxamine and dimethyloxoglycine (DMOG). Additionally, both CAIX and Cap43 were induced in HIF-1 alpha normal but not in knock-out cells. The induction of CAIX and Cap43 by nickel(II), cobalt(II) and desferoxamine, but not by DMOG, can be abolished by 0.1 mM of ascorbic acid added to culture media. These data suggest that nickel exposure may result in depletion of cellular ascorbate, leading to a shift in the iron oxidation status. CAIX and Cap43 could be also induced by high cell culture density. Here we show that the density-dependent induction of Cap43 and CAIX may be associated with the depletion of ascorbate in dense cultures.

1642 THE BENZENE METABOLITES HYDROQUINONE AND BENZOQUINONE INCREASE C-MYB ACTIVITY IN HD3 CELLS: AN INSIGHT INTO BENZENE MEDIATED LEUKEMOGENESIS.

J. Wan[1] and L. M. Winn[1, 2]. [1]*Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada and* [2]*School of Environmental Studies, Queen's University, Kingston, ON, Canada.*

Benzene, a ubiquitous environmental toxicant, has been proposed to lead to carcinogenesis. While the mechanism behind benzene mediated leukemogenesis remains unknown, it is generally accepted that benzene exerts its toxicity after being metabolized in the body by cytochrome P450s. During blood cell development, the transcription factor c-Myb plays an important role such that mice lacking expression of this protein die *in utero* and are anaemic. Furthermore, overexpression of c-Myb prevents differentiation of induced erythrocytes. Therefore we hypothesize that c-Myb may be involved in benzene-mediated leukemogenesis. To investigate this hypothesis, cells were transiently transfected with a luciferase reporter gene containing the chicken mim-1 promoter and then exposed to 0-100 μM of hydroquinone and benzoquinone for up to 24 hours. Our results show that the benzene metabolites benzoquinone and hydroquinone can significantly increase c-Myb activity at 24 hours but not at 6 hours in a dose dependant fashion. Previous studies indicate that exposure to catechol, but not benzene or phenol, can increase c-Myb activity. In light of these past results, this study supports the hypothesis that benzene must be metabolized before mediating its toxicity and provides insight into benzene's actions on the c-Myb signalling pathway as a potential mechanism of leukemogenesis. (Support:CIHR and PREECAN)

1643 ACTIVATION OF DOWNSTREAM RAS EFFECTORS IN LUNG LESIONS FOLLOWING DOXYCYCLINE (DOX) REGULATED EXPRESSION OF MUTANT HUMAN KI-*ras* IN A BITRANSGENIC MOUSE MODEL.

H. S. Floyd[1], C. L. Farnsworth[2], N. D. Kock[1], J. L. Little[1], S. T. Dance[1] and M. S. Miller[1]. [1]*Cancer Biology, Wake Forest University, Winston-Salem, NC and* [2]*Cell Signaling Technology, Beverly, MA.*

Previous studies from our laboratory reported the development of a bitransgenic mouse model that expresses the mutant human Ki-*ras*CYS[12] allele in alveolar type II and/or Clara cells in a DOX-regulated, lung-specific manner. When DOX was administered in the drinking water for up to 12 months, macroscopically visible lung lesions were first detected by 3 months, showing an increase in number and size during the course of DOX treatment. Microscopically, hyperplasia could be seen as early as 12 days and had progressed to adenomatous lesions by 6 months. Interestingly, none of the tumors progressed beyond the adenoma stage even after 12 months of DOX treatment. Withdrawl of DOX for 1 month resulted in a lack of proliferative lesions, suggesting tumor regression in the absence of Ki-*ras* expression. A 2-fold activation of both the RAS and RAL pathways in DOX-treated mice was detected by measuring the binding of tissue lysates to the Ras Binding Domain of Raf or Ral Binding Protein, respectively. Immunohistochemical analyses of the lung tissues demonstrated elevated phosphorlylation of downstream RAS effectors. Increased phosphorlyation of Erk, p90 ribosomal S6 kinase, ribosomal S6 protein, and the transcription factor CREB was detected with antibodies specific for phosphorylated residues of these proteins. The highest levels of activated signaling proteins were seen in bronchiolar epithelium, cells surrounding blood vessels, and the hyperplasic or adenomatous tissue. These results suggest that mutant RAS-mediated activation of the RAF-MEK-ERK and possibly the RAL pathways plays a critical role in the early stages of lung tumorigenesis. Continued studies with this *in vivo* model will allow the delineation of the role of these and other RAS effector pathways in the lung neoplastic process. (Supported by NCI grant CA91909 and CA91909-S1)

1644 OVEREXPRESSION OF PKC EPSILON IN THE MOUSE EPIDERMIS LEADS TO POLYMORPHONUCLEAR NEUTROPHIL INFILTRATION AND EPIDERMAL DESTRUCTION AFTER A SINGLE TOPICAL DMBA-TPA TREATMENT.

Y. li, D. Wheeler, H. Anathaswamy, A. Verma and T. D. Oberley. *Molecular and Environmental Toxicology, University of Wisconsin, Madison, WI.*

We have previously reported that FVB/N transgenic mice which overexpress protein kinase C epsilon (PKCε) in basal epidermal cells and cells of the hair follicle developed papilloma-independent metastatic squamous cell carcinoma (mSCC) elicited by an initiation (7, 12-dimethylbenz[a]anthracene, DMBA) - promotion (12-O-tetradecanoylphorbol-13-acetate, TPA) protocol. The present studies were performed to determine early morphological events in PKCε transgenic mouse skin treated with DMBA/TPA. FVB/N wild type mice and PKCε transgenic mice were initiated with a single dose of 100 nmol DMBA, followed by a single topical application of 5 nmol TPA one week later. Dorsal skin samples were fixed for histological examination at 0, 12, 24, 48, 72, and 96 hrs after TPA treatment. At 12 and 24 hrs, polymorphonuclear neutrophils (PMNs) infiltrated into the epidermis in both wild type and PKCε transgenic mice, and the epidermal area involved by PMN infiltration was not significantly different. However, complete epidermal necrosis by 48 h was observed in PKCε transgenic mice, not in their wild type littermates. Epidermal cell regeneration was observed at 72 h post TPA treatment in PKCε transgenic mice. The regenerated skin was disordered and showed extensive hyperplasia and hyperkeratosis. In addition, lack of staining with the differentiation marker keratin 10 showed the regenerated epidermis to be poorly differentiated. These histological changes were not observed in PKCδ overexpressing transgenic or in wild type mice. These data suggested that a single TPA treatment of the dorsal skin of PKCε transgenic mice resulted in epidermal destruction by PMNs, followed by hyperproliferation of cells of the basal layer and hair follicle and resulting in a hyperplastic, poorly differentiated epidermis. Our results may provide insights into the mechanism of the development of papilloma independent mSCC in PKCε transgenic mice.

1645 ACTIVATION OF AP-1 AND PRO/ANTIOXIDANT STATUS IN SKIN OF AP-1 TRANSGENIC MICE DURING CANCER PROMOTION WITH CUMENE HYDROPEROXIDE.

M. Xu[1], E. Kisin[2], A. R. Murray[1], C. Kommineni[2], V. Vallyathan[2], V. Castranova[1, 2] and A. A. Shvedova[1, 2]. [1]*Physiology and Pharmacology, WVU, Morgantown, WV and* [2]*PPRB, NIOSH, Morgantown, WV.*

Organic peroxides, widely used in the chemical and pharmaceutical industries, can act as skin tumor promoters and cause epidermal hyperplasia. They are also known to trigger free radical generation. The present study evaluated the effect of cumene hydroperoxide (Cum-OOH) on the induction of activator protein-1 (AP-1), which is linked to the expression of genes regulating cell proliferation, growth, and transformation. Previously, we reported that topical exposure to Cum-OOH caused formation of free radicals and oxidative stress in skin of vitamin E deficient mice. In addition, *in vitro* studies found that exposure to Cum-OOH reduced levels of GSH in JB6 P^+ cells and caused the induction of AP-1. The present study used AP-1-luciferase reporter transgenic mice to identify whether exposure to Cum-OOH *in vivo* caused activation of AP-1, oxidative stress, depletion of antioxidants and tumor formation during two-stage carcinogenesis. Mice primed with dimethylbenz[α]anthracene (DMBA) were treated topically with Cum-OOH (82.6 μmol) or 12-O-tetradecanoylphorbol-13-acetate (TPA, 17 nmol) twice weekly for 20 weeks. Activation of AP-1 in skin was detected as early as 2 weeks following Cum-OOH and TPA exposures. Maximum AP-1 expression was detected 4 weeks post initiation with Cum-OOH or TPA. No AP-1 activation was found 19 weeks post initiation. Papilloma formation was observed in both the DMBA/TPA and DMBA/Cum-OOH exposed animals, while skin carcinomas were found only in the DMBA/Cum-OOH treated mice. A greater accumulation of peroxidative products (TBARS), inflammation, and decreased levels of GSH, vitamin E and total antioxidant reserves were also observed in the skin of DMBA/Cum-OOH exposed mice. These results suggest that Cum-OOH induced carcinogenesis is accompanied by increased AP-1 activation and changes in antioxidant status.

1646 REDUCTION OF COX-2 EXPRESSION IN THYROID FOLLICULAR EPITHELIAL CELLS DURING DHPN-INDUCED CARCINOGENESIS IN RATS.

T. Imai, M. Hasumura, J. Onose, M. Ueda, T. Takizawa, Y. Cho and M. Hirose. *National Institute of Health Sciences, Tokyo, Japan.* Sponsor: M. Ema.

In thyroid follicular epithelial cells, constitutive expression of cyclooxygenase-2 (COX-2) has been demonstrated. To assess the role of COX-2 during rat thyroid carcinogenesis, we evaluated its expression in the proliferative lesions induced by goitrogens with or without *N*-bis(2-hydroxypropyl)nitrosamine (DHPN)-initiation. DHPN was subcutaneously injected once to 40 male F344 rats at age of 6-week. One week after the initiation, goitrogens such as propylthiouracil (PTU) and sulfadimethoxine (SDM) were administered in drinking water for 4 or 10 weeks, and then thyroid samples were collected for histopathology and immunohistochemistry for COX-2. Goitrogen alone groups were also placed. At week 4, multiple focal follicular cell hyperplasias and adenomas were frequently observed in DHPN + PTU and DHPN + SDM groups. In DHPN + SDM and SDM alone groups, severe inflammation with fibrosis in the thyroid capsule with migrated follicular epithelial cells into the capsule were also observed. At week 10, increased incidences and multiplicities of focal hyperplasias and adenomas in DHPN + PTU and DHPN + SDM groups were observed. In addition, invasive adenocarcinomas to capsule were also detected in 3 of 10 rats of the DHPN + SDM group. These carcinomas were suggested to originate in the focal hyperplasias and adenomas adja-

cent to the capsule. In the proliferative lesions observed in DHPN + PTU and DHPN + SDM groups as well as the migrated follicular epithelial cells in DHPN + SDM and SDM alone groups, COX-2 reactivity was frequently reduced or negative. These results suggested that capsular inflammation and reduction of COX-2 expression in the preneoplastic and neoplastic lesions play roles in the development of invasive adenocarcinomas and promotion/progression of rat thyroid carcinogenesis, respectively.

1647 INHIBITION OF ESTROGEN RECEPTOR NEGATIVE MDA-MB-453 AND BT-474 BREAST CANCER CELL GROWTH BY ARYL HYDROCARBON RECEPTOR AGONISTS.

L. Kotha and S. Safe. *Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.*

MDA-MB-453 and BT-474 are estrogen receptor (ER) negative breast cancer cell lines that over express epidermal growth factor receptor 2 (EGFR2, erbB2) and exhibit high constitutively active kinase activities. Treatment of both cell lines with 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) induced CYP1A1 expression and proteosome-dependent degradation of the aryl hydrocarbon receptor (AhR). Low but significant induction of CYP1A1 was also observed for the selective AhR modulators (SAhRMs) 6-methyl-1, 3, 8-trichlorodibenzofuran (MCDF) and 3'3-diindolylmethane (DIM). However these compounds did not downregulate AhR levels. TCDD (10 nM), MCDF (2 and 5 μM), and DIM (10 and 20 μM) all significantly inhibited MDA-MB-453 and BT-474 cell proliferation but did not significantly affect the percent distribution of the cells in G_0/G_1, S or G_2/M phases of the cell cycle. TCDD and the SAhRMs had minimal effects on the expression of erbB2 and phospho-erbB2, mitogen activated protein kinase 1/2 (MAPK1/2) or phospho-MAPK1/2, whereas the MAPK inhibitor UO126 inhibited cell proliferation and phosphorylation of MAPK. These data coupled with results obtained for other activated kinase pathways demonstrate that TCDD and SAhRMs uniquely inhibit growth of ER-negative MDA-MB 453 and BT-474 breast cancer cells through kinase-independent pathways. (Supported by NIEHS ES09106 and ES04176).

1648 ESTROGEN THROUGH CALCIUM MEDIATED SIGNALING INDUCES CELL GROWTH IN BREAST CANCER CELLS.

M. K. Singh and D. Roy. *Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL.*

Natural estrogen, 17βestradiol (E2) and its catechol metabolite 4-hydroxyestradiol (4-OH-E2), are implicated to be involved in the development of breast cancer. The mechanism by which E2 and 4-OH-E2 participate in breast carcinogenesis is not clear. In this study we have examined the effects of E2 and 4-OH-E2 on the proliferation of MCF-7 cells and intracellular ROS production. Cells were treated with varying concentration of E2 and 4-OH-E2 in DMEM-F12. Cell growth was assessed by cell count and sulforhodamineB assay, and cell proliferation by measuring BrdU incorporation. Intracellular ROS production was measured by a micro-fluorometric assay using 2, 7-dichlorofluorescin diacetate after treatment with different concentration of E2 and 4-OH-E2 with and without co treatment with calcium chelator BAPTA/AM . E2 (100pg/ml) and 4-OH-E2 (10ng/ml) treatments increased cell number by 200% and 250%, respectively. Co treatments of E2 and 4-OH-E2 with BAPTA revealed that BAPTA inhibited the stimulatory growth produced by E2 and 4-OH-E2 treatments. Both E2 and 4-OH-E2 increased intracellular ROS production in a dose dependant manner. BAPTA inhibited E2 and 4-OH-E2-induced increases in intracellular ROIs production. These data suggest that estrogen through regulating calcium signaling pathway stimulates the growth of MCF-7 breast cancer cells.

1649 ESTROGEN-INDUCED UPREGULATION OF CRE CONTAINING GENES IN BREAST CANCER CELLS.

Q. H. Felty and D. Roy. *Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL.*

The second messengers calcium (Ca2+) and cyclic AMP (cAMP) activate nuclear target genes containing the CRE binding motif through signaling within the cytoplasm. The CRE motif is present in the promoters of genes that encode growth factors and proteins involved in cell cycle progression. Both cAMP/Ca2+ activate the transcription factor CREB which in turn binds the CRE response element leading to transcription of various target genes. Estrogen-dependent tumors account for the majority of breast cancer cases in women. A hierarchy of organ tissue response to the endogenous estrogen 17-?-estradiol (E2) has shown that calcium metabolism is the most sensitive followed by epithelial cell growth. E2 has been demonstrated to rapidly activate adenylate cyclase responsible for cAMP synthesis as well as increase intracellular [Ca 2+]. Although E2 modulation of intracellular cAMP/Ca2+ levels are known, the role that cAMP/Ca2+ play in controlling the cell growth of estrogen dependent breast cells is not clear. In this study we present data that demonstrates E2 controls the transcription of cAMP/Ca2+ responsive genes, which may play a role in the paracrine or autocrine stimulation of breast cancer cell growth. Super GEArray analysis of cAMP/Ca 2+ genes at 6h revealed a 3.5- and 2.9 fold-induction of TGF ?3 and the substance P receptor in MCF-7 breast cancer cells when treated with 100pg/mL E2 for 6h. Interestingly, the substance P receptor is distributed in the breast, uterus, and brain tissue. Whether increases in mRNA levels of substance P receptor and TGF ?3 correlate to changes in estradiol stimulated breast tumor cell growth remains to be elucidated. The biological significance of our findings from this study may provide a basis for the development of a breast cancer therapy, which targets cAMP/Ca 2+ signaling pathways.

1650 LONG TERM EXPOSURE OF HUMAN MAMMARY EPITHELIAL CELLS TO HEXACHLOROBENZENE (HCB) INDUCES A PROCARCINOGENIC PHENOTYPE.

R. M. Audet, S. Girard, G. Lassonde and M. Charbonneau. *INRS-Institut Armand-Frappier, Universite du Quebec, Montreal, QC, Canada.*

Organochlorines are environmental lipophilic contaminants found in breast tissue with a debated role in breast cancer development (BCD). HCB, a widespread organochlorine in the environment, is virtually present in 100% of North American women. Epidemiological studies indicated that some breast cancer patients have higher HCB levels. We observed that long term exposure of human mammary epithelial cells to HCB increases the phosphorylation of c-erbB2 (neu). Cells with such epigenetic alterations can be considered procarcinogenic taking into account the bad prognostic role of c-erbB2 in BCD. In order to further evaluate the potential of HCB in causing epigenetic changes akin to a procarcinogenic phenotype, cultures of a non-tumorigenic human mammary epithelial cell line, MCF10-A, were chronically exposed to 20 μM HCB (0.1% in DMSO) or to DMSO alone. Following approximately 25 passages of continuous exposure to HCB, an increase in proliferation was observed. These cells showed an increase in the phosphorylation of the integrin-linked kinase (ILK), with a decrease in Akt/PKB levels. E-Cadherin, an important cell adhesion molecule, was also decreased. Control cells sub-cultured on Matrigel® showed a distinctive branching pattern after 24h. However, HCB-exposed cells exhibited a rounded shape with minimal cellular contacts. This is suggestive of a decrease in intercellular communication and cell adhesion ; the latter is supported by the decrease in E-Cadherin expression in these cells. Moreover, after 10 d in culture HCB-exposed cells migrated differently into the Matrigel® layer as compared to controls. Overall, data suggest that HCB induces a procarcinogenic phenotype characterized by reduced intercellular communication and that this may result from overactivation of ILK, known to be a down-stream target of c-erbB2, leading to down-regulation of Akt, which controls E-cadherin expression. This work brings biological plausibility to the involvement of environmental contaminants in BCD. (Supported by PREECAN)

1651 MAPPING AND GENOMIC ANALYSIS OF RESISTANCE TO AZOXYMETHANE-INDUCED COLORECTAL CANCER.

D. J. Barrick[1], J. Uronis[2] and D. Threadgill[2, 1]. [1]*Curriculum in Toxicology, UNC-Chapel Hill, Chapel Hill, NC and* [2]*Genetics, UNC-Chapel Hill, Chapel Hill, NC.*

Colorectal cancer (CRC) is the 3rd most diagnosed malignancy in the United States with approximately 1 in 17 people expected to develop the disease. Although some incidences of CRC occur in dominantly inherited patterns, the majority of CRCs are classified as "sporadic" (i.e. non-familial). However, many incidences of sporadic CRC occur in individuals with heightened genetic susceptibility. The chemical carcinogen azoxymethane (AOM), an alkylating agent that is a specific colorectal carcinogen in rodents, induces tumors that are pathologically similar to human colorectal tumors. Moreover, there are strain specific differences in susceptibility. Earlier work in this laboratory screened 32 inbred strains of mice for susceptibility to AOM-induced CRC. These studies revealed that A/J mice are extremely sensitive to AOM as nearly 100 percent of animals developing colorectal tumors, while SPRET/Ei mice are resistant. A better understanding of the AOM model using these two strains may provide clues to genetic predisposition to CRC. To further investigate genetic differences in susceptibility to sporadic CRC, we have taken two approaches. First, comparative genome hybridization (CGH) studies have identified chromosomal aberrations in colorectal tumors from AOM-exposed A/J mice. Potential amplifications were detected in the distal ends of chromosomes 9, 10, 14, 16, and 17, while deletions were observed on chromosomes 1 and 19. Second, we are in the process of mapping genetic loci responsible for resistance of the SPRET/Ei strain to AOM-induced colon cancer. While nearly 100 percent of ASPRET F1 mice are tumor-free, over 40 percent of progeny from a backcross of F1

mice to A/J parents ((ASPRET)A N2) develop AOM-induced colorectal tumors, implying that SPRET/Ei resistance genes are dominant. Using di-nucleotide microsatellite markers, we are scanning the genome of the (ASPRET)A N2 mice and have thus far identified three putative loci which demonstrate significant correlation to colon tumor resistance. Together, these studies will reveal potential CRC modifier genes. DJB T32 ES07126

1652 MAPPING RAT MAMMARY CANCER SUSCEPTIBILITY LOCI THAT CONTROL N-METHYL-N-NITROSOUREA-INDUCED MAMMARY CARCINOGENESIS IN FISCHER 344 RAT.

H. Zarbl[1,2], L. Jing[1], A. M. Mikheev[1], H. Xie[1], Y. Gao[1], X. Ren[1], J. Lew[1] and X. Zhang[1]. *[1]Human Biology and Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA and [2]Center for Ecogenetics and Environmental Health, NIEHS/ University of Washington, Seattle, WA.*

Rat strain differ dramatically in their susceptibility to mammary carcinogens such as 7, 12-dimethylbenz[a]anthracene (DMBA), N'-nitroso-N'-methylurea (NMU), and ionizing radiation. The Copenhagen (Cop) and Wistar-Kyoto strains are remarkably resistant while pubescent females of most other rat strains are susceptible to mammary carcinogenesis, although they vary with respect to tumor incidence and latency. Genetic crosses among susceptible and resistant strains have been used to genetically map and identify genes that control sensitivity and susceptibility to mammary carcinogenesis. However, genome wide linkage studies have failed to detect a major suppressor locus. Instead the data suggested that susceptibility is a multigenic trait controlled by multiple Quantitative Trait Loci (QTL), termed Mammary Cancer Susceptibility (Mcs) loci. None of the gene(s) comprising these loci locus has been identified to date. In the present study, we focussed on genetic mapping of the Mcs loci that conferred susceptibility to NMU-induced mammary carcinogenesis. We generated ~600 N2 backcross progeny using the highly resistant Cop strain and the susceptible F344 strains. Linkage analysis and interval mapping in this model demonstrated that susceptibility in the F344 strain segregated as a recessive Mendelian trait. The Mcs locus mapped to a ~10 centimorgan (cM) interval (LOD score ~ 9) close the centromere on the long arm of chromosome 12. A second locus mapped to the long arm of rat chromosome 1 (LOD~4). We are using comparative genomics, gene expression profiling and function genomics approaches to identify candidate genes within these loci. Identification of the putative Mcs genes in the rat model will undoubtedly lead to enhanced understanding of human breast cancer risks, and possibly lead to new strategies for risk assessment and early detection.

1653 GENISTEIN AND ESTROGEN REGULATION OF ANDROGEN RECEPTOR AND EXTRACELLULAR REGULATING KINASES IN RAT PROSTATE. C E HARPER, C A LAMARTINIERE. DEPARTMENT OF PHARACOLOGY AND TOXICOLOGY, UNIVERSITY OF ALABAMA AT BIRMINGHAM, BIRMINGHAM, AL.

C. E. Harper. *Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL.*

Prostate cancer is the second leading cause of death in men. Asian men consuming a diet high in soy have a lower incidence of hormone-dependent cancers. The primary isoflavone and phytoestrogen of soy, genistein, has been shown to suppress prostate cancer in rodent models. We have hypothesized that genistein elicits its effects *via* crosstalk between sex steroid and growth factor signaling. Thirty-five day old male Sprague-Dawley CD rats were given one subcutaneous injection of 500 mg genistein/kg BW, 500 ug estradiol benzoate (EB)/kg BW, or dimethylsulfoxide (DMSO). The rats were killed 16 hours after treatment. Dorsolateral and ventral prostates were dissected from each rat and Western blot analyses were performed to evaluate sex steroid and growth factor signaling. The dorsolateral prostate has been reported to be the embryologic homologue to the human prostate while the human does not have a ventral prostate. In the dorsolateral prostate, EB, but not genistein, significantly decreased phosphorylated extracellular regulating kinases 1 and 2 (P-ERKs 1 and 2). Neither genistein nor EB caused significant changes to androgen receptor (AR), epidermal growth factor receptor (EGFR) and total ERKs 1 and 2 in the dorsolateral prostate. In the ventral prostate, genistein and EB treatments resulted in decreases in AR and P-ERKs 1 and 2 and no significant effect on total ERKs 1 and 2 and EGFR. The action of genistein appears to be prostate tissue selective and there is a difference in the action of genistein and estrogen in the dorsolateral prostate. The embryologic origin of prostate tissue appears to dictate the action of genistein and estrogen. Understanding the selective action of genistein and estrogen should reveal how genistein suppresses prostate cancer and estrogen causes toxicity in the prostate.

1654 INHALATION DELIVERY OF AEROSOL CONTAINING PEI-GLUCOSE-PTEN COMPLEX INDUCED CHANGE OF PROTEIN TRANSLATION IN KRAS KNOCKOUT LUNG CANCER MODEL MICE.

H. Kim[1], I. Park[2], C. Cho[2], G. Beck[3], N. Colburn[3] and M. Cho[1]. *[1]Laboratory of Toxicology, College of Veterinary Medicine, Seoul National University, Seoul, South Korea, [2]Laboratory of Biomedical Polymer and Tissue Engineering, College of Agricultural Biotechnology, Seoul National University, Seoul, South Korea and [3]Basic Research Laboratory, National Cancer Institute, Frederick, MD.*

Difficulties of long-term survival of lung cancer patients treated with conventional therapies require the need for novel approaches and gene therapy holds promise in this area. Several genes are known to have anti-tumor activities and have been used as genes of delivery, however, a number of problems such as low efficiency and specificity hinder the application of gene therapy. Such traditional problems have re-emerged the aerosol gene delivery as a *via*ble and a noninvasive approach to lung cancer therapy. In this study, glucose conjugated polyethyleneimine (PEI-Glu) was used as a carrier particle. Aerosols containing PEI-Glu and recombinant plasmid pcDNA3.0-PTEN complex were transferred into Kras knockout lung cancer model mice through nose-only inhalation. Western blo revealed that aerosol gene delivery was successful. PTEN and Erk proteins were highly expressed in the mouse lung. PTEN delivery also induced increasde of Bax, c-Myc, p21, p65. Also, PI3K expression was increased significantly. However, increase of mutant p53 and decrease of Akt1, PDK1, eIF4E, eIF4E-BP1, p70S6K, phosphorylated mTOR, and phosphorylated p70S6K were observed. Our data strongly suggest that our carrier system is compatible with *in vivo* gene delivery and could be applicable to gene therapy. Supported by BK21 grant.

1655 PREVENTION BY METHIONINE OF DICHLOROACETIC ACID-INDUCED LIVER CANCER AND DNA HYPOMETHYLATION IN MICE.

M. A. Pereira, W. Wang, P. M. Kamer and L. H. Tao. *Pathology, Medical College of Ohio, Toledo, OH.*

Dichloroacetic acid (DCA) is a mouse liver carcinogen. One of the epigenetic mechanisms for carcinogens is the ability to induce DNA hypomethylation. Methionine has been shown to prevent DNA hypomethylation induced by carcinogens. To test the involvement of DNA hypomethylation in the carcinogenic activity of DCA, we determined the ability of methionine to prevent DCA-induced DNA hypomethylation and liver tumors. Female B6C3F1 mice were administered DCA in their drinking water and 0, 4.0 and 8.0gm/kg methionine added to their AIN-76A diet. After 8 weeks of exposure, DCA increased the liver/body weight ratio and caused DNA hypomethylation, glycogen accumulation and peroxisome proliferation. Methionine prevented completely the DNA hypomethylation, reduced by only 25% the glycogen accumulation and did not alter the increased liver/body weight ratio and the proliferation of peroxisomes induced by DCA. DCA induced foci of altered hepatocytes and liver adenomas. The multiplicity of foci of altered hepatocytes/mouse was increased from 2.41± 0.38 to 3.40 ± 0.46 by 4.0gm/kg methionine and decreased to 0.94 ± 0.24 by 8.0gm/kg methionine suggesting that methionine slowed the progression of foci to tumors. The low and high concentrations of methionine reduced the multiplicity of liver tumors/mouse from 1.28 ± 0.31 to 0.167 ± 0.093 and 0.028 ± 0.028, i.e. by 87 and 98%, respectively. Thus, the prevention of liver tumors by methionine correlated best with its prevention of DNA hypomethylation indicating that DNA hypomethylation was critical for the carcinogenic activity of DCA. This research was supported by a grant from the USEPA's Science to Achieve Results (STAR) program Grant number R82808301.

1656 LACK OF MODIFICATION OF MEIQX RAT LIVER CARCINOGENESIS BY CAFFEINE INDUCTION OF CYP1A2.

H. Kandori[1,2], M. Kuribayashi[1,3], M. Asamoto[1], S. Suzuki[1] and T. Shirai[1]. *[1]Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan, [2]Takeda Chemical Industries Ltd., Drug Safety Research Laboratories Hikari Branch, Hikari, Japan and [3]Ono Pharmaceutical Co. Ltd., Safety Research, Fukui, Japan.*

2-Amino-3, 8-dimethylimidazo[4, 5-f]quinoxaline (MeIQx), a heterocyclic amine in cooked meat and fish, is speculated to be associated with human carcinogenesis. MeIQx is known to be metabolically activated by several enzymes, especially CYP1A2. In order to evaluate modifying effects of CYP1A2 inducers on MeIQx hepatocarcinogenesis, caffeine or diethylnitrosamine (DEN) was given together with MeIQx to rats. Modification was assessed using a medium-term liver bioassay system. After initiation of hepatocarcinogenesis in F344 rats with a single dose of DEN, MeIQx was given together with or without 0.1% caffeine or 0.01% DEN in

the diet or drinking water, respectively for 6 weeks. First, we analyzed the numbers and areas of glutathione S-transferase (GST-P) positive liver foci, putative preneoplastic lesions, after immunohistochemical staining. Then, mRNA expression levels of several metabolic enzymes were compared between GST-P positive foci and the surrounding liver tissue. As a result, DEN given together with MeIQx showed a synergistic promoting effect on development of GST-P positive foci. However, no modification was found with caffeine, although it up-regulated CYP1A2 mRNA expression. DEN is reported to also be a CYP1A2 inducer but we could not confirm this. In the GST-P positive foci, phase II detoxifying enzymes were up-regulated. In the background tissue of rat livers after exposure to DEN as a CYP inducer, specific expression of extracellular matrix-related genes including sparc and laminin receptor 1 was detected. These results suggest that modifying effects of caffeine on MeIQx through CYP1A2 are limited because caffeine also up-regulates various phase II detoxifying enzymes. Moreover, our findings indicate that changes in extracellular matrix-related genes in the surrounding tissue are important for progression of GST-P positive foci.

1657 CURCUMIN PROTECTS AGAINST 2-AMINO-1-METHYL-6-PHENYLIMIDAZO[4, 5-B]PYRIDINE (PHIP) CARCINOGENECITY THROUGH MODULATION OF ITS METABOLISM.

R. Thimmulappa[1], M. Knize[2], K. Mai[1], J. S. Felton[2] and S. Biswal[1]. *[1]Johns Hopkins University, Baltimore, MD and [2]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA.*

Colorectal cancer is the second leading cause of death among Americans. Epidemiological evidences suggest the association of colon cancer with increased exposure of heterocyclic amines (HA) due to consumption of well-done cooked red meat and other dietary carcinogens. PhIP, the most abundant HA, has been reported to be a carcinogen in rodents and an etiological factor for colon and breast cancer. PhIP is activated by cytochrome P4501A2 (CYP1A2), and detoxified by phase 2 enzymes such as UDP-glucuronosyltransferase (UGT), glutathione-S-transferase (GST). Curcumin, a yellow pigment from Curcuma longa, is an important component of the spice turmeric. which shows anti-inflammatory, antioxidant properties and is being developed as a cancer chemopreventive agent. This study was designed to test the efficacy of curcumin to alter the PhIP metabolism. There was significant increase in hepatic GST and UGT levels as well as inhibition of CYP1A2 in rats fed with 1% curcumin for 14 days. Administration of PhIP by gavage (1mg/kg body weight, single dose) to rat fed on 1% curcumin resulted in significant increase in the PhIP-glucuronide metabolites such as N2-OH-PhIP-N2-glucuronide, N2-OH-PhIP-N3-glucuronide, and PhIP-N2-glucuronide compared to PhIP-treated group fed on control diet as measured by LC/MS/MS. Recent studies have demonstrated that Nrf2, a bzip transcription factor plays a pivotal role in induction of phase 2 enzymes. Administration of PhIP to the Nrf2-wild type and Nrf2-deficient mice fed on curcumin for 14 days resulted in higher levels of PhIP-glucuronide metabolites in urine of Nrf2-wildtype mice type than Nrf2-deficient mice indicating that mechanism of curcumin function might be through activation of Nrf2 In conclusion, data indicate that curcumin can protect against PhIP carcinogenecity by enhancing its detoxification (Supported by Howard/Hopkins Partnership grant).

1658 CURCUMIN ENHANCES EGCG-MEDIATED CYTOTOXICITY *IN VITRO* AND MODULATES LIVER ENZYMES *IN VIVO*.

S. Valentine, M. J. Le Nedelec and R. J. Rosengren. *Pharmacology & Toxicology, Univeristy of Otago, Dunedin, New Zealand.*

Both curcumin and epigallocatechin gallate (EGCG), are potent inhibitors of ERα negative breast cancer cell proliferation. The aim of this work was to determine if curcumin and EGCG would be synergistically cytotoxic in the ERα negative breast cancer cell line, MDA-MB-231. Cells were plated at 20, 000 cell/well in a 24 well plate in DMEM media. 24 hours later cells were treated with either 2-8 μmol curcumin, 20-40 μmol EGCG or a combination of the two. Control cells were treated with 0.005% DMSO. After 3, 5 or 7 days cell number was determined by the sulforhodamine B assay. All values are expressed as mean ± SE of at least 3 independent determinations performed in duplicate. At 3 days, either 4 or 5 μmol curcumin plus 25 μmol EGCG resulted in synergistic cytotoxicity, causing 20% greater cell death than the highest individual treatment (46±3% vs 24±4%). 5 days of treatment was even more effective with 3 μmol curcumin and 20 μmol of EGCG causing 11±4% and 34±2% cell death, respectively. While in combination the treatment was cytotoxic to 65±2% of cells, therefore showing even greater synergism. These results demonstrate that the administration of EGCG and curcumin *in vitro*, at concentrations similar to human plasma levels obtained during clinical trials, produce synergistic cytotoxicity in MDA-MB-231 cells. Additionally we have previously shown that EGCG modulates the activity of drug metabolizing enzymes *in vivo*. To determine if curcumin would act in a similar manner, female Swiss Webster mice were dosed with DMSO control, 200 mg/kg or 400 mg/kg curcumin p.o. for 1 and 2 weeks. Liver microsomes and cytosol were prepared 24 hours after the last dose. Results showed that curcumin did not alter hepatic GSH stores, but 400 mg/kg (2 week) increased GST activity. Furthermore 200 mg/kg (2 week) decreased CYP1A activity. Results indicate that the combination of curcumin and EGCG *in vivo* are likely to have greater effects on enzyme systems important in chemoprevention.

1659 COMPARATIVE EFFECTS OF NNK AND RESVERATROL ON INOS EXPRESSION AND INITIATION OF TUMORIGENESIS IN THE LIVER OF FEMALE A/J MICE.

R. H. Nsaif, O. M. Philip and C. S. Mehta. *College of Pharmacy and Health Sciences, Texas Southern Univesity, Houston, TX.*

4-(Methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) is an established potent lung tumorigen in rodents and humans, but there is a paucity of date regarding its action on the liver. Resveratrol (trans-3, 4', 5-trihydroxystillbene), a polyphenolic phytoalexin found in red grapes and fruits, is a known anti-inflammatory and anti-tumorigenic agent. The potential effects of resveratrol and NNK on iNOS expression and initiation of tumorigenesis were compared in the liver of A/J mice. The first group, served as control, was allowed free access to food and water ad libitum. The second group of 15 female A/J mice (5-6 weeks old) was administered resveratrol (50 ppm) in the diet for 28 weeks. The third group received NNK (9.1mg/mouse) in the drinking water for 7 weeks; this dose of NNK was sufficient to initiate tumorigenesis in the liver. The experiment was terminated 28 weeks later. Levels of iNOS and nitric oxide were determined by western blot and Griess reaction. Liver tissues were stained with H&E stain for tumor assessment. It was observed that administration of NNK significantly ($p < 0.010$) induced iNOS expression in the liver, whereas resveratrol had no effect on the levels of iNOS when compared to the control group. Measurement of nitric oxide production in urine by the Griess reaction showed that resveratrol significantly ($p < 0.004$) increased the production of nitric oxide, while NNK significantly ($p < 0.004$) reduced that production when compared to the control group. NNK caused an abnormal increase in cell size and induced cellular aggregation in the liver, but resveratrol had no effect on cell size when compared to that of the control group. In conclusion, effects of resveratrol on NNK-induced iNOS expression and initiation of tumorigenesis needs additional study in the liver.

1660 EPIGENETIC MECHANISMS OF ORGANIC TUMOR PROMOTERS AND THE ANTICARCINOGENIC ROLE OF RESVERATROL.

B. L. Upham, J. P. Scott, J. M. Carbone, L. Lee, A. M. Rummel and J. E. Trosko. *Pediatrics & Human Development, National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI.*

Dicumyl and benzoyl peroxides act as tumor promoters in SENCAR mice, while di-t-butyl-peroxide does not. Tumor promotion requires the removal of growth suppression by decreasing gap junction intercellular communication (GJIC) and the induction of mitogenic intracellular pathways. N-acetylcysteine was needed to prevent cytotoxicity of benzoyl- but not dicumyl peroxide. Dicumyl and benzoyl peroxides both reversibly inhibited GJIC and transiently activated mitogen-activated protein kinase (MAPK), specifically extracellular receptor kinase, in WB-F344 rat liver epithelial cells, while the non-tumor promoting di-tert-butyl peroxide did not inhibit GJIC or activate MAPK. The consumption of red wine has been linked to the lower incidence of cancer and heart disease among the French population and the active component is thought to be the antioxidant resveratrol. Pretreatment with resveratrol prevented the GJIC-inhibitory effects by dicumyl but not benzoyl peroxide. These results clearly indicate that specific signaling and not general oxidative events are involved in the promoting effects of organic peroxides and anticarcinogenic effects of the antioxidant, resveratrol. Supported by the #P42 ES04911-07.

1661 MOLECULAR CLONING OF CYTOCHROME P450 2 AND 3A FROM LARGEMOUTH BASS.

A. McNally, T. Knowles and D. S. Barber. *Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL.*

The largemouth bass (Micropterus salmoides, LMB) is a ubiquitous freshwater fish that is ecologically and economically important in freshwater ecosystems in Florida and much of the continental United States. Toxicity from exposure to environmental chemicals has been proposed as the cause of declining bass populations in several

Florida ecosystems. Despite the importance of biotransformation reactions in toxicity, very little is known about the ability of LMB to metabolize xenobiotics. The cytochrome P450 superfamily of enzymes are the primary mediators of phase I xenobiotic metabolism and lack of information on LMB P450 has hampered metabolism studies. We designed degenerate primers for CYP3A and CYP2 based on amino acid alignment of these proteins from numerous phyla including fish, mammals, and invertebrates. PCR amplification of cDNA prepared from male largemouth bass liver using these primers resulted in four products identified as P450. Two 886 bp fragments were obtained with the 3A primers that shared 73% and 81% identity with CYP 3A isoforms from Fundulus heteroclitus but were only 60% identical to each other. Using the CYP2 primers, a 666 bp fragment with 78% identity to CYP 2N1 and a 666 bp fragment with approximately 80% identity to CYP 2P isoforms from F. heteroclitus were obtained. This work provides the foundation to study the regulation of LMB phase I metabolic genes by environmental chemicals.

1662 ROLE OF USF AND NF-1 PROTEINS IN THE BASAL EXPRESSION OF HUMAN CYP1A2.

M. J. Narvaez[2], G. V. Pickwell[1] and L. C. Quattrochi[1]. [1]*Medicine, University of Colorado Health Sciences. Ctr., Denver, CO and* [2]*Pharmaceutical Sciences, University of Colorado Health Sciences. Ctr., Denver, CO.*

Cytochrome P450 1A2 (CYP1A2) is a major cytochrome P450 present in the adult human liver. This enzyme is involved in the metabolism of many therapeutic compounds and shows high catalytic activity towards procarcinogens, such as arylamines, dietary heterocyclic amines, and aflatoxin B1. To better understand the molecular mechanisms of CYP1A2 regulation, we have characterized a region of the promoter (+3 to -176) which contains consensus E-box and NF-1 sites. Both sites were shown to specifically bind nuclear proteins from the human hepatoma cell line HepG2. Supershift analysis with specific antibodies to USF-1 and USF-2 indicated that the USF1/2 dimer interacts with the E-box motif. Analysis with specific antibodies to NF-1 proteins showed that some NF-1 protein members interact with the NF-1 motif. Site-directed mutagenesis of the USF and NF-1 binding sites was used to examine the role of these sites in basal CYP1A2 expression. Mutation of the E-box site resulted in approximately 50% reduction in basal reporter gene activity, while mutation of the NF-1 site resulted in approximately 80% reduction in activity. These results further demonstrate the complex regulatory mechanisms governing the constitutive expression of human CYP1A2. This research was supported by grant NIHGM 54477.

1663 NONYLPHENOL ATTENUATES P450 INDUCTION BY TCPOBOP IN FVB/NJ MICE.

J. P. Hernandez and W. Baldwin. *Biological Sciences, University of Texas at El Paso, El Paso, TX.*

4-Nonylphenol (NP) binds and activates both the estrogen receptor (ER) and the pregnane-X-receptor (PXR), and 7-day exposure to NP has been shown to induce CYP2B and 3A family members. However, recent work demonstrated that gestational treatments and chronic treatments (32 weeks) of mice with 4-nonylphenol (NP) reduced some CYP3A family members and attenuated induction of CYP2B family members, potentially by down-regulating RXRα, PXR's dimerization partner. Therefore, we are interested in determining if NP could reduce the ability of FVB/NJ mice to respond to CAR or PXR ligands. Mice were treated for six weeks with honey as vehicle control or 50mg/kg/day NP in honey. At the end of six weeks control and NP-treated mice were split further into groups, and vehicle control mice were injected for three days with corn oil vehicle, dexamethasone (PXR inducer), or 1, 4-bis [2-(3, 5-dichloropyridyloxy)] benzene (TCPOBOP)(CAR inducer). NP treated mice were also split into more groups and injected with corn oil, dexamethasone, or TCPOBOP. Mice were euthanized and microsomes were prepared for immunoblot analysis of CYP3A1 and CYP2B6, which are transcriptionally activated by CAR and PXR. Immunoblots demonstrate TCPOBOP significantly induced CYP2B6, and that this induction was reduced by more than 50% lower in mice pre-treated with NP prior to TCPOBOP injections. NP had no significant effect on CYP3A induction by TCPOBOP. Furthermore, testosterone hydroxylation assays indicate that chronic treatment with NP can reduce TCPOBOP induction of P450s, suggesting that long-term exposure to NP may decrease an organism's ability to respond to toxicants. Mice treated with dexamethasone in conjunction with NP are currently being examined. The mechanism by which NP reduces an organism's ability to respond to toxicants is being investigated, and may include a reduction in RXRα, or an increase in co-repressors such as short heterodimeric protein, which represses steroid hormone receptor activity and is inducible by estrogens.

1664 DEVELOPMENT OF CHEMILUMINESCENT ASSAYS FOR MEASURING CYTOCHROME P450 ISOFORMS.

K. Roberts[1, 2], R. Morgan[1], C. Morris[1] and S. Woodhead[1]. [1]*Molecular Light Technology Research, Cardiff, CF14 5DL, United Kingdom and* [2]*Cardiff University, School of Biosciences, PO Box 911, Cardiff, CF10 3US, United Kingdom.* Sponsor: A. Smith.

Hybridisation protection assays (HPA) assays have been optimised to measure rat cytochrome p450 (CYP) 1A1 and 1A2 mRNA transcripts. These assays utilise short DNA oligonucleotides labelled with a chemiluminescent acridinium molecule as a probe to quantify the levels of mRNA transcripts. A hydrolysis step, that destroys any unhybridised probe or mismatched probe-target duplexes, is included making this technique ideally suited to measure specific mRNA transcripts where there are closely related gene products. The AE molecule reacts rapidly with hydrogen peroxide under alkaline conditions to produce light and the intensity of emitted light is a function of the amount of target present. As little as 0.1 fmol of transcript can be accurately quantified by this method. The assays were used to measure both the CYP1A1 and 1A2 levels, independently, in the rat hepatoma cell line H4IIE. When H4IIE cells were exposed to increasing concentrations of benzo(a)pyrene (0, 5, 10, 20 & 30mM) for 24 hours, CYP1A1 levels increased 50 fold in treated cells compared to untreated. An increase in expression of both 1A1 and 1A2 was seen in cells exposed to 30mM benzo(a)pyrene for 0, 6, 12 & 24 hours though a decrease was seen at 48 hours. Assays for b-actin and cyclophilin B, whose transcription levels can be monitored as internal control genes, were also designed. In conclusion, the HPA technique has been used successfully to measure changes in gene expression of specific CYP isoforms. The measurement of the specific mRNA from individual members of the CYP family will provide the ability to predict the potential of new compounds to activate certain cellular pathways and therefore provide mechanistic and toxicological information.

1665 DEVELOPMENTAL CYTOCHROME P450 EXPRESSION IN THE MOUSE LUNG: SUSCEPTIBILITY DIFFERENCES TO METABOLICALLY-ACTIVATED PULMONARY CYTOTOXICANTS.

A. Taff[1, 2], M. Bartosiewicz[1], D. Rocke[3], K. Ruggiero[4], K. Butler[5], A. Buckpitt[1] and C. Plopper[2]. [1]*Molecular Biology, School of Veterinary Medicine, University of California, Davis, CA,* [2]*Anatomy, Physiology, Cell Biology, School of Veterinary Medicine, University of California, Davis, CA,* [3]*Applied Science, University of California, Davis, CA,* [4]*CSIRO Mathematical and Informational Sciences, Canberra, ACT, Australia and* [5]*Primary Industries Research, Victoria, VIC, Australia.*

Earlier studies demonstrate substantial differences in susceptibility of adult and early postnatal animals to metabolically activated pulmonary toxicants. With the rise in incidence of chronic lung diseases in children and adults there is increasing public health concern about the effects of these substances on the lung. One chemical of particular concern is naphthalene, a polyaromatic hydrocarbon of consequence in the environment. To determine which other factors account for the higher susceptibility of neonates to toxicants like naphthalene, we have compared gene expression profiles using RNA isolated from mouse lungs. Tissue was harvested from neonatal (day 0), juvenile (day 7), weanling ng (day 21), and adult (day 56) mice. Total RNA was extracted from fresh tissue with Trizol, and quantified. CDNA was prepared from purified mRNA samples, fluorescent dye probes created, hybridized onto microarray slides, and read. Glass slides were spotted with 10, 000 mouse-specific 50mers purchased from MWG. Preliminary data reveal that CYP450 gene expression is generally upregulated with increasing age, with one notable exception: CYP2F2 which decreases approximately 25 % from neonatal to adult age ($p < .01$). In the mouse, naphthalene is predominantly metabolized by CYP2F2. This suggests that differential naphthalene toxicity seen at distinct developmental stages may be due to decreased levels of CYP2F2. Changes in injury/repair processes and their associated genes may also be important in delineating specific naphthalene toxicity at unique ages (analyses in progress). Supported by NIEHS ES06700, ES04311 and ES05707.

1666 SYNERGISTIC INDUCTION OF CYP3A4 EXPRESSION BY RIFAMPICIN AND TCDD IN PXR-ENHANCED HEPG2 CELLS.

X. Gu[1], T. Sheng[1], S. Ke[1], P. E. Thomas[2], W. Xie[3] and Y. Tian[1]. [1]*Vet. Physiology and Pharmacology, Texas A&M University, College Station, TX,* [2]*EOHSI, Rutgers University, Piscataway, NJ and* [3]*Center for Pharmacogenetics, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA.*

Cytochrome P450 3A4 is responsible for biotransformation of 50-60% of clinical drugs in the human liver. Rifampicin, a common clinical drug, is a putative ligand of pregnane X receptor (PXR) which activates the transcription of CYP3A4 upon binding of PXR to the regulatory regions of CYP3A4. TCDD (2, 3, 7, 8-tetra-

chlorodibenzo-p-dioxin), an ubiquitous environmental contaminant of public concern, exerts its toxic effects primarily by binding to the aryl hydrocarbon receptor (AhR) which acts as a transcription factor for cytochrome P450 1A1. In order to analyze the CYP3A4 inducers in cell culture, we developed cell lines by stable co-transfection of human PXR and luciferase reporter gene into HepG2 cells. The luciferase reporter gene was constructed using a combination of enhancer (-7836 to -7208) /promoter (-362 to +53) modules from CYP3A4 gene. The reporter gene is highly responsive to classic CYP3A4 inducer, rifampicin, in these PXR-enhanced cells. Interestingly, we found that rifampicin and TCDD show synergistic induction of the luciferase reporter gene. To investigate if rifampicin and TCDD exert synergistic effect on transcription of CYP 3A4 gene in natural state, we use real-time PCR to quantitate the relative levels of CYP3A4 mRNA in PXR-enhanced HepG2 cell line in response to rifampicine and/or TCDD treatment. The results indicate a synergistic induction of CYP3A4 expression by rifampicin and TCDD, suggesting AhR may be involved in the transcriptional regulation of CYP3A4. Supported in part by NIEHS grants ES09859 and ES09106.

1667 ALTERED HEPATIC CYTOCHROME P450 ENZYME EXPRESSION IN A CHOLESTATIC MOUSE MODEL.

S. M. Bandiera[1], E. G. Hrycay[1], D. Forrest[2], R. Wang[2] and V. Ling[2]. *[1]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada and [2]British Columbia Cancer Research Center, British Columbia Cancer Agency, Vancouver, BC, Canada.*

The biosynthesis, metabolism, and transport of bile acids in liver are tightly regulated. Cytochrome P450 (CYP) enzymes play a major role in the biosynthetic pathway and may catalyze bile acid metabolism. The sister of p-glycoprotein (spgp), also known as the bile salt export pump, mediates biliary bile acid secretion. Spgp-knockout mice lacking the spgp gene are mildly cholestatic, display enhanced bile acid hydroxylation, and develop severe cholestasis when fed a cholic acid (CA)-supplemented diet. The purpose of the present study was to investigate hepatic expression of individual CYP enzymes in spgp-/- mice to ascertain whether bile acids regulate CYP gene expression and to explore the role of CYP enzymes in bile acid metabolism. Female wild-type (WT) and spgp-/- mice were maintained on a normal diet or WT mice were fed a diet containing 0.5 % CA for a period of 4 days, after which they were killed and hepatic microsomes prepared. Immunoblot analysis was carried out using antibodies directed against various CYP enzymes. Total CYP content was lower by 38% and 20% in spgp-/- and CA-fed WT mice, respectively, relative to WT control mice. When expressed on a per mg protein basis, hepatic levels of Cyp1a2 were decreased in spgp-/- and CA-fed WT mice by 56% and 42%, respectively, compared to control mice. Levels of Cyp 2b9 and Cyp 2b10 were also diminished in spgp-/- mice by 29% and 36%, respectively. Moreover, hepatic expression of one Cyp3a enzyme was reduced by almost 70%, whereas expression of a second Cyp3a enzyme was increased in spgp-/- mice. NADPH-CYP-reductase levels remained unchanged. In conclusion, results of immunoblot analysis indicate that hepatic CYP expression profiles are altered markedly in the spgp-/- cholestatic mouse model. Experiments are ongoing to determine which CYP enzymes catalyze synthesis of the tri- and tetrahydroxylated bile acid metabolites that accumulate during hepatic cholestasis.

1668 EXPRESSION OF CYP1A1 AND 1B1 MNRA IN BLOOD LYMPHOCYTES FROM TWO POPULATIONS IN SLOVAKIA COMPARED TO TOTAL PCBs LEVELS AND TEQS IN BLOOD.

M. van den Berg[1], R. Fernandez Canton[1], A. Kocan[2], H. T.Besselink[3], T. Sanderson[1] and B. Brouwer[4]. *[1]Institute for Risk Assessment Sciences, Utrecht, Netherlands, [2]National Reference Center for Dioxins and Related Compounds, Bratislava, Slovakia, [3]Biodetection Systems B.V., Amsterdam, Netherlands and [4]Institute for Environmental Studies, Amsterdam, Netherlands.*

Cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) are enzymes that are inducible by low levels of halogenated polycyclic aromatics such as chlorinated dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) through activation of the aryl hydrocarbon receptor (AhR). The possible relationship between CYP1A1 or 1B1 mRNA levels in lymphocytes and dioxin and/or PCBs blood levels was examined using real time quantitative PCR analysis. CYP1A1 and 1B1 mRNA levels were determined in two different human populations in Slovakia; one highly PCB contaminated area and one representing background exposure. PCB blood levels were measured using gas chromatography-mass spectrometry and total dioxin equivalents (TEQs) using the DRE-Calux® assay. The average of total PCBs was greater in the contaminated population (1-35 mg/kg lipid) than background (1-5 mg/kg lipid). Fifteen individuals from the contaminated area had PCB levels 7 times higher than those to be considered background in both populations. The average of TEQs in blood was not different between the two populations. A distinct relationship was observed between intraindividual CYP1A1 and CYP1B1 expressions ($r_{=0.647}$, p(0.005, n = 268), reflecting the similar pathway of gene regulation mediated through the AhR. No relationship could be found between the gene expressions and total PCB levels; the same was true when adjusted for smoking and gender. However, stratified analyses showed that TEQs values were associated with CYP1A1 expression in the group of men living in the contaminated area (p(0.001; n=91). In conclusion, this study shows that CYP1A1 expressions in blood lymphocytes might be a useful biomarker for exposure to dioxin like compounds at levels that are close to background exposure.

1669 HISTONE DEACETYLATION EFFECTS OF THE CYP1A1 PROMOTER ACTIVITY, PROLIFERATION AND APOPTOSIS OF CELLS IN HEPATIC, PROSTATE AND BREAST CANCER CELLS.

K. Min, K. Joung, M. Cho, J. An, D. Kim and Y. Y. Sheen. *Pharmacy, Ewha Womans University, Seoul, seoul, South Korea.* Sponsor: Y. Cha.

We have studied the mechanism of action of TCDD on CYP1A1 promoter activity in both Hepa-1 and MCF-7 cells using transient transfection system with p1A1-Luc reporter gene. When HDAC inhibitors, such as trichostatin A, HC toxin and a novel HDAC inhibitor, IN2001 were cotreated with TCDD to the cells transfected with p1A1-Luc reporter gene, the basal promoter activity of CYP1A1 was increased by HDAC inhibitors. Also, in MCF-7 human breast cancer cells, HDAC inhibitors, such as IN2001 and trichostatin A increased the basal activity of CYP1A1 promoter but TCDD stimulated CYP1A1 promoter activity was not changed by HDAC inhibitors. And, in stably-transfected Hepa-1 cells with p1A1-Luc, HDAC inhibitors increased the basal promoter activity only. Also, we have investigated the effects of HDAC inhibitors on the human breast and prostate cancer cells in terms of cell proliferation and apoptosis based on SRB assay. IN2001 as well as trichostatin A inhibited the MCF-7, MDA-MB-231, MDA-MB-468, T47D, ZR75-1, PC3 cell growth dose-dependently. The growth inhibition of these cells with HDAC inhibitors was associated with profound morphological change, which suggests the HDAC inhibitors induced apoptosis of cells. The result of cell cycle analysis after 24h exposure of IN2001 showed G2/M cell cycle arrest in MCF-7 cells and apoptosis in T47D and MDA-MB-231 cells.[This work was supported by the grant from the ministry of environmental science]

1670 EFFECTS OF QUERCETIN AND AMENTOFLAVONE ON CYP1 EXPRESSION IN RL95-2 ENDOMETRIAL CARCINOMA CELLS.

Z. R. Master and K. L. Willett. *Pharmacology, University of Mississippi, Oxford, MS.*

Endometrial cancer is the most common gynecological malignancy in women. To model this disease and identify potential chemopreventatives, RL95-2 endometrial cancer cells derived from a moderately differentiated adenosquamous endometrial carcinoma were used. RL95-2 cells are estrogen- and Ah-receptor positive, and CYP1 genes are induced by BaP and TCDD exposure. Previous work in our laboratory screened a series of flavonoids for their ability to inhibit recombinant human microsomal CYP1-mediated ethoxyresorufin-O-deethylase (EROD) activity. Quercetin (QT) and amentoflavone (AF) were identified for further study. RL95-2 cells were induced with TCDD (10 nM/24 hrs). To investigate the CYP1 inhibition pattern in intact induced cells, 30 min prior to EROD assays, quercetin and amentoflavone (0.5 to 5 μM) were added. Quercetin (IC50 = 1.9 μM) inhibited EROD activity in a dose-dependent manner. In intact cells, EROD measurements are not CYP1 isoform specific. Thus, western blots on cell microsomes prepared after 24 hr dosing with DMSO, TCDD (10nM), QT (5 μM), AF (5 μM), TCDD + QT and TCDD + AF were done. While immunoreactive CYP1B1 was non-detectable, CYP1A1 protein was increased 7-fold by TCDD alone and 14-fold by TCDD + AF, relative to DMSO control. Quercetin alone did not increase CYP1A1 protein above control levels, but CYP1A1 was increased 2.4-fold by AF alone. CYP1A1 levels in the TCDD + QT cotreated cells were decreased 41% compared to the TCDD alone. Because CYP1 family P450s have been implicated in both detoxifying chemotherapeutic drugs and activating xenobiotics to genotoxic intermediates, finding compounds that inhibit CYP1-mediated metabolism is an important goal in cancer and chemoprevention research. To determine the relative inhibition of CYP1A1 and CYP1B1 at the message level, we are also doing quantitative real time RT-PCR. CYP1A1 enzyme activity and protein levels were inhibited by quercetin in these endometrial cells which suggests that quercetin may be efficient in preventing the metabolic activation of carcinogens. (Supported by American Assoc. of Colleges of Pharmacy)

1671 NO RELATIONSHIP BETWEEN NF-κB ACTIVATION BY PPARα AGONISTS AND TCDD-MEDIATED INDUCTION OF *CYP1A1*.

D. E. Machemer[1], A. Galijatovic[1], D. J. Beaton[1], Z. Li[1], M. Karin[1] and R. H. Tukey[1, 2]. *[1]Department of Pharmacology, University of California San Diego, La Jolla, CA and [2]Department of Chemistry & Biochemistry, University of California San Diego, La Jolla, CA.*

Cytochrome P450 1A1 (Cyp1a1) catalyzes the hydroxylation of a variety of xenobiotic compounds and is strongly induced by halogenated and polycyclic aromatic hydrocarbons through activation of the aryl hydrocarbon receptor (AhR).

Induction of *Cyp1a1* and NF-κB activation appear to be linked in terms of function and regulation. Peroxisome proliferators stimulate NF-κB and production of inflammatory mediators in the liver, and thus may exert a regulatory influence on *Cyp1a1*. To further elucidate the relationship between peroxisome proliferators, the inflammatory cascade, and *Cyp1a1*, TCDD-mediated induction of *Cyp1a1* was investigated in wild-type, IKKβ$^{+/-}$, TNFr1$^{+/-}$, and TNFr1$^{-/-}$ C57Bl/6 mice. Embryonic fibroblasts from IKKβ$^{+/-}$ C57Bl/6 mice exhibit a 50% reduction in IKK activity and a 90% reduction in NF-κB DNA binding activity. Signalling through TNFr1 has been shown to be responsible for 85-90% of NF-κB DNA binding and transcriptional activity of an NF-κB reporter gene. Mice were treated with PPARα agonist Wy-14643 (100 mg/kg), 16 μg/kg TCDD, or both compounds. Liver Cyp1a1 protein levels and enzymatic activity were quantitated. Cyp1a1 protein levels and enzymatic activity were not significantly different in IKKβ$^{+/-}$ mice when compared to IKKβ$^{+/+}$ mice. In both IKKβ$^{+/+}$ and IKKβ$^{+/-}$ mice, addition of Wy-14643 alone did not induce Cyp1a1, and Wy-14643 did not affect the TCDD inducibility of Cyp1a1 protein or enzymatic activity. TCDD-mediated induction of Cyp1a1 protein and enzymatic activity were not altered in TNFr1$^{+/-}$ and TNFr1$^{-/-}$ mice when compared to TNFr1$^{+/+}$ mice. Regardless of TNFr1 genotype, Wy-14643 alone did not induce Cyp1a1, and Wy-14643 did not affect the inducibility of Cyp1a1 protein or enzymatic activity by TCDD. This suggests that TCDD-mediated induction of *Cyp1a1* is not affected by peroxisome proliferators or by a significant loss of NF-κB activity *in vivo*. (Supported by USPHS grant ES10337)

1672 EFFECT OF EPIGALLOCATECHIN GALLATE AND EPICATECHIN GALLATE ON CYP450 ISOFORMS IN THE MALE SWISS WEBSTER MOUSE.

M. G. Goodin and R. J. Rosengren. *Pharmacology and Toxicology Department, University of Otago, Dunedin, New Zealand.*

Epigallocatechin gallate (EGCG) is a potent cancer-preventative agent. Specifically, EGCG has been shown to inhibit prostate tumor growth in a male athymic nude mouse model. However, no mechanism for this action has been determined. Therefore, the effect of EGCG and epicatechin gallate (ECG) on enzymes relevant to both the development of prostate cancer and carcinogen bioactivation were evaluated in the male Swiss Webster mouse. Mice were dosed with EGCG or ECG (25 or 50 mg/kg, ip) for 7 days and cytosolic extract and microsomes were prepared on day 8. All treatments were well tolerated by the mice. However, after 5 days of EGCG (50 mg/kg) one mouse was euthanised because it appeared ill and lost 4 g of body weight. This mouse had severe hepatotoxicity as indicated by an ALT of 1995 IU/L and extensive histological evidence of hepatic necrosis. All other mice had normal weight gain and normal levels of ALT activity. Enzymatic results demonstrated that EGCG and ECG (50 mg/kg) decreased CYP1A catalytic activity and polypeptide levels, while CYP3A catalytic activity and polypeptide levels were increased in response to EGCG (50 mg/kg). Specifically, EGCG and ECG inhibited CYP1A catalytic activity by 43 and 29% while CYP3A catalytic activity was induced by 52%, compared to vehicle control (1.19 vs. 0.78 nmol/mg/min). ECG (25 and 50 mg/kg), but not EGCG, inhibited prostatic aromatase activity by 31% compared to vehicle control. Hepatic COMT remained unchanged following catechin treatment. In conclusion, EGCG and ECG may play a cancer-preventive role in prostate cancer due to the induction of CYP3A and inhibition of aromatase, respectively. However, the results with EGCG (50 mg/kg) are tempered by the fact that this dose can produce hepatotoxicity in susceptible mice. A similar hepatotoxic profile occurs in BALB/c mice. Interestingly, this effect is species-specific as high doses of EGCG are not hepatotoxic in male rats.

1673 ENDOGENOUS REGULATION OF CYP1A1 INDUCTION IN HEPA 1c1c7 CELLS IS MEDIATED BY CALCIUM-DEPENDENT ADHESION.

L. M. Van Pay[1], M. A. Pickart[2, 3], C. R. Ivarie[3] and B. Allen-Hoffmann[1, 2, 3]. *[1]Molecular and Environmental Toxicology, University of Wisconsin, Madison, WI, [2]Biotechnology Training Program, University of Wisconsin, Madison, WI and [3]Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI.*

Previous studies in our laboratory have shown that disruption of cell-cell and cell-substratum interactions in Hepa 1c1c7 cells leads to an AhR-dependent induction of CYP1A1 mRNA levels in the absence of xenobiotics. In an effort to identify suspension-sensitive regulatory elements we performed deletion analysis of the 5' region of the human CYP1A1 promoter. These studies revealed that the xenobiotic responsive elements and GC box are essential for suspension-induced transcription, while the negative regulatory element (NRE) did not appear to play a role in the suspension mediated response. To specifically disrupt cadherin-mediated cell-cell adhesion, cells were shifted from high to low calcium-containing media. Steady state levels of CYP1A1 mRNA increased in wild-type Hepa 1c1c7 cells following a cadherin-disrupting shift from 1.8 mM calcium media to 0.05 mM calcium media. Additionally we show that β-catenin, an intracellular adherens junction protein that binds to cadherins, coimmunoprecipitates with the AhR in wild-type Hepa 1c1c7 cells. We determined the expression patterns of adherens junction proteins in Hepa 1c1c7 wild-type, Class I and Class II mutant cell lines. All three cell lines express comparable levels of N-cadherin and β-catenin, however the expression of β-catenin was greatly reduced in the Class I mutants as compared to the other two cell lines. These data suggest that cadherin-mediated cell-cell adhesion may function in the endogenous regulation of CYP1A1 gene expression in Hepa 1c1c7 cells.

1674 EFFECT OF THIABENDAZOLE ON RAT HEPATIC XENOBIOTIC METABOLISING ENZYME ACTIVITIES.

B. G. Lake, C. Meredith, M. P. Scott and R. J. Price. *BIBRA International Ltd.,, Carshalton, Surrey, United Kingdom.*

The aim of this study was to examine the effect of the anthelmintic agent and fungicide thiabendazole (TB) on some markers of rat hepatic xenobiotic metabolism. Male Sprague-Dawley rats were fed control diet or diets containing 102-5188 ppm TB for 28 days. As a positive control for induction of hepatic xenobiotic metabolism, rats were also fed diets containing 1457 and 10155 ppm butylated hydroxytoluene (BHT). Treatment with TB and BHT resulted in dose-dependent increases in relative liver weight. TB was found to be a mixed inducer of cytochrome P450 (CYP) forms in the CYP1A and CYP2B subfamilies. The administration of high doses of TB resulted in the induction of 7-ethoxyresorufin O-deethylase and 7-pentoxyresorufin O-dependentylase activities and CYP1A2 and CYP2B1/2 apoprotein levels. Levels of hepatic CYP mRNAs were determined by real-time quantitative reverse transcription-polymerase chain reaction methodology. Treatment with 5188 ppm TB produced 169-, 25-, 41- and 25-fold increases, respectively, in CYP1A1, CYP1A2, CYP2B1 and CYP2B1/2 mRNA levels In contrast to TB, BHT was a CYP2B form inducer in rat liver, increasing 7-pentoxyresorufin O-dependentylase activity and CYP2B1/2 apoprotein levels. Treatment with 10155 ppm BHT produced 3115- and 972-fold increases, respectively, in CYP2B1 and CYP2B1/2 mRNA levels, whereas CYP1A1 and CYP1A2 mRNA levels were increased <4-fold. Both TB and BHT induced GSH S-transferase (GST) activities towards a range of substrates. GST activity towards 1-chloro-2, 4-dinitrobenzene as substrate was induced 3.1- and 3.9 -fold, respectively, by treatment with 5188 ppm TB and 10155 ppm BHT. In addition, TB and BHT produced 248- and 102-fold increases in GSTP1 mRNA levels, whereas GSTT1 mRNA levels were only increased around 4-fold. In summary, these results demonstrate that TB induces both phase I and II xenobiotic metabolising enzymes in rat liver. (Supported by the UK Food Standards Agency).

1675 CYP2E1 GENOTYPE AND PHENOTYPE IN A POPULATION OCCUPATIONALLY EXPOSED TO VOLATILE ORGANIC CHEMICALS (VOCS).

A. Mendoza-Cantu[1], F. Castorena-Torres[1], M. Bermudez[1], R. A. Reyes[1], L. Lopez-Carrillo[2] and A. Albores[1]. *[1]Cinvestav-IPN, Mexico City, D.F., Mexico and [2]Instituto Nacional de Salud Publica, SS, Cuernavaca, Mor., Mexico.*

VOCs effects were studied in 103 employees of two print workshops. Environmental toluene and xylene levels exceeded maximum exposure level (MEL). The mean toluene concentration in an 8 h increased 52% compared to concentrations at the beginning of the working shift, whereas xylene levels increased 90%. As for toluene, there is a significant relationships between the toluene in the environment/ urinary hypuric acid with lymphocytary mRNA. Another statisctically significant was the relationship between methyl hypuric acid and the 1-hydroxy-chlorzoxazona(1-OHCZ)/chlorzoxazona (CZ). Although polymorfism did not modify toluene, there is a trend suggesting that individuals showing the c1 allele of the RsaI/PstI, polymorphism are more responsive to toluene mediated mRNA icrese than those showing the allele c2. Our results suggest that VOCs regulate CYP2E1 at different levels, i.e., toluene directly regulates mRNA content whereas xylene increases enzyme activity mainly (Supported by: EC contract: ERB IC18-CT980341 and Conacyt project M0061-M9602).

1676 CYP1A2 GENO- AND PHENOTYPE IN A POPULATION OF THE COAL REGION IN NORTHERN MEXICO.

F. Castorena[1], A. Mendoza-Cantu[1], M. Bermudez[1], L. Lopez-Carrillo[2], J. E. Salinas-Moreno[3] and A. Albores[1]. *[1]Cinvestav-IPN, Mexico City, D.F., Mexico, [2]Instituto Nacional de Salud Publica, SS, Cuernavaca, Mor., Mexico and [3]Secretaria de Salud, Sabinas, Coah., Mexico.*

CYP1A2 regulation by polycyclic aromatic hydrocarbons (PAHs) and the polymorphism influence was investigated in 46 masculine volunteers in the Coal Regio of Northern Mexico. PAHs exposure was estimated by the urinary excretion of 1-hydroxy pyrene (1-OHP) and CYP1A2 phenotype was assessed by the caffeine meta-

bolic ratio (CMR) and CYP1A2 mRNA in peripheral lymphocytes. Genotype was evaluated in the promotor region (-2964) and Intron 1. A 1-OHP gradient was observed in the population studied. The genotype distribution was: for the intron 1 C/C (0), C/A (45) and A/A (55%), and for the promotor G/G (37.5), G/A (39.5) and A/A (23%), both distributions were in agreement to the Hardy-Weimberg equilibrium model, and showed that, according to the CMR, a greater enzyme activity was observed in the A/A individuals compared to C/A ($p<0.001$). No correlation was observed between polymorphism in the promotor region and the parameters evaluated. These results suggest that PAHs affect the CYP1A2 phenotype in individuals with an A/A polymorphism in the intron 1 (Supported by: EC contract: ERB IC18-CT980341 and Conacyt project M0061-M9602).

1677 EFFECT OF SOY DIET ON EXPRESSION OF ADRENAL CYTOCHROMES P450 (CYP) 1A1, 1B1, 11A, AND STEROIDOGENIC ACUTE REGULATORY PROTEIN (STAR) IN RATS AFTER ADMINISTRATION OF 7, 12-DIMETHYLBENZANTHRACENE (DMBA).

X. Fu[1], B. Blaydes[1], S. Cooper[1], J. R. Latendresse[2], L. Muskhelishvili[2], L. J. Hennings[2] and B. Delclos[1]. *[1]Biochemical Toxicology, NCTR, Jefferson, AR and [2]Pathology Associates International, Jefferson, AR.*

CYP1A1 and CYP1B1 are important in DMBA bioactivation. Although dietary soy has been shown to modulate the induction of CYP1A1 and 1B1 by DMBA in rat liver and mammary, the effects of soy on DMBA adrenal toxicity and enzymes potentially related to this toxicity have not been thoroughly investigated. In this study, the effect of a soy-containing diet on mRNA expression of CYP1A1 and CYP1B1 as well as two enzymes involved in steroid hormone synthesis in the adrenal, CYP11A and StAR, were examined. In contrast to CYP1A1, but like CYP1B1, constitutive expression of CYP11A and StAR are high in rat adrenal cortex and regulated *via* cAMP. Female Sprague-Dawley rats were placed on either soy-containing NIH-31 diet or soy- and alfalfa-free 5K96 diet from postnatal day (PND) 21 until sacrifice. On the first day of diestrus when the animals were PND 50 ± 5, rats received either an oral dose of 80 mg/kg DMBA or sesame oil, the vehicle, and sacrificed at 24 h after treatment. Serum was collected at necropsy and corticosterone levels were measured and found not to be altered by DMBA. DMBA-induced apoptotic changes in adrenal glands, confirmed by TUNEL assay and activation of caspase 3, were more apparent in rats fed NIH-31 diet. mRNA expression was analyzed by real time RT-PCR. In comparison to controls, rats treated with DMBA in both dietary groups had greatly induced adrenal CYP1A1 but significantly lower CYP1B1. DMBA treatment also caused significantly lower expression of CYP11A and StAR relative to vehicle controls only in rats fed NIH-31 diet. It appears that different mechanisms underlie regulation of CYP1A1 and CYP1B1 expression by DMBA in rat adrenal gland and that dietary soy may affect DMBA-induced apoptosis and enzyme expression in rat adrenal.

1678 ROLE OF CYTOCHROME P4501A2 IN THE METABOLIC ACTIVATION OF 3-METHYLCHOLANTHRENE TO GENOTOXIC METABOLITES THAT PREFERENTIALLY BIND TO CYP1A1 PROMOTER *IN VITRO* AND MODULATE CYP1A1 GENE EXPRESSION.

B. Moorthy[1], K. Muthiah[1], S. R. Kondraganti[1], G. Zhou[2] and W. Jiang[1]. *[1]Pediatrics, Baylor College of Medicine, Houston, TX and [2]School of Rural Public Health, Texas A&M University system, Houston, TX.*

Cytochrome P4501A (CYP1A) enzymes play important roles in the bioactivation of 3-methylcholanthrene (MC) to genotoxic metabolites. Here, we tested the hypothesis that CYP1A2 plays a role in the metabolism of MC to metabolites that may covalently bind to CYP1A1 promoter and modulate CYP1A1 gene expression. Liver microsomes (2 mg) from MC-treated female wild-type (C57BL/6J) (WT) mice or CYP1A2-null (KO) mice were incubated at 37 ^{0}C for 2 h with MC (10 μM) in the presence of NADPH and plasmid DNA containing human CYP1A1 promoter (pGL3-1A1) (50 μg). The plasmid DNA was re-precipitated and DNA adducts were analyzed by ^{32}P-postlabeling. MC, in the presence of WT or KO microsomes, induced formation of 5 adducts that were not seen in the absence of NADPH. The adduct levels were 3-fold lower in the presence of KO microsomes, suggesting that CYP1A2 played an important role in the bioactivation of MC. In order to determine if MC adducts were located on the CYP1A1 promoter, the 1.6 kb CYP1A1 promoter insert was released from the pGL3-1A1 construct by restriction enzyme digestion, followed by gel electrophoresis and elution of the promoter (1.6 kb) and vector (5.2 kb) fragments. Postlabeling analyses of the promoter and vector fragments revealed that 85% of the adducts were present in the promoter fragment, suggesting that the MC-DNA adducts were preferentially formed in the promoter region. Transient transfection of the adducted plasmids into rat hepatoma (H4IIE) cells for 16 h, followed by treatment of the cells with MC (1 μM) for 8 h resulted in 35% lesser induction of reporter (luciferase) gene expression, compared to that observed when control plasmids were used in transfection experiments. In conclusion, our results suggest an important role for CYP1A2 in the formation of sequence-specific MC-DNA adducts, which in turn attenuate CYP1A1 gene expression. (Supported by NIH grant ES09132.)

1679 ARSENITE DECREASES PHENOBARBITAL- AND RIFAMPICIN-MEDIATED INDUCTION OF CYP3A4 BY DIFFERENT MECHANISMS IN CULTURED HUMAN HEPATOCYTES.

T. L. Noreault[1], V. E. Kostrubsky[2], S. G. Wood[3], R. C. Nichols[3, 6], S. C. Strom[4], H. W. Trask[3], S. A. Wrighton[5], J. M. Jacobs[3], P. R. Sinclair[3, 1] and J. F. Sinclair[3, 1]. *[1]Pharmacology & Toxicology, Dartmouth Medical School, Hanover, NH, [2]Department of Safety Sciences, Pfizer, Ann Arbor, MI, [3]Research, VA Medical Center, White River Junction, VT, [4]Pathology, University Pittsburgh Med. Ctr, Pittsburgh, PA, [5]Research Laboratories, Lilly, Indianapolis, IN and [6]Microbiology/Immunology, Dartmouth Medical School, Indianapolis, NH.*

In rodents, the receptor CAR mediates the induction of CYP3A by phenobarbital (PB), while the receptor PXR mediates the induction of CYP3A by dexamethasone. In studies with reporter constructs, however, human PXR (hPXR) is responsive to PB. We have previously reported that, in cultured rat hepatocytes, arsenite decreases induction of CYP3A protein by dexamethasone and PB with little to no decrease in CYP3A mRNA. These findings indicate that arsenite acts by a post-transcriptional mechanism and does not alter the interaction of CAR or PXR to its ligands or co-factors. Here we investigated the effect of arsenite on induction of CYP3A4 by either rifampicin or PB in cultured human hepatocytes. In PB-treated cells, arsenite decreased CYP3A4 protein and metabolism of testosterone to 6beta-hydroxytestosterone, an activity associated with CYP3A. There was no significant decrease in CYP3A4 mRNA, similar to our findings for rat CYP3A. In contrast, in rifampicin-treated cells, arsenite decreased induction of CYP3A4 mRNA in parallel with decreases in CYP3A4 protein and the formation of 6beta-testosterone. Since hPXR is responsive to rifampicin, the results suggest that hPXR is more sensitive to inhibition by arsenite than rat PXR. However, since induction of CYP3A4 mRNA by PB is not decreased by arsenite, human CAR may be less sensitive to arsenite than hPXR. Thus, PB may utilize the human CAR receptor to induce CYP3A when PXR is inhibited. We are currently investigating the effect of arsenite on expression of reporter constructs of CAR and PXR. This work was supported by NIH (ES010426).

1680 EXPRESSION OF AH RECEPTOR, ARNT, CYP1A1, AND CYP1B1 IN RAT MAMMARY EPITHELIA *IN VITRO* IS EACH SUBSTANTIALLY ELEVATED BY SPECIFIC EXTRACELLULAR MATRIX INTERACTIONS THAT PRECEDE DUCTAL FORMATION.

M. L. Larsen[1], R. S. Pollenz[2] and C. R. Jefcoate[1]. *[1]Pharmacology, University of Wisconsin, Madison, WI and [2]Biology, University of South Florida, Tampa, FL.*

Cytochrome P4501B1 (CYP1B1), the major constitutively expressed CYP in the rat mammary gland, is induced by Ah-receptor (AhR) ligands, while CYP1A1 is usually only expressed after such induction. Each form contributes to carcinogenic activation of polycyclic aromatic hydrocarbons (PAHs). CYP1B1, AhR, and Arnt were very weakly expressed in rat mammary epithelial cells (RMEC) when grown on plastic, even after AhR activation by TCDD. RMEC cultured on Matrigel (predominately laminin) or on a floating gel of collagen I demonstrated tubular organization and substantially increased basal CYP1B1 and induced CYP1A1 expression, in parallel with large increases in AhR and Arnt. Collagen I, collagen IV, and laminin, plated as thin films, were all unable to reproduce the tubular organization of RMEC or the increases in AhR, Arnt, or CYP expression. However, a thick, adherent collagen I bed recapitulated the substantial increases in AhR, Arnt, basal CYP1B1, and induced CYP1A1 expression, but without supporting tubulogenesis. These increases in protein expression were observed within 24h of RMEC dispersal on Matrigel, and substantially prior to tubulogenesis (3- to 5 days). Increases in AhR, Arnt, and CYP expression were closely paralleled by enhanced expression of beta-catenin and E-cadherin, components of cell-cell adhesion complexes. Synthetic peptides that selectively antagonize E-cadherin function and integrin-ECM interactions each reduced tubular formation without diminishing AhR, Arnt, and CYP expression. These data demonstrate that extracellular matrix surface adhesion interactions, which may also subsequently promote tubule morphogenesis, mediate AhR and Arnt expression, which, in turn, enhances CYP expression. Tubule morphology is not needed for this expression sequence and is more demanding on adhesion interactions.

1681 ACTIVITIES OF CYP2E1 AND 1A2 IN PATIENTS WITH HEPATITIS C (HC) AND HC-ASSOCIATED PORPHYRIA CUTANEA TARDA (PCT/HC).

S. I. Shedlofsky[1, 3], R. T. Tosheva[1], R. A. Blouin[1], C. M. Charriez[1], C. K. Hallberg[2] and K. Anderson[2, 3]. *[1]University of Kentucky, Lexington, KY, [2]University of Texas Med. Branch, Galveston, TX and [3]General Clinical Research Centers, Lexington,, KY.*

Liver injury in HC very likely involves increased hepatic oxidative stress with hepatic cytochromes P450 activities probably contributing to it. We report initial results on the activities of two P450 isoforms known to leak reactive oxygen, and other markers for oxidative stress, in patients with HC, PCT/HC and controls. METHODS: Chlorzoxazone and theophylline oral clearances were used as markers of CYP2E1 and 1A2, respectively; urines were collected for analysis of F2-isoprostanes; blood samples were collected for hyaluronic acid (HA) and procollagen propeptide III (PPIII). 24 patients with HC who had not been treated recently for their infection, 11 patients with PCT/HC, and 10 controls have been studied to date. Correlations were made between CYP 2E1 and 1A2 and histological activity and fibrosis scores, urinary F2-isoprostanes, HA and PPIII. For CYP1A2, smokers were analyzed separately from non-smokers. All PCT patients were smokers. RESULTS: CYP2E1 activities for HC patients with mild, moderate or severe histological injury were 5.28 +2.05(n=10), 3.43 +1.54(n=8) and 4.64+2.13(n=6) respectively and were not statistically significant. Results for CYP 1A2 activities in the above groups were divided in smokers and non-smokers and are as follows: mild 0.31 +0.16 (n=2), moderate 0.30 +0.07 (n=4) and severe 0.25 +0.03 (n=4) for non-smokers and mild 0.45 +0.19 (n=8), moderate 0.90 +0.30 (n=4) and severe 0.69 +0.30 (n=2) for smokers. Results in patients with PCT/HC for both CYP1A2 and 2E1 were not statistically significant from HC or controls. Histological scores and levels of HA (r=0.347), PPIII (r=0.214) and F2-isoprostanes (r=0.048) show weak or low correlation. CONCLUSIONS: These preliminary results do not show good correlations between degree of liver injury and measures of oxidative stress in HC or PCT/HC. However, due to large variability in P450 activities, results from a larger number of subjects will be needed

1682 IDENTIFICATION OF CYP2E1-DEPENDENT GENES INVOLVED IN CARBON TETRACHLORIDE INDUCED LIVER INJURY: A MODEL FOR CHEMICAL TOXICITY MEDIATED BY FREE RADICALS.

S. Avasarala and S. Lee. *Biochemistry, The Chinese University of Hong Kong, Shatin, Hong Kong, Hong Kong.* Sponsor: K. Chan.

The hazardous effects of chemicals are of great concern and widely studied in an effort to determine the mechanisms underlying their toxicity and carcinogenicity. Chemicals such as carbon tetrachloride (CCl_4) and acetaminophen induce liver injury through free radical mechanism. CCl_4 is considered as a prototype for better understanding of free radical mediated hepatotoxicity. The biochemical basis of CCl_4 induced lipid peroxidation has been well studied but molecular mechanism underlying this injury remain to be elucidated. The hepatotoxicity of CCl_4 is mainly mediated by cytochorme P4502E1 (CYP2E1) that belongs to P450 (P-450s) superfamily of hemoproteins, which carry out oxidative metabolism of many endogenous and xenobiotic chemicals. Previous studies in our laboratory demonstrated that mice, which lack CYP2E1 expression, were resistant to liver damage after i.p. administration of CCl_4 (1 ml/kg), thus indicating that CYP2E1 is the major factor involved in CCl_4 induced liver injury. In order to depict the molecular mechanism underlying this event, CYP2E1-null mice were used as an *in vivo* model and fluorescent differential display (FDD) method was employed to identify CYP2E1-dependent genes. Approximately thirty-five differentially expressed FDD fragments have been excised, reamplified, subcloned and sequenced. The expression levels of genes related to metabolism of lipids, inflammatory response and hepatocyte damage were confirmed by northern blotting. Further characterization of these genes for their temporal and tissue expression may help us to identify their roles in CCl_4 induced hepatotoxicity thus giving clear insight into the molecular biology of free radical mediated liver injury. (Supported by a grant from the Research Grants Council of the HKSAR project No.CUHK4135/01M).

1683 NEUROPROTECTIVE EFFECTS OF CYP2D6 OVEREXPRESSION IN DIFFERENTIATED PC12 CELLS.

R. D. Harbison[1], M. Banasik[1, 2], T. Stedeford[1, 2], N. Matoh[3], S. Tanaka[3], M. Takehashi[3] and K. Ueda[3]. *[1]Department of Environmental and Occupational Health, University of South Florida, Tampa, FL, [2]Laboratory of Toxicology and Risk Assessment, Polish Academy of Sciences, Gliwice, Poland and [3]Laboratory of Molecular Clinical Chemistry, Institute for Chemical Research, Kyoto University, Uji, Japan.*

Clonal pheochromocytoma cell lines overexpressing cytochrome P450 2D6 (CYP2D6) were established. CYP2D6 was localized in the endoplasmic reticulum, and its enzymatic activity in the microsomal fraction was confirmed by using high performance liquid chromatography analysis with [guanidine-14C]debrisoquine as a substrate. Overexpression of CYP2D6 protected both actively dividing and differentiated cells against the toxic effects of 1-methyl-4-phenylpyridinium ion at the concentration range of 20 to 40 microM, as assessed by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. The production of reactive oxygen species in the mitochondria was suppressed. The cytotoxicity of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine was unchanged in both actively dividing and differentiated cells overexpressing CYP2D6 versus mock-transfected controls at concentrations up to 500 microM. These results suggest that the lowered enzyme activity of CYP2D6 in individuals, termed poor metabolizers may represent a risk factor from exposure to select neurotoxicants.

1684 EFFECT OF THE PROTOTYPICAL INDUCER RIFAMPICIN ON CYTOCHROME P450 3A INDUCTION IN LONG-TERM CULTURES OF HUMAN HEPATOCYTES.

C. M. Chandler, G. Zhang, R. Clark, C. L. Crespi and D. M. Stresser. *Discovery Labware, BD Biosciences, Woburn, MA.*

The demand for pharmacological and toxicological use of primary cultured human hepatocytes is increasing. However, high quality primary human hepatocytes suitable for these studies are available only in limited supply. The goal of this study was to examine if long-term cultures of hepatocytes are suitable for cytochrome P4503A induction studies. The use of long-term cultures would thus greatly increase the experimental window for induction studies, permit repeat experiments with material from the same donor and allow for a more efficient use of this scarce resource. Hepatocytes were isolated from livers of 5 donors and maintained in hepatocyte culture medium for different periods up to 4 - 9 weeks before culture quality had significantly deteriorated or became unresponsive to inducer. Morphological examination showed that cuboid shape of hepatocytes and monolayer appearance could be maintained during culture however, culture quality declined with time in culture as expected and varied between donors. CYP3A induction was studied weekly or bi-weekly using 20 mM rifampicin (RIF), a prototypical inducer for CYP3A4. After the final day of the three-day treatment, the activity of testosterone 6b-hydroxylase was determined. Treatment with RIF caused a minimum 7-fold and maximum 120-fold induction in testosterone 6b-hydroxylase activity, depending on donor or length of time in culture. In all five donors, RIF-induced CYP3A fold-induction at or near the final week in culture was comparable to that in the 1st week of culture. In general, the fold-induction response was higher after 2 weeks of culture due to a decreased basal level of CYP3A activity. Our results demonstrate that long-term cultures of human hepatocytes are suitable for CYP3A induction testing and that culture use within one week of isolation is not required.

1685 THE USE OF IMMORTALIZED HEPATOCYTES IN METABOLISM AND INDUCTION STUDIES.

K. C. Lyon, M. Czerwinski, P. Toren, M. Perry and A. Parkinson. *XenoTech LLC, Lenexa, KS.*

Ea1C-35 and Fa2N-4 are two SV40-immortalized human hepatocyte cell lines developed by Multicell Technologies (Warwick, RI). Czerwinski et al (Drug Metabolism Reviews 35, suppl 2, #462, 2003) has previously demonstrated that multiple cytochrome P450 (CYP) enzymes are inducible in the Fa2N-4 cells. In the current study, we investigated the CYP activity in vehicle and enzyme inducer-treated cultures to characterize the function of the major nuclear receptor pathways in the two cell lines. Using LC/MS/MS methods, we demonstrated that both cell lines metabolize CYP-specific substrates for CYPs 1A2 (phenacetin), 2B6 (bupropion), 2C9 (diclofenac), 2C19 (S-mephenytoin), 2D6 (dextromethorphan) and 3A4 (midazolam). In addition, we examined the response of the cell lines to compounds that regulate enzyme induction through specific nuclear receptor pathways. Treatment of the Ea1C-35 and Fa2N-4 cells with aryl hydrocarbon receptor agonists including 3-methylcholanthrene, βnaphthoflavone and omeprazole caused an increase in CYP1A2 activity. Likewise, dosing of Ea1C-35 and Fa2N-4 cells with pregnane X receptor agonists such as rifampin, hyperforin and ciglitazone caused an increase in CYP3A4 activity. In summary, these cell lines demonstrate hepatocyte-like CYP-mediated metabolism and induction through distinctive nuclear receptor pathways. The use of the cell lines present a promising alternative to primary cultures of human hepatocytes for evaluating both metabolite stability and enzyme induction of new chemical entities.

1686 VALIDATION OF POOLED CRYOPRESERVED HUMAN HEPATOCYTES AS A MODEL FOR METABOLISM STUDIES.

G. Zhang, C. M. Chandler, A. Dandeneau, C. L. Crespi and D. M. Stresser. *Discovery Labware, BD Biosciences, Woburn, MA.*

Primary human hepatocytes are one of the most important systems for xenobiotic metabolism and toxicity studies. However, the demand for fresh human hepatocytes far exceeds current supply. Cryopreserved human hepatocytes represent a con-

venient and biologically relevant model to mitigate this issue. The aim of the study was to validate a pool of cryopreserved human hepatocytes as a model for drug metabolism studies. Four lots of hepatocytes were selected based on previously characterized *via*bility, P450 isoform- and uridine diphosphate glucuronosyltransferase (UGT)-activity. After enrichment of *via*ble cells using a percoll purification, hepatocytes (0.5x106 cells/ml) were incubated with 25 uM 7-ethoxycourmarin (7-EC) in Williams Medium E in a 24-well plate for different times up to 240 minutes. The formation of 7-hydroxycoumarin (7-HC), and its glucuronide (7 HCG) and sulfate (7-HCS) were measured using HPLC. The cell *via*bility was monitored over the culture period by both trypan blue exclusion and the leakage of lactate dehydrogenase. Cell *via*bility was well maintained during the entire 240 minute incubation period (essentially unchanged from initial 77% *via*bility). Formation of all three metabolites (7-HC, 7HCG and 7-HCS) was linear with time of incubation except that 7-HCS was not detected at the first incubation time point (30 min). After 240 min of incubation, the total metabolism from the three metabolites was 11.6 nmol/106 cells, an average of 12.7 and 10.4 nmol/106 cells from two separate experiments. In other experiments, the metabolic stability of 10 compounds was studied and compared to literature values. Our results demonstrate the suitability of this pool for *in vitro* drug metabolism studies.

1687 THE ONTOGENY OF HEPATIC CYTOCHROME P450S IN SPRAGUE-DAWLEY RATS.

B. T. McPhail, S. Muralidhara and J. Bruckner. *University of Georgia, Athens, GA.*

Physiological and biochemical changes that occur as an infant grows have the potential to substantially alter the toxicokinetics and adverse effects of solvents, pesticides and other chemicals. Limited information is available on the ontogeny of P450s in both humans and rodents. The objective of our project was to thoroughly characterize the maturation of several hepatic P450s that are involved in the metabolic activation of halogenated solvents and in the inactivation of pyrethroid insecticides. Groups of Sprague-Dawley rats of mixed sexes, ranging in age from 5 to 60 days, were sacrificed. Livers were removed, homogenized, and the microsomes isolated for measurement of total P450 levels and p-nitrophenol hydrolyase (CYP2E1), ethoxyresorufin O-deethlyase (CYP1A1/2) and pentoxyresorufin O-deethylase (CYP2B1/2) activities. Activities of P450s were very low in the liver of neonatal rats, but increased substantially within the first 15 days of life. Twenty-one-day-old pups exhibited maximal CYP2E1 activity of 2.75 nmol/mg protein. CYP2E1 diminished slightly by 30 days of age, but CYP1A1/2 activity rose dramatically (i.e., from 1000 to 6000 nmol/mg protein). CYP2B1/2 remained relatively constant, while total P450 levels gradually increased from birth to puberty. Our findings indicate that activities of CYP1A1/2, 2B1/2, and 2E1 in rats are very low at birth, rise to maximums that exceed adult levels by 20-30 days of age and then progressively diminish through sexual maturity.

1688 ORAL ADMINISTRATION OF BENZO(A)PYRENE IN THE INTACT MOUSE: DETOXICATION BY INDUCIBLE CYP1A1 IS MUCH MORE IMPORTANT THAN METABOLIC ACTIVATION.

D. W. Nebert, H. Dong, T. P. Dalton, M. L. Miller, S. Derkenne, C. Curran and S. Uno. *Environmental Health and Center for Environmental Genetics, University of Cincinnati, Cincinnati, OH.*

There is a general rule in pharmaceutical companies that, if any test drug coming through the pipeline shows induction of CYP1 proteins (*i.e.* AH receptor activation), it is immediately dropped for fear of its likelihood to cause cancer. Benzo[a]pyrene (BaP) must be metabolically activated to exert its toxic effects. Numerous studies performed *in vitro* and in cell culture systems have shown unequivocally that the CYP1A1 enzyme metabolically activates BaP into toxic and DNA-binding intermediates that are associated with mutations and cancer. We therefore sought to confirm the role of CYP1A1 in metabolic activation of BaP, comparing *Cyp1a1(-/-)* knockout with *Cyp1a1(+/+)* wild—type mice——following oral BaP at doses of 125, 12.5 and 1.25 mg/kg/day. *Cyp1a1(-/-)* mice at the 125-mg dose die within 30 days whereas *Cyp1a1(+/+)* mice appeared healthy 1 year later. The rate of BaP clearance was 4-fold greater in wild-type than knockout mice. The cause of death in *Cyp1a1(-/-)* mice receiving oral BaP was immunotoxicity, including toxic chemical depression of the bone marrow; effects on the *Cyp1a1(-/-)* mouse's immune system were detected even at oral BaP doses of 12.5 and 1.25 mg/kg/day. DNA post-labeling studies demonstrated statistically significantly (P<0.0001) higher BaP-DNA adduct levels in *Cyp1a1(-/-)* than *Cyp1a1(+/+)* in liver, small intestine, spleen and marrow at the 125 and 12.5 mg/kg/day BaP doses, and in spleen even at the 1.25 mg/kg/day dosage. We conclude that, despite previous studies *in vitro* and in cell culture to the contrary, the present data indicate that——in the intact animal——inducible CYP1A1 is much more important in detoxication and protection against oral BaP toxicity than in mice lacking CYP1A1. Whether the protection comes primarily from the liver, the GI tract, or something in the context of the immune system——remains to be determined.——***Supported, in part, by NIH grants P30 ES06096, R01 ES06321 and R01 ES08147.***

1689 ABILITY OF FLAVONOIDS IN ST. JOHN'S WORT TO DECREASE CYP1 ACTIVITIES.

A. M. Chaudhary and K. L. Willett. *Pharmacology, The University of Mississippi, University, MS.*

As with many available herbal preparations, components of St. John's wort are not completely characterized with respect to their interaction with drug metabolizing enzymes. CYP1B1 is a cytochrome P450 gene involved in metabolizing both polycyclic aromatic hydrocarbons and estradiol to potentially carcinogenic intermediates. CYP1B1 is also over expressed in human cancer cells. Finding compounds that inhibit CYP1B1-mediated metabolism is an important goal in cancer and chemoprevention research. In order to identify flavonoids that specifically inhibit recombinant CYP1B1 compared to CYP1A1, nine flavonoids were screened for their ability to inhibit ethoxyresorufin-O-deethylase (EROD) activity. Rutin, hyperoside, and homoeriodictoyl did not inhibit in either system whereas quercetrin, kaempferol, myrecetin and isoquercetin did not specifically inhibit CYP1B1 relative to CYP1A1. Quercetin (IC50s: 1A1= 4.6 μM; 1B1 = 3.25 μM) and amentoflavone (IC50s: 1A1= no inhibition; 1B1 = 4.2 μM) did exhibit some specificity of inhibition and were selected for further study in 22Rv1 prostate cancer cell. Prostate cancer is the most common cancer in US men and second leading cause of cancer death in men. While TCDD induced EROD activity (19 ± 1.8 pmol/mg/min), quercetin and amentoflavone alone did not induce activity relative to DMSO control treated cells. TCDD induced cells (1nM/24 hr) were then dosed with different concentrations (0.5-5μM) of flavonoids. After 30 min, EROD assays were done. Relative to TCDD alone, quercetin inhibited EROD activity (IC50: 3.74 ± 0.3 μM) in dose dependent manner in 22Rv1 prostate cells. In contrast, amentoflavone did not inhibit EROD activity. In intact cells EROD is not CYP isoform specific. Therefore, qRT/RT PCR and western blots are being used to determine the relative inhibition of CYP1B1 and CYP1A1 in the prostate cells. By distinguishing relative roles of cytochrome P450s (specifically CYP1B1 vs. CYP1A1) in different human cancer cell lines, methodologies can be developed to provide better diagnostics and possibly new therapies against cancer formation. (Supported by AACP)

1690 THEOPHYLLINE METABOLISM AND PHARMACOKINETICS IN CYP1A2(+/+) WILD-TYPE AND CYP1A2(-/-) KNOCKOUT MICE.

S. Derkenne, C. Curran, T. P. Dalton, H. G. Shertzer and D. W. Nebert. *University of Cincinnati, Cincinnati, OH.*

Theophylline is widely used in the treatment of chronic obstructive pulmonary diseases. Plasma pharmacokinetics and renal excretion of theophylline and its metabolites (1-methyl- and 1, 3-dimethyluric acid, 3-methylxanthine) were investigated in Cyp1a2(+/+) wild-type C57BL/6J and Cyp1a2(-/-) knockout mice (C57BL/6J). Following a single i.p. dose (8 mg/kg), urine was collected during the next 24 h and sequential blood samples were drawn. Some mice were treated with i.p. 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD; 10 μg/kg) 24 h prior to theophylline administration. Plasma and urine concentrations of theophylline and its metabolites were determined by high-performance liquid chromatography. We confirmed that this dose of theophylline (8 mg/kg i.p.) gave linear pharmacokinetics by testing 4 doses. The half-life of theophylline elimination from blood was more than 4 times longer in Cyp1a2(-/-) than Cyp1a2(+/+) mice (172 ± 33 vs 40.4 ± 8.8 min, mean ± SD) and more than 10 times different after TCDD treatment (152 ± 28 vs 13.5 ± 5.0 min). Theophylline clearance was concomitantly 4 times slower in Cyp1a2(-/-) than Cyp1a2(+/+) mice (2.6 ± 0.4 vs 10 ± 1.5 ml/min/kg) and more than 10 times different after TCDD treatment (3.4 ± 0.94 vs 32 ± 7.7 ml/min/kg). Thus, the total area under the plasma theophylline concentration-time curve (AUC) was 3 times larger in Cyp1a2(-/-) than in Cyp1a2(+/+) mice (2346 vs 794 mg min/ml); TCDD treatment increased this difference to 8-fold (2046 vs 255 mg min/ml). The urinary 1-methyluric acid is 8 times greater in Cyp1a2(-/-) mice after TCDD treatment (3.0 ± 0.5 vs 0.37 ± 0.10, percent of injected amount of drug); in contrast, there is little change in Cyp1a2(+/+) mice after TCDD treatment (17 ± 1.6 vs 15 ± 4.2). We conclude that CYP1A2 (which is TCDD-inducible) is the major enzyme metabolizing theophylline; there is a second enzyme, however, and it is TCDD-inducible and responsible for a small amount of 1-methyluric acid formation. ——-Supported, in part, by NIH grants P30 ES06096, R01 ES06321 and R01 ES08147.

1691 DECREASED CYP1A1-DEPENDENT ENZYME ACTIVITY AND PROTEIN LEVELS IN HEPG2 CELLS EXPOSED TO BENZO(A)PYRENE IN THE PRESENCE OF 1-NITROPYRENE.

S. Cherng[1, 2], S. Hsu[3], J. Yang[4] and H. Lee[1]. [1]*Institute of Toxicology, Chung Shan Medical University, Taichung, Taiwan,* [2]*Food Science and Nutrition, Hung Kuang University, Taichung, Taiwan,* [3]*Department of Education & Research, Taichung Veterans General Hospital, Taichung, Taiwan and* [4]*Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan.* Sponsor: P. Howard.

The genotoxicity of polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs may be influenced by interaction of the compounds at the level of enzyme activity. Limited information was available concerning the effect of 1-nitropyrene (1-NP) on benzo[a]pyrene (BaP)-induced genotoxicity in mammalian cells. In this study, the human hepatoma cell line HepG2 was simultaneously treated with 1-NP and BaP to investigate the effects of chemical interaction on the genotoxicity of this binary mixture. The BaP-DNA adduct levels, evaluated by 32P-postlabeling, decreased in the presence of added 1-NP in a dose-dependent manner. Our results showed that the inhibition of BaP-DNA adduct formation by 1-NP was due to decreased cytochrome P450 1A1 (CYP1A1)-linked aryl hydrocarbon hydroxylase activity. Furthermore, northern blot analysis showed that 1-NP slightly attenuated BaP-induced CYP1A1 mRNA expression, but western blot analysis revealed a large decrease in the level of CYP1A1 protein. Analysis of *in vitro* proteolysis demonstrated that the addition of 1-NP enhanced CYP1A1 protein degradation. The proteolysis of CYP1A1 protein was inhibited by the addition of an antioxidant, dithiothreitol. These findings suggested that a posttranslational mechanism is involved in loss of CYP1A1 protein, causing the decrease of BaP-DNA adduct levels in the presence of binary mixtures of 1-NP and BaP.

1692 ROLE OF MOUSE CYP2E1 IN THE O-HYDROXYLATION OF P-NITROPHENOL: COMPARISON OF ACTIVITIES IN *CYP2E1* (-/-) AND WILDTYPE MICE.

K. K. Wolf[1], S. G. Wood[3], J. L. Bement[3], P. R. Sinclair[3, 2, 1], S. A. Wrighton[4], E. Jeffery[5], F. J. Gonzalez[6] and J. F. Sinclair[3, 2, 1]. [1]*Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH,* [2]*Biochemistry, Dartmouth Medical School, Hanover, NH,* [3]*VA Medical Center, White River Junction, VT,* [4]*Lilly Research Laboratories, Indianapolis, IN,* [5]*Food Science and Human Nutrition, University of Illinois, Urbana, IL and* [6]*Laboratory of Drug Metabolism/ Disposition, National Cancer Institute, Bethesda, MD.*

Selective enzymatic activities are used to identify the role of an individual form of CYP in a particular biotransformation. p-Nitrophenol O-hydroxylation (PNPH) has been widely used as a measure of CYP2E1. However, rat and human forms of CYP3A have also been shown to catalyze this activity. In mice, the contribution of CYP3As and CYP2E1 to PNPH is not known. Here we compared hepatic microsomes from *Cyp2e1* (-/-) and wild type mice to investigate the contribution of constitutive and alcohol-induced levels of murine CYP2E1 and CYP3A to PNPH. In untreated mice, hepatic levels of PNPH were much greater in wildtype mice compared to *Cyp2e1* (-/-) mice, suggesting a major role of CYP2E1 in catalyzing PNPH. Hepatic levels of PNPH were not significantly different between males and females, even though females have dramatically higher levels of CYP3A. Treatment with ethanol in combination with isopentanol resulted in induction of CYP3A proteins in wildtype and knockout mice, and CYP2E1 protein in wildtype mice. The alcohol treatment increased PNPH in hepatic microsomes from wildtype mice but not from knockout mice. Our findings suggest that constitutive and alcohol-induced forms of mouse CYP3A have little to no role in PNPH. Therefore, in untreated and alcohol-treated mice, PNPH can be used as an unambiguous assay for CYP2E1.

1693 STUDY OF METABOLIC INTERACTIONS OF FIPRONIL AND SOME CYP3A4 SUBSTRATES.

J. Tang, A. Usmani, E. Hodgson and R. L. Rose. *Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.*

Fipronil, a highly active and broad spectrum insecticide from the phenyl pyrazole family, is a potent disruptor of the ?-aminobutyric acid (GABA)-gated chloride channel in the insect central nervous system. It owes its insect selectivity to the fact that it is less potent in binding the GABA receptor in mammals. Since its discovery about a decade ago, fipronil has been used against a variety of agricultural, domestic and veterinary insect pests. Despite its wide range of use, however, mammalian metabolism of fipronil has not been extensively investigated. The human metabolism of fipronil has been studied in our laboratory using human liver microsomes (HLM) and cytochrome P450 (CYP) isoforms. CYP3A4 has been identified as the major enzyme catalyzing fipronil metabolism in human. Because of broad substrate specificity of CYP3A4, fipronil may interact with many other CYP3A4 substrates. This study investigated metabolic interactions of fipronil with testosterone or diazepam, two other CYP3A4 substrates. Fipronil was incubated with testosterone or diazepam in HLM and metabolites were analyzed using HPLC. When 1.25, 5 or 20 ?M fipronil incubated with a serial concentrations of testosterone (0-200 ?M), activation of fipronil S-oxidation was observed using HLM as the enzyme source. Inhibition of testosterone 6?-hydroxylation was observed in HLM when incubating testosterone (4, 20, or 100 ?M) with fipronil (0-160 ?M). There were no significant effects of diazepam (0-400 ?M) on metabolism of fipronil (5, 20 or 80 ?M). However, when 6.25, 25 or 100 ?M concentrations of diazepam were incubated with fipronil (0-160 ?M), diazepam hydroxylation was not significantly affected although demethylation of diazepam was inhibited. These results demonstrate that fipronil has the potential to interact with other CYP3A4 substrates (Supported by NIOSH Grant OH07551-ECU).

1694 N-DEALKYLATION OF N-ETHYL-N-(2-HYDROXYETHYL)PERFLUOROOCTANESULFONAMIDE (N-ETFOSE) BY RAT LIVER MICROSOMES AND BY EXPRESSED RAT AND HUMAN CYTOCHROMES P450 (CYPS).

L. Xu[1], A. S. Seacat[2], J. L. Butenhoff[2] and M. W. Anders[1]. [1]*Pharmacology and Physiology, University of Rochester, Rochester, NY and* [2]*3M Co., St. Paul, MN.*

N-EtFOSE is a N-substituted perfluorooctanesulfonamide that is degraded to PFOS. Male rat hepatic microsomal fractions catalyze the NADPH-dependent dealkylation of N-EtFOSE to N-(2-hydroxyethyl)perfluorooctanesulfonamide (FOSE alcohol), which is dealkylated to perfluorooctanesulfonamide (FOSA). The nonselective CYP inhibitors 1-octylamine and 1-benzylimidazole and the NADPH-cytochrome P450 reductase inhibitor diphenyliodium chloride blocked the biotransformation of N-EtFOSE to FOSE alcohol, indicating a role for the CYPs. The objective of this study was to identify the major rat and human CYPs that catalyze the N-dealkylation of N-EtFOSE. Inhibition of N-dealkylase activity by several CYP isoform-selective inhibitors was first examined in rat hepatic microsomal fractions. The N-deethylation of N-EtFOSE was inhibited 90% by disulfiram (CYP2E1), 70% by furafylline (CYP1A2), orphenadrine and diphenhydramine (CYP2B1), and cimetidine (CYP2C11), and 55% by clotrimazole (rat CYP3A2). Studies with expressed rat CYPs showed that CYP3A2, CYP2C11, and CYP2B1 catalyzed the N-deethylation of N-EtFOSE at rates of 15.6, 14.7, and 6.6 pmol/min/nmol P450, respectively. Studies with expressed human CYPs showed that hCYP2C19, hCYP3A4, and hCYP3A5 catalyzed the N-deethylation of N-EtFOSE at rates of 267, 23.6, and 204 pmol/min/nmol P450, respectively. Incubation of FOSE alcohol with the same series of expressed rat and human CYPs showed that rat CYP2C11 and hCYP2C19 catalyzed the N-dealkylation of FOSE alcohol at rates of 0.69 and 1.55 nmol/min/nmol P450, respectively. Rat CYP3A2, CYP1A2, and CYP2B1 catalyzed the N-dealkylation of FOSE alcohol at rates of 98, 39, and 30 pmol/min/nmol P450, respectively. These results establish a role for rat CYP3A2, CYP2C11, and CYP2B1 and human hCYP2C19 hCYP3A4/5 in the biotransformation of N-EtFOSE and FOSE alcohol. (Supported in part by the 3M Co.)

1695 COMPARATIVE HEPATIC MICROSOMAL ENZYME STUDIES IN COMMERCIALLY RAISED GAMEBIRDS.

K. A. Cortright and A. L. Craigmill. *Environmental Toxicology, University of California-Davis, Davis, CA.*

Species differences in drug metabolism can affect therapeutic efficacy and residues in food animals. For example, Webb et al. (2000) has shown that it is safe to treat bobwhite quail with fenbendazole but the therapeutic index in this species is much lower than for other gamebird species. Using hepatic microsomes, we have characterized the kinetics of hepatic cytochrome P450 (CYP)1A and 3A-like activities in chicken, pheasant and quail, using the fluorescent substrates ethoxyresorufin and 7-benzyloxy-(trifluoromethyl)-coumarin (BFC). Inhibition studies were also conducted with prototypical inhibitors of CYP1A2, furafylline, and CYP3A, ketoconazole and erythromycin. Pheasant had the highest ethoxyresorufin-O-deethylase (EROD) activity compared to chicken and quail, 418 versus 46.8 and 15.08 pmoles product/mg x min (quail pooled), respectively. The calculated IC50's for furafylline toward EROD activity were 39uM, 40uM and >100uM for bobwhite quail, chicken, and pheasant, respectively. Kinetic parameters for oxidation of BFC (a CYP3A-selective substrate) were estimated. The apparent Vmax in chicken, pheasant and bobwhite quail was 1628, 7915, and 28347 pmoles product/mg x min, respectively. The apparent Km was 5.0, 4.2 and 34.3uM in chicken, pheasant and bobwhite quail, respectively. The IC50's for ketoconazole inhibition of BFC (20uM) metabolism were 98uM, 100uM and >400uM for chicken, bobwhite quail and pheasant, respectively. Chicken and quail were similar to rats and sheep in their sensitivity to ketoconazole inhibition (IC50's 130 and 106uM, respectively), while pheasant was again much less sensitive. Erythromycin showed very minor inhibi-

tion at most inhibitor and substrate concentrations tested. At 2 uM BFC it increased metabolism in a dose-dependent manner in the pheasant and the quail, but not in the chicken. CYP1A and CYP3A metabolize the majority of therapeutic drugs. These species differences in metabolic capacity may be related to the greater sensitivity of bobwhite quail to fenbendazole.

1696 CYTOCHROME P450 ENZYME ACTIVITIES IN EMBRYONIC TURKEY LIVER.

C. E. Perrone[1], L. Leung[2], J. Kao[2], H. Yasmeen[2], G. L. Fisher[2] and G. M. Williams[1]. *[1]Pathology, New York Medical College, Valhalla, NY and [2]Drug Safety and Metabolism, Wyeth Research, Collegeville, PA.*

Previous experiments in our laboratory showed that embryonic turkey liver has functional phase I and phase II biotransformation enzymes capable of bioactivation of selected pro-carcinogens (Toxicologist 72(S-1): 155, 2002). To further assess whether turkey embryos could be useful as a model to obtain data on chemical-induced toxicity and carcinogenicity with human relevance, we examined embryonic turkey liver microsomal fractions for activities of seven major human drug-metabolizing cytochrome P450s by fast-gradient liquid chromatography tandem mass spectrometry (LC-MS/MS) (Drug Metab. Dispos. 29(1): 23-29, 2001). Using a cocktail consisting of specific probes for human CYP450s (ethoxyresorufin [CYP1A2], coumarin [CYP2A6], taxol [CYP2C8], dicofenac [CYP2C9], S-mephenytoin [CYP2C19], bufuralol [CYP2D6], and midazolam [CYP3A4]), we detected activities for all probes except for CYP1A2 and CYP2C19. Corresponding activities for CYP enzymes were similar in liver microsomes from control, phenobarbital (PB)- and 3-MC-treated turkey embryos, suggesting that induction had not occurred except for CYP1A2 activity which was only detected in 3-MC-treated embryos. The distribution of enzyme activities for the different substrates in embryonic turkey liver microsomes appeared to be quite similar to that in a pool of human liver microsomes determined in our laboratory. We conclude that embryonic turkey liver possesses P450 enzyme activities that can metabolize probes specific for different human P450 enzymes. Thus, turkey embryos could be utilized to assess potential chemical toxicity to humans.

1697 MODULATION OF HEPATIC AND PULMONARY CYTOCHROME P4501A1 EXPRESSION BY HYPEROXIA AND INHALED NITRIC OXIDE IN THE NEWBORN RAT: IMPLICATIONS FOR LUNG INJURY.

X. I. Couroucli, Y. Wei, W. Jiang, L. Evey and B. Moorthy. *Pediatrics, Baylor College of Medicine, Houston, TX.*

Inhaled nitric oxide (INO), in conjunction with supplemental oxygen, is used in the treatment of neonatal pulmonary hypertension associated with hypoxic respiratory failure. In this study, we tested the hypothesis that exposure of newborn rats to INO, hyperoxia or hyperoxia + INO would modulate expression of pulmonary and hepatic cytochrome P450 (CYP)1A1 isoform, in relation to acute lung injury. Newborn Fisher 344 rats were maintained in room air or exposed to INO (50 ppm), hyperoxia (> 95% oxygen), or hyperoxia + INO (20 or 50 ppm) for up to 168 h, and catalytic activities, apoprotein contents (western blotting), and mRNA levels (RT-PCR) of pulmonary and hepatic CYP1A1 were determined. Animals given hyperoxia + INO (20 or 50 ppm) were more susceptible to acute lung injury, as determined by lung weight/body weight ratios, than those exposed to hyperoxia or INO alone. Hyperoxic rats given 20 ppm INO displayed lesser lung injury than those exposed to 50 ppm INO. Hepatic and pulmonary CYP1A1 expression at the protein and mRNA level was significantly induced by hyperoxia or INO for 48 h, compared to air-breathing controls, followed by decline at later time points. On the other hand, while no significant alteration in CYP1A1 expression was noticed in animals given hyperoxia + INO (20 or 50 ppm) for up to 48 h, CYP1A1 levels declined after 72-168 h of exposure. The decline of CYP1A1 induction was accompanied by increased lung injury. Immunohistochemical studies revealed localization of CYP1A1 expression in the epithelial and endothelial cells of the lung. In conclusion, our results suggest that modulation of CYP1A1 expression contributes to the development of acute lung injury by hyperoxia and INO, and that lower dose of INO in conjunction with hyperoxia may be beneficial to infants suffering from pulmonary hypertension. (Supported by NIH grant HL KO8 04333.)

1698 DECREASED ACETAMINOPHEN TOXICITY IN LUNG AND KIDNEY, BUT NOT NASAL MUCOSA, OF MICE WITH LIVER-SPECIFIC KNOCKOUT OF THE NADPH-CYTOCHROME P450 REDUCTASE GENE.

J. Gu, H. Cui, M. Behr, L. Zhang, Q. Zhang, Y. Weng, W. Yang and X. Ding. *Wadsworth Center, New York State Department of Health, Albany, NY.*

Acetaminophen (AP) overdose causes toxicity in liver and extrahepatic tissues. Although it is well established that cytochrome P450 (CYP) enzymes play a critical role in the metabolic activation of AP, it is not yet clear whether AP toxicity in extrahepatic tissues depends on hepatic biotransformation. The aim of this study is to determine whether extrahepatic AP toxicity is altered in a mouse model with liver-specific deletion of the NADPH-cytochrome P450 reductase (CPR) gene. The liver CPR-knockout (null) and the wild-type (WT) mice were given a single i.p. injection of AP at 400 mg/kg, and blood samples were collected for determination of serum AP concentrations. Serum alanine aminotransferase and tissues histopathology for liver, lung, nose, and kidney were also determined. As expected, acute AP hepatotoxicity was observed in WT, but not the null mice. However, the null mice also had reduced toxicity in the nasal mucosa, lung, and kidney, although the anticipated decreases in hepatic AP metabolism would increase circulating AP levels in the null mice. Pharmacokinetic analysis indicated that AP clearance in the null mice was significantly faster than in the WT controls, which suggests occurrence of compensatory increases of other biotransformation enzymes that are active in AP metabolism in the null mice. Nevertheless, after increasing AP dose to 600 mg/kg in the null mice to maintain comparable circulating AP levels in the two strains, we still observed a reduced AP toxicity in the lung and kidney, but not in the nasal mucosa of the null mice. These results indicate that AP toxicity in the nasal mucosa is not dependent on hepatic metabolic activation, and that AP toxicity in the lung and the kidney may be at least partly caused by liver-derived AP metabolites.

1699 INJURY PATTERNS IN THE NASAL PASSAGE FROM INHALED NA ARE RELATED TO AIRFLOW PATTERNS AND IN SITU METABOLISM OF NA IN SPRAGUE-DAWLEY RATS.

M. G. Lee[1], S. Camacho[2], A. R. Buckpitt[2] and C. G. Plopper[1]. *[1]Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, Davis, CA and [2]Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, Davis, CA.*

Naphthalene (NA) is a bio-activated environmental cytotoxicant for rodent respiratory tract. In the rat nasal passage, NA injury was confined within the olfactory mucosa regardless of route of exposure. Since cell types of the olfactory mucosa are known to be phenotypically homogeneous, it was assumed their metabolic capacity was also homogeneous. However, only a limited region of the olfactory mucosa was injured by inhaled NA whereas injury from NA i.p. occurred across the olfactory mucosa. To test whether this characteristic injury pattern from inhaled NA is relevant to previous defined patterns of nasal airflow, male Sprague-Dawley rats were exposed to filtered air, 2 ppm NA, or 25 ppm NA for 4 h. Injury was assessed 24 h after exposure by high-resolution histopathology. The region where the highest nasal airflow exists was injured by either dose of NA without any significant differences in the degree of cellular injury. At 25 ppm, however, injury was spread across broader region of the olfactory mucosa. In an effort to identify the metabolic environment of the rat nasal passage, we performed immunohistochemistry against cytochrome P450 2F, the key enzyme for generating toxic metabolites of NA. Cytochrome P450 2F was present primarily within the olfactory mucosa in the nasal passage. Recently, companion studies revealed a high rate of NA metabolism in the olfactory mucosa (Km = 84 μM and Vmax = 24 nmoles/nmole P450/min). Overall, this study demonstrated that the injury pattern associated with NA exposure in the rat nasal passage was correlated with nasal airflow patterns as well as in situ metabolism of NA. It is notable that we detected NA injury at 2 ppm NA for 4 h, a concentration which is much lower than the current OSHA standard for humans (10 ppm for 8 h time-weighted average (TWA)). Supported by NIEHS ES04311, ES06700 and ES05707.

1700 TARGETED DISRUPTION OF THE OLFACTORY MUCOSA-SPECIFIC CYP2G1 GENE: IMPACT ON ACETAMINOPHEN TOXICITY IN THE LATERAL NASAL GLAND, AND TISSUE-SELECTIVE EFFECTS ON CYP2A5 EXPRESSION.

H. Cui, X. Zhuo, J. Gu, M. Behr, P. Swiatek, Q. Zhang, Y. Xie, D. Collins and X. Ding. *Wadsworth Center, New York State Department of Health, Albany, NY.*

CYP2G1 is a cytochrome P450 monooxygenase expressed uniquely in the olfactory mucosa (OM). We have generated Cyp2g1-null mice to identify the roles of CYP2G1 in the biology and the tissue-specific toxicity of xenobiotic compounds in the nose. Homozygous Cyp2g1-null mice are *via*ble and fertile; they show no evidence of embryonic lethality, morphological abnormality, or developmental deficits; and they seem to have normal olfactory ability. However, OM microsomes from Cyp2g1-null mice were found to have significantly lower activities than microsomes from wild-type mice in the metabolism of testosterone and progesterone (~60% decrease), and in the metabolic activation of coumarin (>70% decrease). Unexpectedly, a significant reduction in the expression of the Cyp2a5 gene was found in the liver, the lateral nasal gland (LNG), and, to a lesser extent, the kidney of adult Cyp2g1-null mice. The loss of CYP2G1 expression, and the associated decrease in the hepatic expression of CYP2A5, did not decrease systemic clearance, extent of hepatotoxicity, or OM toxicity of acetaminophen (AP). However, the

LNG, which is the major site for the production of odorant binding proteins, was protected from AP (at 400 mg/kg) toxicity in the Cyp2g1-null mice. Paradoxically, the LNG did not have detectable CYP2G1, and the decrease in LNG CYP2A5 expression in the Cyp2g1-null mice was not accompanied by decreases in microsomal AP metabolism. We hypothesize that OM CYP2G1 (through a paracrine pathway) or LNG CYP2A5 may indirectly influence resistance of the LNG to chemical toxicity, possibly by regulating gene expression in the LNG through steroid hormones or other endogenous CYP substrates and their metabolites.

1701 MECHANISM-BASED INACTIVATION OF HUMAN PULMONARY CYTOCHROME P450 2F1 BY PNEUMOTOXIN 3-METHYLINDOLE.

J. S. Kartha, K. W. Skordos, D. L. Lanza and G. S. Yost. *Pharmacology and Toxicology, University Of Utah, Salt lake city, UT.*

3-Methylindole (3-MI) is a pneumotoxin that requires P450-catalyzed metabolic activation (dehydrogenation), to an electrophilic methylene imine to elicit toxicity. Previous studies have shown that the human pulmonary cytochrome P450 2F1 enzyme and its goat analog, P450 2F3, selectively catalyze the dehydrogenation of 3-MI without the formation of any oxygenated metabolites. In addition, previous work with 2F1 have shown that concentrations of 3-MI above 300μM strongly inhibited formation of the dehydrogenated product, indicating that 3-MI could be a mechanism-based inhibitor of 2F1. The purpose of this study was to determine if 3-MI could inhibit 2F1 and 2F3 in a time- and concentration-dependent manner. In the presence of NADPH, P450 2F1 lost approximately 58 % of its 7-ethoxycoumarin O-deethylase activity following a 30 minute pre-incubation with 3-MI. Pre-incubations done with 3-MI in the absence of NADPH did not show comparable loss of activity. The kinetic constants for the inactivation of P450 2F1 by 3-MI were determined using the Kitz-Wilson plot. The concentration required for half-maximal inactivation KI, was found to be 49μM and the maximal rate of inactivation kinact was 0.01/min.The partition ratio, which is the number of 3-MI molecules metabolized per molecule of 2F1 inactivated, was approximately 280.Thus, 3-MI inactivated 2F1 slowly, but at a reasonably low concentration. Exogenous nucleophiles such as glutathione did not protect P450 2F1 from 3-MI mediated inactivation, suggesting that the reactive intermediate remained within the active site of the enzyme. Preliminary data show that 3-MI could be inhibiting the 7-ethoxy coumarin deethylase activity of 2F3 in a time- and concentration-dependent manner. In conclusion, this study presents evidence that 3-MI acts as a mechanism-based inhibitor of human CYP2F1, which may be the most important enzyme in the bioactivation of pneumotoxicants like 3-MI, or carcinogens like naphthalene, benzene and styrene. This research was supported by NHLBI grant HL13645.

1702 INVESTIGATION OF THE IRREVERSIBILITY OF CYP450 INHIBITION CAUSED BY M-XYLENE AND METABOLITES IN RAT LUNG AND NASAL MUCOSA.

A. Vaidyanathan and R. Schatz. *Pharmacology Sciences., Northeastern University, Boston, MA.*

Xylene (XYL) is used as a solvent in paints, cleaning agents and gasoline. Exposure occurs primarily by inhalation. Due to the volatility and lipophilicity of the xylenes, the lung and nasal mucosa are primary target organs. m-XYL is oxidized *via* cytochrome P450 (CYP450) isozymes to 3-methylbenzyl alcohol (3-MBA) and subsequently to m-tolualdehyde (m-ALD). The major CYP450 isozymes involved in the metabolism of the xylenes in the lung and nasal mucosa (target tissues) include CYPs 2B, 2E1 and 4B1. m-XYL alters cytochrome CYP450 activity in an organ and isozyme specific manner. The purpose of this work was to determine if the interaction of the reactive metabolite of m-XYL, m-ALD, to CYP450 was reversible. To determine the role of m-ALD in m-XYL induced CYP inhibition in these respiratory tissues, hydrazine was used to adduct the unstable aldehyde intermediate and prevent it from binding to CYP450. There was a time-dependent inhibition by m-XYL and metabolites of CYPs 2B, 2E1 and 4B1 in the lung and nasal mucosa. CYP 2B, 2E1 and 4B1 inhibition caused by these compounds was not reversible upon overnight dialysis. The presence of hydrazine in preincubation mixtures prevented inhibition caused by m-XYL, 3-MBA or m-ALD of CYPs 2B, 2E1 and 4B1 in the lung and nasal mucosa. This irreversible binding by m-ALD may represent a mechanism by which xylene expresses its *in vivo* toxicity.

1703 TOXICOGENOMICS PROJECT IN JAPAN - OBJECTIVE AND PROPOSAL.

T. Urushidani[1,2], J. Kanno[1], T. Miyagishima[2] and T. Nagao[3,2]. *[1]Cell. & Mol. Toxicol., National Institute of Health Sciences(NIHS), Tokyo, Japan, [2]Toxicogenomics Project, NIHS, Tokyo, Japan and [3]Director General, NIHS, Tokyo, Japan.* Sponsor: S. Tsuda.

In the worldwide pharmaceutical market, the entry of new chemicals is decreasing in spite of the progress of technology in finding the seeds for new drugs. Many candidate drugs drop out in the stage of non-clinic and clinic. A considerable percentage of dropouts is due to toxicity. It was commonly recognized by the Japanese pharmaceutical industry and the government that predicting toxicity in the early stage of drug development was indispensable, and that the construction of a database based on toxicogenomics technology using it along with bioinformatics technology seemed to be the most effective plan. Along this line, The Ministry of Health Labor and Welfare, NIHS, and the working group of the Japan Pharmaceutical Manufacturers Association began to draft a research project where both the nation and private companies joined. The project, for the 5 years from 2002 to 2007, has just started. Half of the amount for the entire budget, about 5 billion yen, is from the national budget, while the remaining half is from the 17 companies which participated in the project within the Japan Pharmaceutical Manufacturers Association. The main features and advantages of the project are: 1) the quantification of "absolute content of mRNA per one cell" is attained by using the Affymetrix GeneChip, 2) of the total group of 150 chemicals including considerable number of "the drug of which clinical trial was terminated or of which marketing was ceased due to the fact that human toxicity emerged, even though the presence of toxicity was undetected or neglected in non-clinical tests", 3) various toxicological data with high quality have links to the gene expression data in the database, and 4) the bridging of the interspecies is considered. The final goal of the project is to construct a large-scale database of these chemicals on rats and to develop a system which will forecast the toxicity of new chemicals in the early stage of drug development. This paper reports the outline and the present state of the project.

1704 USING TRANSCRIPT PROFILING FOR PREDICTIVE TOXICOLOGY: OPPORTUNITIES AND CHALLENGES.

R. A. Jolly, K. M. Goldstein, T. Wei, J. Colet, H. Gao, T. P. Ryan, C. E. Thomas, H. B. Harlow, K. Kramer, S. Patwardhan, S. T. Estrem and J. L. Stevens. *Exploratory Toxicology, Lilly Research Labs, Indianapolis, IN.*

The development predictive toxicology databases based on transcript profiles represents a promising application for microarrays in pharmaceutical safety assessment. In principle, a predictive toxicology database contains transcript profiles from model toxicants (training set) with known mechanisms of injury. The hypothesis is that the training set can be used to predict the biological properties of a novel chemical entity based on a tissue transcript profile alone. To test this hypothesis, male SD rats were treated with a single dose of five prototypical hepatotoxicants and sacrificed 4, 24, 48 and 168 hours post dose. A dose response was characterized using minimally or non-toxic dose (low dose) and a high dose that caused a discernable lesion. Transcript profiles were generated and evaluated in liver. Preliminary analysis using hierarchical clustering (HCS) and principle component analysis (PCA) of hepatic transcript profiles showed that distinct transcript classes were generated. To determine the predictive value of the data set, we used a supervised k nearest neighbor (kNN) analysis. High dose samples were categorized, a priori, as HEPATOTOXIC (HPTX); low dose and vehicle samples were categorized as NON- HEPATOTOXIC (N-HPTX). Samples were randomly divided into training and test sets. The training set samples were designated HPTX or N-HPTX based on clinical chemistry and histology results while samples in the test set were classified as HPTX or N-HPTX by kNN analysis. Prediction rates were assessed after one thousand randomized iterations. Accuracy was approximately 80% but was variable with high rates of false negatives noted. When early time points and low dose toxicant samples (these samples also showed fewer transcript changes) were excluded the prediction rates improved with a dramatic decrease in false negatives. Thus, the dramatic effects of dose and time on accuracy of prediction, highlight the limitations of this type of analysis.

1705 FORMULATION OF RNA PERFORMANCE STANDARDS FOR REGULATORY TOXICOGENOMIC STUDIES.

B. A. Rosenzweig[1], E. Mansfield[1], P. Pine[1], F. D. Sistare[1], J. C. Fuscoe[2] and K. Thompson[1]. *[1]Division of Applied Pharmacology Research, CDER, USFDA, Laurel, MD and [2]Center for Functional Genomics, NCTR, USFDA, Jefferson, AR.*

Genome-wide measurement of gene expression has the potential to be highly informative for non-clinical assessments of drug safety and action, but there is acknowledged variability among users and between platforms due to differences in probe sequence selection, protocol non-uniformity, and other issues associated with this technology. To enable regulatory reviewers to objectively evaluate the quality and content of gene expression data, it is important to identify performance characteristics of well-conducted microarray experiments. To this end, we have initiated a collaborative research project to test the feasibility and value of using a set of mixed tissue samples to evaluate the ability of different laboratories and microarray platforms to detect designed-in differences in gene expression between two complex biological samples. To construct the mixed tissue samples, total RNA was isolated from four tissues (brain, liver, kidney, and testis), pooled across sets of 8 male Sprague-Dawley rats, and assayed on Affymetrix RAE230A arrays. Probes for tis-

sue-selective genes that exhibited a range of expression levels and performed consistently across pools prepared from different animal shipments and sources were identified as potential benchmark calibration genes. Two samples composed of different defined proportions of total RNA from the four tissues were prepared and tested to assure consistent detection of benchmark gene expression at the input ratios. To establish which metrics are most informative for assessing expression array assay performance within a given platform, sets of mixed tissue samples will be evaluated at multiple sites, on Affymetrix, Agilent, and Amersham arrrays, and on an in-house spotted oligonucleotide array at NCTR. Incorporation of measures of assay performance, such as a mixed tissue standard, into toxicogenomic data submissions could help assure regulators that high quality data is being submitted to support a regulatory review.

1706 LASER MICRODISSECTION AND ITS APPLICATION IN TOXICOGENOMICS.

W. Hu, M. Taurino, M. Wojke, T. Monticello and Z. Jayyosi. *Drug Safety Evaluation, Aventis Inc., Bridgewater, NJ.*

Gene expression analysis using total RNA isolated from whole organs may not identify specific gene modulations attributed to toxicity related to individual cell type. When sampling whole organs, gene expression responses from the specific cellular site of injury may be 'diluted' with adjacent unaffected cell population. The goal of this study was to develop, validate, and utilize a reliable laser microdissection (LMD) technique for the isolation of RNA for cell-specific gene expression studies. The Leica AS LMD system was utilized to capture cells from cryosections (8mm thick) of rat kidney. Several variables were evaluated: 1) optimal RNA isolation protocol; 2) time from tissue slide preparation to LMD; and 3) optimal number of cell profiles. Several RNA isolation protocols were evaluated; optimal results (yield and purity) were obtained with the Qiagen RNeasy Micro kit. A time-course experiment (30 sec - 4 min) was performed to determine the maximal amount of time the sample is stable to permit light microscopic evaluation until LMD. Quality RNA was still present up to the 4 min post-cryosectioning time-point. Similar RNA quality was obtained from all sample sizes (500 to 40, 000 cells), and increasing amounts of RNA were obtained with increasing cell number. Upon validation results, the expressions of 14 differentially regulated genes were evaluated from RNA isolated from whole rat kidney or LMD-derived samples of specific regions using RT-PCR. Comparable gene expression levels were found from both samples indicating that LMD did not alter the gene expression profile within various cellular components. In addition, cell-specific RNA expression profiling, using the Affymetrix RGU34A chip, obtained by LMD, was performed to delineate the responses of proximal tubules affected by a kidney toxicant from the responses of adjacent non-affected cell types or control tissue; these responses were also compared to those obtained from the entire kidney. In conclusion, results from this study provided a full scope validation of LMD technique and its application in the field of toxicogenomics.

1707 EXPRESSION ANALYSIS OF NOVEL BIOMARKERS OF NEPHROTOXICITY USING LASER CAPTURE MICRODISSECTION (LCM) AND IMMUNOHISTOCHEMISTRY (IHC).

L. A. Obert[2], J. W. Davis[1], F. Goodsaid[1], K. Milford[2], P. Louro[2], M. Geraci[2], R. J. Smith[1] and I. Y. Rosenblum[1]. *[1]Molecular Toxicology, Schering-Plough, Lafayette, NJ and [2]Pathology, Schering-Plough, Lafayette, NJ.*

Previous studies have identified clusterin, osteopontin (OPN) and hepatitis A virus cellular receptor-1 (HAVcr-1), a homologue of kidney injury molecule-1 (KIM-1) as novel genomic markers of nephrotoxicity in cynomolgus monkeys. Those results provided the first data confirming gene expression changes associated with rodent nephrotoxicity in a non-human primate model. In order to further analyze these novel biomarkers LCM was employed to analyze their localized expression in kidneys of female cynomolgus monkeys dosed with the aminoglycoside antibiotic gentamicin (10 mg/kg) and/or everninomicin (30 or 60 mg/kg), an experimental oligosaccharide antibiotic, for seven days. Monkeys receiving both drugs demonstrated proximal tubular injury as early as Day 1, consistent with a potentiation of toxicity resulting from co-administration of both test articles. By Day 7, monkeys dosed with 60 mg/kg everninomicin alone developed similar lesions, while the group exposed to both compounds had more extensive tubular damage. Gene expression within LCM-selected regions correlated with whole tissue expression changes especially at the anatomical site of damage in the kidney. Cortical tubules were the primary site of HAVcr-1 expression changes. Clusterin and OPN were more uniformly expressed throughout the kidney; although there was a higher correlation between expression in the cortex and the whole tissue than in medullary tubules or glomeruli. The presence of these putative markers in the urine has been proposed as proteomics markers of nephrotoxicity in rodents and humans. IHC was used to analyze in situ protein expression of these markers. Preliminary IHC analyses have confirmed that protein expression correlated with whole tissue and LCM gene expression analysis. The results shown here provide evidence that candidate genomic biomarker expression was localized to specific anatomic locations using LCM and protein expression was confirmed by IHC.

1708 QUANTIFYING GENE EXPRESSION NETWORKS: IDENTIFYING NETWORK STRUCTURE.

T. Yamanaka[1], H. Toyoshiba[1], N. Walker[1], F. Parham[1], J. Martinez[1], H. Sone[1] and C. Portier[1]. *[1]Laboratory of Computational Biology and Risk Analysis, National Institute of Environmental Health Sciences, Research Triangle Park, NC and [2]LCBRA, NIEHS, Research Triangle Park, NC.*

Gene expression arrays have revolutionized biology by generating vast amount of data quantifying the level of mRNA expression. Consequently biology is currently undergoing a shift to an informative and quantitative science. One of the major unresolved issues in the analysis of gene expression data is the identification and quantification of gene regulatory networks. Several methods have been proposed for identifying gene networks. However, almost all of these don't fully incorporate statistical methods into the analyses in the sense that they fail to quantify the likelihood that the network is a gbesth network and the strength of the links between genes in the network. We present a new method for analyzing gene expression to infer genetic interactions. We use Bayesian networks as a mathematical tool. Our approach is based on a rigorous Bayesian statistical framework. The analytical approach we present uses the actual measured observations on gene expression and incorporates prior distributions for all parameters in the gene interaction network model. The method encompasses model selection theory in Bayesian regression to find gene network structures suitable for given datasets. By introducing rigorous statistical methods into the estimation problem for gene interaction networks, important issues such as the statistical power for identifying the correct model and the uncertainty in model parameters can be easily assessed. Computer simulations demonstrate that the proposed method is capable of extracting knowledge from datasets with a limited number of small replicates as is the current case for most microarray studies. This lack of experimental replication is a common issue when analyzing expression data. The performance of our algorithm is evaluated by a dataset from HPL1A lung airway epithelial cells after exposure to TCDD for 24 hours. We estimated a hypothetical network from this dataset and argue its validity both biologically and statistically.

1709 ESTROGENICITY OF THE DIETARY INDOLE, 3, 3'-DIINDOLYLMETHANE, IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*).

S. C. Tilton and D. E. Williams. *Environmental and Molecular Toxicology and The Marine and Freshwater Biomedical Sciences Center, Oregon State University, Corvallis, OR.*

Indole-3-carbinol (I3C) is a glucosinolate hydrolysis product from cruciferous vegetables that has been found to have chemoprotective effects in several animal models. However, I3C given long-term postinitiation modulates liver carcinogenesis similar to the actions of 17β-estradiol (E2) so that it promotes hepatic tumors in trout and rat models, but inhibits them in mice. 3, 3'-Diindolylmethane (DIM) is the major acid condensation product of I3C observed in the stomach after dietary consumption and in the liver after absorption. We have found DIM to be estrogenic in precision trout liver slices and bind to the estrogen receptor with an EC_{50} of 110 μM and a relative binding affinity 17-fold higher than I3C. Hepatic gene expression profiles were analyzed in juvenile rainbow trout fed dietary E2, DIM, I3C, β-naphthoflavone (a known cytochrome P450 inducer) or 0.2% DMSO vehicle for 12 days. Transcript profiles were compared between treatment groups and controls using custom Atlantic salmon cDNA arrays to determine the expression level of approximately 3700 genes, including 3119 unique *Salmo salar* elements and 438 *Oncorhynchus mykiss* elements (ESTs). Genes known to be controlled by E2, such as vitellogenin (VTG), vitelline envelope, and binding proteins were found to be upregulated by both DIM and E2 while other genes, such as those involved in the acute immune response, were downregulated. Gene expression correlated well with levels of hepatic zona radiata, VTG and CYP1A proteins measured in these fish. This data suggests that DIM is estrogenic in trout models and regulates gene expression similarly to E2 *in vivo*. Supported by NIH grant ES03850 and ES07060.

1710 ESTROGEN-INDUCED GENE EXPRESSION IN HUMAN UTERINE LEIOMYOMA AND NORMAL UTERINE SMOOTH MUSCLE CELL LINES.

C. Swartz[1], C. Afshari[2], L. Yu[1], K. Hall[1] and D. Dixon[1]. *[1]NIEHS, Research Triangle Park, NC and [2]Amgen, Thousand Oaks, CA.*

Uterine leiomyomas are widely prevalent, yet benign reproductive tract neoplasms of women. Their growth, and perhaps development, are widely believed to be under the influence of steroid hormones (estrogen and progesterone) although the

exact mechanism of this influence remains unclear. Numerous growth factors, cytokines, and other signal transduction factors have been implicated as effectors in the pathogenesis of uterine leiomyoma, either as downstream components of hormone initiated pathways or as effectors on their own. Interactions between these various pathways have been difficult to establish. Global gene expression analysis platforms, such as cDNA microarray, provide a means for tracking activities of multiple pathways simultaneously. In this study, we used cDNA microarrays to identify genes and pathways involved in the response of uterine leiomyoma (UtLM) and normal uterine smooth muscle (UtSMC) cells to estrogen. UtLM and UtSMC cells were treated with 10^{-8}M17β-estradiol or ethanol (vehicle control) for 24 hours and differential gene expression between treated and control cells was assessed through competitive hybridization to cDNA arrays using fluorescent labels. Expression changes for selected genes were confirmed by northern blot and either western blot or immunocytochemistry. IGF-1 was up-regulated in estrogen-treated UtLM cells. Also, two other genes that are potentially involved in the IGF-1 pathway were differentially expressed. A-myb, a transcription factor which promotes cell cycle progression, was up-regulated, and MKP-1, a dual specificity phosphatase that dephosphorylates mitogen-activated kinase, was down-regulated. Of these three genes, only MKP-1 was differentially expressed (also down-regulated) in UtSMC cells. These results demonstrate some novel genes that may play a role in the growth of uterine leiomyoma and strengthen the case for involvement of the IGF-1 pathway in the response of uterine leiomyoma cells to estrogen.

1711 DIFFERENTIAL GENE EXPRESSION BY ANDROGENS AND ESTRADIOL BY MICROARRAY ANALYSIS IN THE LARGEMOUTH BASS (MICROPTERUS SALMOIDES).

J. L. Blum[2], P. Larkin[4], K. J. Kroll[3] and N. D. Denslow[1, 3]. *[1]Biochemistry and Molecular Biology, University of Florida, Gainesville, FL, [2]Graduate Program in Pharmacology, University of Florida, Gainesville, FL, [3]Biotechnology Program, University of Florida, Gainesville, FL and [4]EcoArray, LLC, Alachua, FL.*

The purpose of this study was to compare the gene expression profiles between largemouth bass treated with estradiol, dihydrotestosterone, or 11-ketotestosterone by cDNA microarray. These steroid hormones principally produce their effects through interaction with their respective nuclear receptors, and in concert with other transcription factors, control gene expression. Genes were cloned using differential display, cDNA subtraction, or directed cloning methods. These genes were arrayed on nylon membranes and hybridized with radiolabeled liver cDNA from treated or control fish. The gene profiles for each of the treatments were distinct from one another suggesting that it will be possible to distinguish hormonally active agents in the environment that behave as androgens or estrogens. The genes that are most up-regulated by estradiol include genes involved in egg production such as three vitellogenins, two choriogenins, aspartic protease and protein disulfide isomerase. On the other hand, the androgens upregulate a different set of genes, including several ESTs. While vitellogenin 2 is upregulated by all treatments, the level of induction was 500 fold for estradiol but only 10-fold for dihydrotestorenone and 2-fold for 11-keto-testosterone. Other estrogen up-regulated genes were not induced by the androgens. In fact the EST clone 24-1 that was up-regulated by estradiol was down-regulated by the androgens. It may be important to have multiple biomarkers, or gene fingerprints, to clearly distinguish the classes of endocrine acting compounds in the environment.

1712 DOSE RESPONSE ANALYSIS OF FEMORAL CHANGES IN GENE EXPRESSION ELICITED BY ETHYNYL ESTRADIOL USING CDNA MICROARRAYS.

J. Burt[1, 3], L. D. Burgoon[2, 3], D. R. Boverhof[1, 3], Y. Sun[1, 3] and T. R. Zacharewski[1, 3]. *[1]Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, [2]Pharmacology & Toxicology, Michigan State University, East Lansing, MI and [3]Institute of Environmental Toxicology, Michigan State University, East Lansing, MI.*

To investigate estrogenic effects on bone, changes in the global gene expression in femoral tissue were examined in immature, ovariectomized C57BL/6 mice treated with 17α-ethynyl estradiol (EE), a synthetic, orally active estrogen. Mice were gavaged with 0.1, 1.0, 10, 100 or 250 μg/kg of EE or vehicle and femurs were harvested after 24 hours. cDNA microarrays representing 6376 clones (4858 unique genes) were used to compare gene expression profiles of EE treated animals to vehicle controls using a common reference design. This design results in two independent labelings per sample, with appropriate dye swaps, and 3 biological replicates were conducted. The data were normalized using a general linear mixed model (GLMM) and gene effects examined using a separate gene-specific GLMM. A list of 202 significant genes was compiled using a model-based t-statistic filtering approach with an absolute value t-score cut-off of 4.0 and a subset verified using real-time PCR. *k*-means clustering was performed and the data were best described with 6 separate clusters: the majority of the genes only being affected at the high doses of EE. Among these genes, many are involved in transcriptional regulation, signal transduction and apoptosis. Of interest was the up-regulation of osteoprotogerin (OPG), a regulator of apoptosis. Previous studies have shown that OPG is estrogen responsive and *in silico* analysis identified two imperfect estrogen response elements in the region downstream of the transcriptional start site. Comparative analysis with liver dose response expression patterns found many pathways in common, including lipid metabolism and transport, and oxidative stress. These results corroborate previous reports that bone is responsive to estrogen and may provide insights into estrogen's role in maintaining bone homeostasis. Supported by ES011271

1713 THE USE OF GENE EXPRESSION PROFILING FOR IDENTIFYING POTENTIAL BIOMARKERS OF REPRODUCTIVE TOXICOLOGY.

L. Nelms[1], R. E. Chapin[2], B. Lu[1], S. J. Curry[2], M. B. Wilhelms[3], M. R. Elwell[3], D. Pelletier[1] and M. P. Lawton[1]. *[1]Molecular and Investigative Toxicology, Pfizer, Groton, CT, [2]Investigative Developmental Toxicology, Pfizer, Groton, CT and [3]Pathology, Pfizer, Groton, CT.*

More sensitive clinical markers of damage to the male reproductive system are needed. Testicular toxicity in rodents and other preclinical species is currently determined by a histologic evaluation of tissues from treated animals. In humans, an ejaculated sperm count has been the gold standard for many years, because sperm output is a direct result of spermatogenesis. However, sperm count has some drawbacks: it is highly variable across individuals and from one sample to another within a single individual, and it reflects damage that happened weeks or months previously. We are using emerging "omics" technologies to identify clinically relevant, "leading" biomarkers of testis toxicity. To find candidate biomarkers, rats were treated with well-described toxicants to produce varying degrees of testis damage. Serum was profiled by 2D gels (proteomics), urine by NMR (metabonomics), and various tissues (testis, prostate, liver, and whole blood) by gene expression profiling (genomics). For this communication, male Sprague-Dawley rats were dosed with the well-known testicular toxicant, 1, 3-dinitrobenzene (DNB) at 0, 2, 5, or 10 mg/kg/day, and samples collected after 2, 4, 7, and 10 days of treatment. The histologic lesions were as described previously: Sertoli vacuolization, and apoptosis of round spermatids and spermatocytes, followed by sloughing of dead and dying germ cells. The degree of these effects varied with dose and duration. Gene expression analysis using Affymetrix GeneChips also revealed changes that varied with time and dose. Experimental validation of candidate markers using a variety of techniques (in situ hybridization, RT-PCR, regulation by other compounds, etc.) will be described.

1714 EXPRESSION PROFILING THE HEPATIC RESPONSE OF RATS TREATED WITH FENOFIBRATE AND FIVE OTHER FIBRATE ANALOGUES.

P. D. Cornwell, A. T. De Souza and R. G. Ulrich. *Molecular Profiling, Rosetta Inpharmatics, Merck Research Labs, Kirkland, WA.*

Peroxisome proliferator-activated receptors (PPAR) are a group of nuclear receptors that mediate a variety of cellular functions. Fibrates (primarily PPARα agonists) are used clinically to treat dyslipidemias but cause peroxisome proliferation, hepatomegaly and hepatic tumors in rodents. The goal of this study was to identify the gene expression changes responsible for the acute effects (*i.e.* hepatomegaly) of fibrates in rats. Male CD rats were treated daily by oral gavage with multiple dose levels of fenofibrate and five other fibric acid analogues for up to two weeks. Only one compound caused any significant increase in serum ALT or AST activity; however, all six compounds caused hepatocellular hypertrophy and significant increases in liver mass : body mass ratios. Liver total RNA from compound treated and vehicle treated animals was hybridized to custom oligonucleotide arrays containing 22, 505 rat sequences. Time-matched pools of liver RNA from vehicle treated animals were used for the reference channel, and all hybridizations were performed in duplicate with the labeling fluors (Cy3 or Cy5) reversed in the second hybridization. Microarray expression profiling revealed that hepatic expression of 1288 genes was related to dose level or length of treatment of all six compounds and was correlated with grade of hepatocellular hypertrophy. Included in this list were changes in gene expression that were consistent with peroxisome proliferation; increased fatty acid transport; increased mitochondrial and peroxisomal β-oxidation; increased uptake of LDL-cholesterol; and decreased glucose uptake, gluconeogenesis and glycolysis. It is likely that these changes are related to the clinical benefits of fibrate drugs, including decreased serum triglycerides and LDL-cholesterol and increased serum HDL-cholesterol. The large number of genes whose expression is treatment and hepatomegaly related, and that have functions associated with the target effects of fibrates leads to the conclusion that hepatomegaly is the result of PPARα activation.

1715 GENE EXPRESSION PROFILE OF HEPATIC STEATOSIS IN PPARα-DEFICIENT MOUSE LIVER AFTER EXPOSURE TO HYDRAZINE.

V. E. Richards, B. Chau and C. A. McQueen. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

Peroxisome proliferator activated receptors (PPAR) are members of the nuclear hormone receptor superfamily. Of the three identified isoforms (α, β/δ and γ), PPARα is involved in the control of hepatic fatty acid and glucose metabolism. A deficiency of PPARα-dependent β-oxidation of fatty acids causes disturbances in lipid and energy homeostasis and is critical in the pathological accumulation of lipids in the liver, referred to as steatosis. Steatosis has been reported as a toxicological endpoint of hydrazine (HD) exposure in rodents. HD is a metabolite of the tuberculostatic drug isoniazid, as well as an industrial compound. In C57Bl/6 mice, HD has been shown to induce the expression of genes regulated by PPARα, including Cyp4a14, Apoa5, Cpt and Hmgcs2. The present study was designed to determine the involvement of PPARα in HD-mediated steatosis. PPARα-deficient mice received an oral dose of HD (0-300 mg/kg). Using a cDNA microarray, hepatic gene expression profiles were investigated 24 hours after a sub-cytotoxic dose of 100 mg HD/kg. Data revealed induction of Prkcz, Apoa4, and Agpat3 and reduction in the expression of Lpl, Abca1, and Gpr68 in PPARα-deficient mice. These genes are reported to coordinate triglyceride-rich lipoprotein homeostasis. Twenty-four hours after 300 mg HD/kg histological studies showed dramatic hepatic lipid accumulation and macrovesicular degeneration in the deficient mice compared to the wild-type. Biochemical assessment revealed a dose-dependent elevation in alanine aminotransferase (ALT) activity and reduction in plasma cholesterol and triglyceride levels. These data suggest HD severely overwhelms the removal of hepatic lipids in PPARα-deficient mice. Moreover, the ability to alter the expression of PPARα-regulated genes is a critical hepatic response to HD. (Supported by R01 ES10047 and P30 ES6694).

1716 EFFECT OF FURAN, CHLOROFORM, DI-(2-ETHYLHEXYL)PHTHALATE (DEHP) AND OXAZEPAM ON BIOMARKERS OF CELL CYCLING AND PROLIFERATION IN MOUSE LIVER.

C. Meredith[1], M. P. Scott[1], A. Barton[2], R. J. Price[1], T. R. Hupp[3], C. R. Elcombe[2] and B. G. Lake[1]. *[1]BIBRA International Ltd.,, Carshalton, Surrey, United Kingdom, [2]CXR Biosciences Ltd.,, Dundee, United Kingdom and [3]University of Dundee, Dundee, United Kingdom.*

The aim of this study was to identify biomarkers of tumor formation by non-genotoxic agents in mouse liver. Male (oxazepam only) and female B6C3F1 mice were treated with both carcinogenic and non carcinogenic doses of the compounds. Mice were given corn oil (control) or the cytotoxins furan (1-15 mg/kg) and chloroform (238 and 437 mg/kg) by gavage 5 days per week for periods of 1, 5 and 26 days. Chloroform was also given in the drinking water at non carcinogenic doses of 200 and 1800 mg/l. Mice were fed control diet or diets containing the mitogens DEHP (30-6000 ppm) and oxazepam (2.5-2500 ppm) for periods of 1, 7 and 28 days. Both furan and chloroform (gavage only) produced dose-dependent hepatotoxicity (increased serum alanine aminotransferase). Treatment with DEHP induced hepatic peroxisome proliferation (palmitoyl-CoA oxidation) and oxazepam induced cytochrome P450-dependent enzymes. At necropsy, total RNA was extracted from liver samples and mRNA levels of selected genes (including c-*fos*, c-*myc*, cyclin D, Gadd45β, p53, bcl2) determined by real-time quantitative reverse transcription-polymerase chain reaction methodology. At day 26, carcinogenic doses of chloroform (238 and 437 mg/kg) produced significant 11 and 47 fold increases, respectively, in c-*myc*. The expression of c-*myc* was also increased by 4 and 15 mg/kg carcinogenic doses of furan, whereas at day 28 neither oxazepam nor DEHP produced significant effects. While carcinogenic doses of DEHP (6000 ppm) and oxazepam (2500 ppm) produced significant increases (10 and 24 fold, respectively) in Gadd45β expression, furan had no significant effect. Overall, certain genes may be useful biomarkers of non-genotoxic carcinogenesis in mouse liver. (Supported by UK Food Standards Agency).

1717 DI(2-ETHYLHEXYL) PHTHALATE INDUCED GENE EXPRESSION CHANGES.

J. S. Wong. *Cell Biology & Neuroscience, Environmental Toxicology Program, University of California, Riverside, CA.*

Increasing chemical use and population growth necessitates an understanding of how pollutants affect the environment, and human and animal health as well. This is particularly true with pollutants such as di(2-ethylhexyl) phthalate (DEHP), a peroxisome proliferator and a plasticizer ubiquitously present in the environment. The National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP CERHR) concluded that there is a serious concern for DEHP's potential detrimental effects on male infant reproductive development. Despite the wide use and known adverse effects of DEHP, its mechanism(s) of reproductive toxicity are poorly understood. To begin to identify potential DEHP targeted pathways relevant to reproductive development, it is important to have a transcriptional profile of genes affected after exposure to DEHP. We did this by assaying gene expression changes in the liver and the testes of C57BL/6 mice after exposure to 1.0% dietary DEHP for 13 weeks using microarray technology. DEHP exposure resulted in the induction or suppression of myriad genes in the liver and the testes. In the liver several affected steroidogenic genes were identified: cyp2b9, cyp7b1, 11b-hydroxysteroid dehydrogenase (11bHSD), and 3b-hydroxysteroid dehydrogenase V (HSD3b5). Additionally, several key genes, either directly or indirectly involved in the development of the gonadal testes, were identified: vanin-1, prostaglandin D synthetase (PGDS), and oligodendrocyte-specific protein (OSP). Currently, detailed studies on selected genes are undertaken to ascertain dose- and time-dependence, and the dependence on the presence of a putative DEHP receptor peroxisome proliferator activated receptor a (PPARa). Taking the data collectively, we postulate that DEHP's mechanism of reproductive toxicity is in part due to its perturbation of genes necessary for important processes critical for proper reproductive development and function: steroid hormone metabolism and testes determination.

1718 CHEMICAL GENOMICS ANALYSIS OF GENE EXPRESSION PROFILES TO HELP ELUCIDATE MOLECULAR MECHANISMS OF ANTI-BREAST CANCER AGENTS.

C. Yang[1, 3], P. Blower[1], K. Cross[1], G. Myatt[1], J. Richards[2], R. Brueggemeier[2] and J. Rathman[3]. *[1]Leadscope, Inc., Columbus, OH, [2]College of Pharmacy, The Ohio State University, Columbus, OH and [3]Chemical Engineering, The Ohio State University, Columbus, OH.* Sponsor: D. Johnson.

Throughput genomic studies are producing large databases of molecular information on cancers and other cell and tissue types. Hence, the opportunity to link these accumulating data to the drug discovery process becomes a real possibility. However, despite the introduction of a new paradigm and methodologies, this large amount of information and significant investment has not led to dramatic increases in productivity in drug discovery. To link the vast amount of genomics information to small molecule discovery, we have in the past correlated the gene expression profiles of NCI 60 cell lines to compound activity patterns of the same cell lines. The genes were classified using a gene hierarchy based on Gene Ontology Consortium. The gene families defined in the hierarchy function as biologically meaningful features for statistical gene mining. The chemical scaffolds were built by extracting common cores of the compounds responsible for the initial correlations with gene expression profiles. These compound scaffolds are used to probe the genes within certain pathways. The set of selected genes and the compounds provide a foundation for building hypotheses based on molecular mechanisms. In this paper, an experimental design to validate the informatics methodology is presented. The activities of the selected compounds were measured by IC50 and EC50 values for breast and colon cancer cell lines. Gene expressions were measured using Affymetrix U133 chips. Data mining results from this experiment will be tested by real time PCR to validate the molecular hypothesis of pairs of genes and compounds.

1719 IDENTIFICATION OF GENE EXPRESSION CHANGE AS A FUNCTION OF DOSE IN A MICROARRAY EXPERIMENT.

K. Dawson[1], J. E. Eckel[1], C. Gennings[1], D. Boverhof[2] and T. Zacharewski[2, 3, 4]. *[1]Biostatistics, VA Commonwealth University, Richmond, VA, [2]Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI, [3]Nation Food Safety & Toxicology Center, Michigan State University, East Lansing, MI and [4]Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.*

Pattern recognition methods are described for cDNA microarray data from a dose-response (D-R) study with immature ovariectomized C57BL/6 mice gavaged with ethynyl estradiol (0, 0.1, 1, 10, 100, and 250 ug/kg). In this study microarrays, representing 6, 528 cDNA clones, were used to assess hepatic changes in gene expression using the common reference design replicated in quadruplicate. A screening algorithm was applied to identify genes associated with change in expression level over dose. For each treatment, replication, and block, data were normalized using loess local regression within each dye swap. A D-R pattern was constructed for each gene by comparing mean responses between adjacent doses. Let E_{ij} denote the mean expression at the ith dose, i=1, 2, .., 6 for the jth gene. When a t-test comparing E_{ij}, $E_{i+1,\,j}$ was significant, the pattern was classified as increasing (p_{ij} = +1) or decreasing (p_{ij} = -1) based on the change in mean expression. Non-significant tests indicated no change (p_{ij} = 0). A pattern set p_j = (p_{1j}, p_{2j}, p_{3j}, p_{4j}, p_{5j}) was then constructed for each gene. Over 80% of the genes fell into the pattern set (0, 0, 0, 0, 0)

indicating constant expression over all doses. Pattern set (-1, 0, 0, 0, 0), associated with a decrease in expression at 0.1 ug/kg which remained constant through higher doses, was associated with 6% of the genes. An increase in expression at 0.1 ug/kg, which then remained constant, was identified in 3% of genes. No change at doses < 250 ug/kg were observed in 1.8% of genes with 0.9% decreasing and 0.9% increasing at that dose. Without first defining forms for the D-R relationships, this algorithm can screen for any pattern in expression over increasing dose. Observed pattern sets are useful in suggesting functional forms for underlying D-R relationships. This work was supported by two grants (#T32 ES07334-01A1 and #R01-) from the NEIHS, NIH.

1720 NORMALIZATION OF MICROARRAY DOSE-RESPONSE DATA.

J. Eckel[1], C. Gennings[1], K. Dawson[1], D. Boverhof[2] and T. Zacharewski[2]. *[1]Department of Biostatistics, Virginia Commonwealth University, Richmond, VA and [2]Department of Biochemistry & Molecular Biology, National Food Safety & Toxicology Center and Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.*

An important underlying assumption of any dose-response study is that the experimental conditions/subjects are similar across levels of the exposure variable, so that changes in the response variable can be attributed to exposure to the agent(s) under study. This assumption is often not valid in the analysis of microarray dose-response data due to experimental factors including differences in labeling or detection efficiencies, and systematic biases in the measured expression levels due to spot location, array or replication factors. Thus, normalization is an important initial step in the analysis of gene expression data where the objective is to balance the individual intensity levels across these experimental factors while maintaining the effect due to exposure to the agent under study. Various normalization strategies have been described in the literature including log centering, analysis of variance modeling, and lowess smoothing for removing intensity-dependent effects. We describe a method that incorporates many of these into a single strategy for use in a dose-response study using a spoke design with dye-swaps between vehicle and each dose group (termed a reference set). The motivation for our normalization strategy is to balance the effects due to dye, location and replication within each dose or vehicle group within each reference set while maintaining the mean within these groups. The method is demonstrated using data from a dose-response study of ethynyl estradiol (0, 0.1, 1, 10, 100, 250 μg/kg) in liver tissue from immature ovariectomized C57/BL6 mice. In this study cDNA microarrys, representing 6, 528 cDNA clones, were used to assess hepatic changes in gene expression using the spoke design that was replicated in quadruplicate. The success of the normalization strategy is demonstrated *via* plots. This work was supported by two grants (#T32 ES07334-01A1 and #R01- ES011271) from the NEIHS, NIH.

1721 USE OF RAY DESIGNS IN EVIDENCE-BASED DECISIONS INVOLVING MIXTURES OF LARGE NUMBERS OF CHEMICALS.

C. Gennings, H. Carter, K. Fisher and R. Carchman. *Solveritas, LLC, Richmond, VA.*

The protection of human health from the adverse effects of cumulative and acute exposure to chemical mixtures is an important issue. The toxicity of a mixture depends in part on the toxicity of the components and how the components interact. In some cases the toxicity of an agent or mixture may be adjusted by selecting/changing industrial or environmental processes that produce the mixture and/or ameliorate the effect by pretreatment or antidote therapy. For example, a chlorination process and an ozonation process in disinfecting drinking water may result in different levels of disinfectant by-products in drinking water. In the case of combination therapy for exposure to nerve threat agents our methods can facilitate the selection of improved treatment. In general, for decision-makers to select/change these processes/treatments, it is important to identify whether or not the components of the mixture, including the unidentified fraction in complex mixtures, combine additively. Fixed-ratio ray designs, which may include the use of single chemical data in addition to data along mixture ray(s), are feasible designs in the analysis of mixtures with many components. Such designs permit a reduction in the amount of experimental effort when the region of interest can be restricted to exposure-relevant mixing ratios. We have developed statistical methodology based on these fixed-ratio ray designs that facilitate testing hypotheses of additivity along specified rays of interest for mixtures of many chemicals. The methods are illustrated with data from a lethality study (Solana et al (1990) Toxicology Applied Pharmacology 102:421-429) of soman exposure in guinea pigs when pretreated with physostigmine and azaprophen. The analysis suggests an antagonistic relationship between these two agents as the level of soman increases indicating azaprophen pretreatment is preferable to the combination in the dose range considered. Further, our methods allow for testing for interactions among subsets of the components in the mixture and for comparison across subpopulations of interest.

1722 IMPROVED DETERMINATION OF REGENERATED SARIN (GB) IN MINIPIG AND HUMAN BLOOD BY GAS CHROMATOGRAPHY-CHEMICAL IONIZATION MASS SPECTROMETRY USING ISOTOPE DILUTION AND LARGE VOLUME INJECTION.

E. M. Jakubowski[1], J. M. McGuire[2], J. L. Edwards[2], R. A. Evans[2], S. W. Hulet[1], B. J. Benton[1], J. S. Forster[1], D. C. Burnett[1], W. T. Muse[1], C. L. Crouse[2], R. J. Mioduszewski[1] and S. A. Thomson[1]. *[1]Toxicology Team, Edgewood Chemical Biological Center, APG-Edgewood, MD and [2]Geo-Centers, APG-Edgewood, MD.*

Recent developments to improve nerve agent biomarker techniques include a method for measuring fluoride regenerated isopropyl methylphosphonofluoridate (Sarin or GB) from human or animal plasma (M.Polhuijs, et al. Toxicol. Appl. Pharmacology, 146, 156-161, 1997). Our efforts extended the fluoride ion regeneration method to be able to determine GB in red blood cell and tissue samples using a gas chromatograph-flame photometric detector (GC-FPD) with a solid sorbent tube sample inlet. However this method is labor intensive and subject to interferences. To improve the analysis, a new GC method was developed that utilizes an autoinjector, a large volume injector port (LVI), positive ion ammonia chemical ionization detection in the SIM mode, and a hexadeuterated-GB stable isotope internal standard. Ethyl acetate extracts from spiked human whole blood samples and from blood/tissue of whole-body GB exposed minipigs were analyzed by the improved method. Results indicated that the method range was 10-5000 pg on column. The detection limit was 3 pg of GB on column despite the complexity of the red blood cell/tissue matrix. Conditions that needed to be optimized for the LVI included injection volume, initial temperature, pressure, and flow rate. Other solvents were evaluated such as methyl acetate, hexane, and methanol but were found to be problematic to the sample preparation or to the LVI. The red blood cell (RBC) fraction of spiked human blood yielded regenerated GB levels comparable to those seen in the plasma when spiked at ng/mL concentrations.

1723 THE INHALATION TOXICITY OF GB VAPOR IN RATS AS A FUNCTION OF EQUILIBRATION TIME FOR TEN MINUTE EXPOSURES.

J. S. Anthony[1], M. V. Haley[1], J. H. Manthei[1], R. A. Way[1], D. C. Burnett[1], B. P. Gaviola[1], D. R. Sommerville[1], R. B. Crosier[1], R. J. Mioduszewski[1], S. A. Thomson[1], C. L. Crouse[2] and K. L. Matson[2]. *[1]Edgewood Chemical Biological Center, Department of the Army, Aberdeen Proving Ground, MD and [2]Geo-Centers Inc., Abingdon, MD.*

Characterizing the toxicity of airborne exposures to chemical warfare agents requires sensitive, accurate and reliable analytical chemistry methods as well as adequate methods for generating and controlling the test atmosphere. In addition to concentration and exposure time, it is necessary to examine what effect the chamber equilibration time (t_{99}) has on measured biological endpoints for shorter duration inhalation exposures (i.e. < 10 minutes). The t_{99} is defined as the time necessary for an exposure chamber to reach 99% of its experimental concentration. Once this value is reached, the chamber concentration will not rise more than an additional 1%, regardless of exposure time. MacFarland (1975) has suggested that for short-term dynamic exposures, exposure time (t) should be greater than (or equal to) 13(t_{99}) (MacFarland's Rule). Although there is no problem in adhering to this guideline for longer exposures (e.g. 60 or 240 min), adherence is not practical for exposures of 10 min or less where chamber dynamics will only allow the t_{99} value be kept to a minimum. The present study examined dose-response (lethality) relationships for GB vapor in rats derived from 10-minute exposures with t_{99} values that do not adhere to MacFarland's Rule, i.e., 2.1, 5.2, or 8 minutes. It was generally concluded that differences in LCT_{50} values collected under the above t_{99} conditions were minimal and would not be considered statistically significant.

1724 CLINICAL SAFETY OF REACTIVE SKIN DECONTAMINATION LOTION (RSDL).

D. A. Tonucci[1], S. Masaschi[1], L. Lockhart[1], M. Millward[1], D. Liu[2], R. Clawson[2], V. Murphy[3], P. O'Dell[4], M. C. Lanouette[5], T. Hayes[6] and C. Sabourin[6]. *[1]Hill Top Research, Cincinnati, OH, [2]Chemical Biological Medical Systems Project Management Office, Ft Detrick, MD, [3]MarCorSysCom, Quantico, VA, [4]O'Dell Engineering, Cambridge, ON, Canada, [5]Canadian Department of National Defense, Ottawa, ON, Canada and [6]Battelle, Columbus, OH.*

A clinical program was designed to assess the dermal safety of a new personal skin decontaminant system. Reactive Skin Decontamination Lotion (RSDL) is a liquid, reactive lotion which removes and destroys chemical warfare agents and toxins from the skin. The clinical program included: a 21-day cumulative dermal irritation study in 30 subjects, a Repeat Insult Patch Test (RIPT; Jordan/King modified Draize design) in 200 subjects and a photo-irritancy/allergenicity study in 30 subjects. For cumulative irritancy 25 μL of RSDL applied to a 1 cm punch of sponge

applicator was patched occlusively for 21 consecutive days. Results from the study indicated that RSDL/applicator was of low irritancy potential versus the positive (0.5 M sodium lauryl sulphate) and negative (normal saline) controls. For allergenicity, 25 µL of RSDL/applicator punch was patched 9 times, with continuous exposure, for a 3-week induction phase followed by a 2-week rest period where subjects received no exposure to RSDL/applicator. A single challenge application at a naive site followed the rest period. RSDL/applicator was not allergenic in the RIPT as indicated by low erythema scores reported during the challenge phase. RSDL was tested for phototoxicity by comparing the skin reaction to a single exposure of RSDL/applicator with or without UVA/B exposure. Finally, photoallergenicity was determined for RSDL in a similar manner as in the RIPT with the exception that subjects were exposed to UV radiation after patch removal during induction and challenge. RSDL was found non phototoxic and non photoallergenic. The cumulative irritation, RIPT and phototox/photoallergy studies indicate that RSDL has a low risk for the development of dermal toxicity. Supported by RSDL/Foreign Comparative Testing Program and conducted under USAMRMC Contract No. DAMD17-99-D-0010.

1725 DETERMINATION OF RADIOPROTECTIVE EFFICACY OF GENISTEIN WHEN ADMINISTERED BEFORE OR AFTER IONIZING RADIATION.

M. R. Landauer and V. Srinivasan. *Radiation Pathophysiology and Toxicology, Armed Forces Radiobiology Research Institute, Bethesda, MD.*

At present there is no way to protect first-responders and remediation workers from the biomedical effects of ionizing radiation. Agents that can protect against ionizing radiation and that can be administered before radiation or shortly after irradiation would permit rescue workers to provide the services needed. In addition to their use against radiological terrorism, chemical radioprotectors have utility in clinical oncology and space travel. Soy phytoestrogens have been reported to have many beneficial health effects including a reduction in osteoporosis and some cancers. The most plentiful isoflavone from soybeans is genistein (4', 5, 7-tri-hydroxy-flavone). Genistein is an antioxidant and protein kinase inhibitor. In this study the radioprotective effects of genistein were evaluated in male CD2F1 mice. The traditional 30-day survival endpoint for the evaluation of radioprotectors was used. Mice received a single subcutaneous injection of either vehicle or 5 mg genistein/mouse at 0.5, 1, or 24 hours before irradiation or 0.5 hours after irradiation. The radiation dose was 9.5 Gy at a dose rate of 0.6 Gy/minute from a cobalt-60 source. Of all the time points tested, genistein provided statistically significant radioprotective efficacy only when administered 24 hours before irradiation. The mechanism(s) for this protection are under investigation.

1726 SEIZURE/STATUS EPILEPTICUS AND ANIMAL TOXICITY INDUCED BY LITHIUM PILOCARPINE CLOSELY MIMICS HIGH-DOSE ORGANOPHOSPHATE EXPOSURE.

M. P. Nambiar[1], L. M. Tetz[1] and R. K. Gordon[1]. [1]*Biochemical Pharmacology, Walter Reed Army Institute of Research, Silver Spring, MD,* [2]*Biochemical pharmacology, WRIAR, Silver Spring, MD and* [3]*Biochemical Pharmacology, WRIAR, Silver Spring, MD.*

In organophosphororus chemical warfare agent exposure, inhibition of neuronal AChE leads to excessive acetylcholine accumulation resulting in hypercholinergy and seizure. Prolonged and repeated seizure (status epilepticus, SE), leads to neuronal damage and neurobehavioral abnormalities. The series of events leading to neurodegeneration following organophosphate exposure are not understood. Thus, there is a requirement for drugs that can protect against OP induced SE and neuropathology. We have developed a rat lithium-pilocarpine animal model and radiotelemetry using bipotential probes to rapidly screen the efficacy of various new generation and next generation anti-epileptic drugs for protection against seizures/SE and the consequent neurobehavioral impairment. Administration of a single dose 220-240 mg/kg pilocarpine, ip, 24 h after 5 mEq lithium injection produce strong seizure/SE. Comparison of seizure/SE produced by lithium pilocarpine to nerve agent to published data of soman shows a close resemblance. The onset of EEG spike activity is observed at 4 min after administration and siezure episodes after 9 min that is followed by SE. Sensitivity regarding gender, quiet period after seizure, frequency, low frequency spikes, clonic jerks of the head & shoulder, repetitive episodes of facial and forelimb clonus, rearing on hind legs, weight loss, brief episodes straub tail, prominent fasciculation of the back and trunk and hunched posture after 24 h were comparable between pilocarpine model and soman exposure. Animal toxicity by survivability and brain histopathology were also similar between pilocarpine and high-dose soman exposure. These data suggest that a single dose injection of pilocarpine following lithium is reminiscent of chemical warfare agent exposure and can be used to rapidly screen potential drugs for post exposure treatment.

1727 REPEATED LOW-LEVEL EXPOSURE TO ORGANOPHOSPHATE VX ACTIVATES BRAIN-DERIVED NEUROTROPHIC FACTOR IN MOUSE BRAIN.

W. E. Chang[1], J. M. Pizarro[1], G. A. Saviolakis[1], E. G. Midboe[2], M. J. Bah[1], A. Alagappan[1], C. L. Robison[1], J. L. Meyerhoff[1], J. D. Shah[1] and L. A. Lumley[1]. [1]*Neurosciences, Walter Reed Army Inst. Research, Silver Spring, MD and* [2]*Pharmacology, USAMRICD, Aberdeen Proving Ground, MD.* Sponsor: J. Yourick.

The highly toxic organophosphorate VX [O-ethyl S-(2(diisopropylamino)ethyl)methyl phosponothionate] is an irreversible inhibitor of the enzyme acetylcholinesterase (AChE). Prolonged inhibition of AChE increases endogenous levels of acetylcholine and is toxic at nerve synapses and neuromuscular junctions. We hypothesized that repeated low-level exposures to VX would affect genes associated with cell survival, neuronal plasticity and neuronal remodeling, including brain derived neurotrophic factor (BDNF). We examined the time course of expression of BDNF mRNA expression in mouse brain following repeated exposure (1/day for 5 days/wk for 2 wk) to low levels of VX (0.2 LD50 and 0.4 LD50). *In situ* hybridization analysis for BDNF mRNA expression showed that there was a significant elevation in BDNF mRNA expression in brain regions including the dentate gyrus, CA3, and CA1 ($P<0.05$) regions of the hippocampal formation, as well as in the piriform cortex, hypothalamus, amygdala and thalamus, 2 hr and 72 hr after the last 0.4 LD50 VX exposure. In addition, 0.2 LD50 VX increased BDNF expression in the piriform cortex and the dentate gyrus 2 hr, but not 72 hr, following the last VX exposure. The greater susceptibility of the piriform cortex and hippocampus to BDNF changes following low dose VX exposure is of interest since these two limbic regions are important for the generation and propagation of seizures. Whether increased BDNF in response to repeated low dose VX exposure is an adaptive response to prevent cellular damage, or is a precursor to impending brain damage, remains to be determined. If elevated BDNF is an adaptive response, exogenous BDNF may be a potential therapeutic target to reduce toxic effects of nerve agent exposure.

1728 LOW-LEVEL EFFECTS OF VX VAPOR EXPOSURE ON PUPIL SIZE IN RATS.

B. J. Benton[1], K. L. Matson[2], C. L. Crouse[2], J. S. Forster[1], E. M. Jakubowski[1], J. S. Anthony[1], J. Scotto[1], J. H. Manthei[1], R. A. Way[1], S. W. Hulet[1], C. E. Whalley[1], D. C. Burnett[1], B. I. Gaviola[1], W. T. Muse[1], D. B. Miller[2], R. J. Mioduszewski[1] and S. A. Thomson[1]. [1]*US Army Edgewood Chemical Biological Command, Aberdeen Proving Ground, MD and* [2]*Geo-Centers, Inc., Gunpowder, MD.*

O-Ethyl S- [2-(diisopropylamino)ethyl] methylphosphonothioate (VX) can present an inhalation, ocular and dermal hazard in vapor or aerosol form. One of the first observable clinical signs of VX vapor exposure is miosis. Therefore, the objective of this study was to characterize the dose-response (miosis) relationship of VX vapor for acute whole-body exposures in rats. Effective dosages (concentration x time) resulting in miosis in 50% of the exposed rats (ECt50) were calculated for 10, 60 and 240- minute exposures. Rats were exposed to low levels of VX vapor in a 750-liter dynamic air flow inhalation chamber. Air-exposed rats served as sham controls. Pupil size changes were assessed with infrared pupillometry. Results indicate that the ECt50s for each exposure duration were not constant but increased with increasing exposure duration. The ECt50s were 1-2 orders of magnitude lower than the ECt50s of at least two other related organophosphorous (OP) compounds, sarin (GB) and cyclosarin (GF). At these concentrations there was no evidence of delayed miotic effects caused by additional inhalation, ocular or dermal penetration of VX.

1729 A COMPARISON OF BASELINE CHOLINESTERASE LEVELS AND THE INHIBITORY RESPONSE TO PYRIDOSTIGMINE IN WHOLE BLOOD, PLASMA, AND RBCS FROM HUMANS AND SEVERAL NONHUMAN PRIMATE SPECIES.

N. A. Niemuth[1], C. T. Olson[1], T. L. Hayes[1], G. van der Zwaag[1], C. Matthews[1], D. E. Lenz[2] and I. Koplovitz[2]. [1]*Medical Research and Evaluation Facility, Battelle Memorial Institute, Columbus, OH and* [2]*US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*

The purpose of this investigation was to compare baseline cholinesterase (ChE) levels and the response to the ChE inhibitor pyridostigmine (PB) in blood, plasma and packed RBCs from humans (HUM), Indian Rhesus (IRH), Chinese Rhesus (CRH), Cynomologus (CYN), and African Green (AFG) monkeys. Baseline ChE and AChE activities were measured in duplicate on a Hitachi 912 clinical analyser using the Boehringer Mannheim modification of the Ellman method. Blood was

spiked with PB to produce final concentrations of 15, 30, or 150 ng/ml. ChE inhibition was measured after approximately one hour. Baseline whole blood ChE levels were similar in IRH, CRH, CYN and AFG monkeys (5.3-6.0 University/mL), but were significantly less than the HUM level (8.4 University/mL). Plasma ChE levels were similar in HUM, IRH, CRH, and CYN (4.3-5.2 University/mL), but were significantly greater than in AFG monkeys (0.6 University/mL). RBC AChE levels were more variable between the individual species. These were highest in humans (10.3 University/ml) lowest in CYN (4.4 University/ml) and mid-level (6.3, 6.9 and 8.1 University/ml) in CRH, IHR, and AFG monkeys, respectively. All species exhibited a strong inhibitory response to PB. In whole blood, the dose-response trend was significantly steeper for AFG than HUM, IRH, CRH, and CYN. Otherwise, similar trends were observed for all species, despite differences in baseline ChE and AChE levels. These results indicate that baseline ChE and AChE, and the *in vitro* inhibitory response to PB differ among primate species; in particular, baseline plasma ChE and whole blood ChE inhibition measured in AG monkeys differed from other primate species.

1730 LOCALIZATION OF SUBSTANCE P GENE EXPRESSION FOR EVALUATING PROTECTIVE COUNTERMEASURES AGAINST SULFUR MUSTARD.

S. L. Casbohm[1], J. V. Rogers[1], M. K. Stonerock[1], J. L. Martin[2], K. M. Ricketts-Kaminsky[2], M. C. Babin[1], R. P. Casillas[1] and C. L. Sabourin[1]. *[1]Medical Research & Evaluation Facility, Battelle, Columbus, OH and [2]US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*

Sulfur mustard [bis(2-chloroethyl)sulfide; SM] is a chemical warfare agent that produces edema and blister formation with a severe inflammatory reaction. The neuropeptide Substance P (SP) contributes to skin inflammation. The mouse ear vesicant model for cutaneous SM injury has been used to evaluate pharmacological agents for countering SM injury. The vanilloid, olvanil has been shown to reduce SM-induced edema and mRNA expression of cytokines and chemokines, suggesting that blocking the inflammatory effects of SP may provide protection against SM-induced dermal injury. This study examined SP expression in skin exposed to SM. Mice were exposed topically to SM (0.16 mg) on the inner surface of the right ear, with or without olvanil pretreatment, and tissues were collected at 1, 10, 30, 60, and 360 min following SM exposure. SP mRNA was localized in skin using *in situ* hybridization. In naive skin, mRNA localization was associated with blood vessels and sebaceous glands. In SM-exposed samples, hybridization was also detected in perivascular dermal cells. SP protein expression was identified immunohistochemically in the ear skin of naive, SM-, olvanil/SM-, and vehicle-treated mice. For the inner surface of the ear, the mean number of SP-positive perivascular cells in the dermis of olvanil-treated/SM-exposed ears was significantly lower than that of SM-exposed ears at both 60 and 360 min. In the outer surface of the ear, the mean number of SP-positive perivascular cells in the dermis of olvanil-treated/SM-exposed ears was significantly lower than that of SM-exposed ears at 360 min. These findings suggest that drugs targeting pro-inflammatory neuropeptides may reduce the severity of SM-induced cutaneous damage. Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-99-D-0010, Task Order 0013.

1731 PROTEOLYTIC CLEAVAGE AND COUNTER-IRRITATING EFFECT OF H2A HISTONE FRAGMENT AGAINST SULFUR MUSTARD-INDUCED SKIN LESIONS.

University. Wormser and B. Brodsky. *Institute of Life Sciences, The Hebrew University, Jerusalem, Israel.*

Skin exposure to sulfur mustard (SM), a potent chemical-warfare agent, results in erythema, edema, and blisters. Previous studies demonstrated the protective effect of topical iodine treatment against SM-induced skin damage. Iodine exerts its beneficial activity by induction of endogenous protective peptide, identified as 9 amino acids fragment of histone H2A. The anti-inflammatory/anti-vesicating activity of the peptide was evaluated by the mouse ear edema model. Intravenous administration of 0.1, 1 and 10 mg/kg of the peptide 5 min prior to SM exposure caused statistically significant reduction of 20%, 23% and 22%, respectively, in mouse ear swelling as compared to control animals injected with saline. The t1/2 of the peptide in mouse and human serum was 7 and 14 min, respectively. The degradation products were separated by HPLC and identified by mass spectra. Similar cleavage sites were observed for both human and mouse serum. It is suggested that the antidotal effect of the peptide might be of therapeutic value in protecting other sensitive tissues such as lung and bone marrow and in treatment of inflammation-associated disorders. (This study was supported by US Army Medical Research and Material Command under Cooperative Agreement DAMD17-03-2-0013.)

1732 REDUCED SULFUR MUSTARD-INDUCED SKIN TOXICITY IN CYCLOOXYGENASE-2 KNOCKOUT AND CELECOXIB-TREATED MICE.

A. Nyska[1], A. Sintov[2], R. Langenbach[3], B. Brodsky[4] and University. Wormser[4]. *[1]Laboratory of Experimental Pathology, NIEHS, Research Triangle Park, NC, [2]Institutes for Applied Research, Ben Gurion University of the Negev, Beer-Sheva, Israel, [3]Laboratory of Molecular Carcinogenesis, NIEHS, Research Triangle Park, NC and [4]Institute of Life Sciences, The Hebrew University, Jerusalem, Israel.*

Sulfur mustard (SM) is a potent vesicant which elicits inflammatory response upon skin exposure. To evaluate the role of COX-1 and COX-2 in SM-induced skin toxicity, we applied the agent to the ears of wildtype (WT), COX-1-deficient, and COX-2-deficient mice. In the latter, SM-induced ear swelling was significantly reduced as compared to WT. Quantitative histopathology revealed no epidermal ulceration in COX-2-deficient mice, whereas the WT showed some degree of severity in this parameter. COX-2-deficient mice showed significant reductions in severity of epidermal necrosis, acute inflammation and hemorrhage as compared to the WT mice. COX-1 deficiency resulted in significant exacerbation in severity of epidermal ulceration and epidermal necrosis as compared to WT strain. Post-exposure intraperitoneal injection of the selective COX-2 inhibitor celecoxib to normal mice resulted in significant reduction in ear swelling at intervals of 40 and 60 min between exposure and treatment. Histopathological evaluation showed significant reductions in subepidermal microblister formation and dermal necrosis as compared to the control group. Topical treatment with a combination of iodine, clobetasol and celecoxib at interval of 20 min between exposure and treatment resulted in significant reduction in ear toxicity. These findings may indicate that a) COX-2 participates in the early stages of SM-induced skin toxicity, b) COX-1 might exert some protective function against this chemical insult, c) topical preparation containing COX-2 inhibitor is effective in reducing SM-induced skin toxicity (This study was supported by US Army Medical Research and Material Command under Cooperative Agreement DAMD17-03-2-0013).

1733 GENE EXPRESSION IN MICE EXPOSED TO LOW AND HIGH LEVELS OF SULFUR MUSTARD.

J. V. Rogers[1], Y. W. Choi[1], R. C. Kiser[1], R. P. Casillas[1], M. C. Babin[1], J. J. Schlager[2] and C. L. Sabourin[1]. *[1]Medical Research & Evaluation Facility, Battelle Memorial Institute, Columbus, OH and [2]Pharmacology Division, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*

Sulfur mustard [bis-(2-chloroethyl)-sulfide; SM] exposure leads to blister formation in skin of exposed individuals. This study examined SM-induced changes in gene expression using cDNA microarrays in skin from mice cutaneously exposed to SM. Ear skin from five mice, paired as SM-exposed right ear and vehicle control left ear at six dose levels (0.005, 0.01, 0.02, 0.04, 0.08, and 0.16 mg), was harvested at 24 h. Alterations in gene expression were analyzed using cDNA microarrays containing 1, 176 genes. Genes were selected on the basis of all mice (N=5) in the same dose group demonstrating a ≥2-fold increase or decrease in gene expression in SM-exposed tissue compared to control skin at all six SM doses. When comparing skin exposed at all six SM doses with controls, a total of 19 genes within apoptosis, transcription factors, cell cycle, inflammation, and oncogenes and tumor suppressors categories were found to be up-regulated; no genes were down-regulated. A comparison of skin exposed to low (0.005 and 0.01 mg) and high (0.08 and 0.16 mg) doses of SM showed differences in the number and category of genes that were up- or down-regulated. Low level SM primarily altered transcription factors and repressors as well as genes involved in the cell cycle (e.g., cdc25B; cyclins) and apoptosis (e.g., GADD45; TNF receptor ligands); whereas high level SM also altered genes related to cytokines, chemokines, growth factors and hormones (IL-1β, MCP-1, MIP-1α, and MIP-2). The results of this study provide a further understanding of the molecular responses to cutaneous SM exposure, and enable the identification of potential diagnostic markers and therapeutic targets for treating exposure to low and high levels of SM. Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-99-D-0010, Task Order 0007.

1734 EFFECTS OF THE CHEMICAL WARFARE AGENT SULFUR MUSTARD ON OXIDATIVE METABOLISM IN CULTURED HUMAN EPIDERMAL KERATINOCYTES.

M. E. Martens. *Biochemical Pharmacology Branch, US Army Medical Res. Inst. Chemical Defense, Aberdeen Proving Ground, MD.* Sponsor: A. Brimfield.

Historically, the effects of sulfur mustard (2, 2'-dichlorodiethyl sulfide, SM) on the mitochondrial pathways involved in oxidation of various metabolic substrates have not been well-characterized. Therefore, we have examined the effects of SM on oxidative metabolism in cultured human epidermal keratinocytes (HEK). Our previous results have shown that, while the oxidation of glucose *via* the Krebs cycle is virtually absent in HEK, the rate of glutamine oxidation is sufficient for it to be a

significant source of energy in these cells. We also demonstrated that oxidation of glutamine and palmitic acid is inhibited by vesicating concentrations of SM (100 - 500 mM). Between 8 - 24 hours after exposure the degree of inhibition at 500 mM SM was severe enough to suggest that irreversible mitochondrial damage had occurred. Here we report the results of time- and concentration-dependence studies of SM-induced inhibition of glutamine oxidation. In these studies, metabolic activity was determined by measuring the rate of 14CO2 production from 14C-glutamine. In controls and at all concentrations of SM used, 14CO2 production was linear between 0 - 18 hours. It then plateaued between 18 - 24 hours, suggesting depletion of one or more vital metabolites, so these data were disregarded when calculating respiratory rates. In these experiments, SM concentrations of 25, 50 and 100 mM had no effect on the metabolic rate, whereas significant inhibition was seen at both 200 and 300 mM SM (72% and 85% inhibited, respectively; $P < 0.05$). Although in our preliminary studies we did see inhibition at 100 mM SM, it was not as severe as that at 300 mM or higher. It may be that 100 mM is at or near a threshold value below which mitochondrial metabolism is not affected. This is similar to the threshold level (67 - 167 mM) we have reported for SM-induced NAD+ depletion and inhibition of anaerobic glycolysis. We concluded from these results that mitochondrial metabolic injury may play an important role in the development of SM-induced pathology.

1735 THE USE OF FLOW CYTOMETRY TO DETERMINE THE EFFECTS OF THE VESICATING AGENTS SULFUR MUSTARD (SM) AND LEWISITE (L) ON HUMAN T CELLS (CD3+).

H. L. Meier. *BiochemPharmacology, USAMRICD, Aberdeen Proving Ground, MD.*
Sponsor: A. Brimfield.

It was learned from the casualties of the Iran-Iraq war that exposure to the vesicating agent SM caused a decrease in the number of circulating white blood cells (WBC). To understand the effects of the vesicant on the WBC, it was decided to study changes induced by vesicants on the subpopulations of leukocytes. Leukocytes were exposed to either SM or L, and the effects of these vesicating agents on CD3+ were investigated by flow cytometry. There were no detectable changes in size, density, or activation of cytotoxic pathways during the first 4 hrs after SM in CD3+, but there was a concentration (conc) dependent decrease in the percent of CD3+. At 24 hrs after SM exposure, CD3+ showed a steep increase in Annexin V+ (AV+) [marker of apoptosis] cells at 10μM SM but required higher conc of SM to demonstrate similar conversion of the CD3+ to Viaprobe (7-AAD+) and propidium iodide (PI+) [markers of necrosis]. L, on the other hand, caused no observable change in the percent of the CD3+ at any of the time points studied but caused increased binding of cytotoxic markers as early as 1 hr after L exposure. L caused a time- and conc-dependent increase in the binding of AV, 7-AAD, and PI to the exposed cells. At 1 and 4 hrs after L exposure, there was a large increase in AV+ cells, PI+ and 7-AAD+ cells at 100 μM L, but at 24 hrs post-exposure to L the large increase in the binding of these markers was at 10 μM. These findings were consistent with the findings that SM initially cause the activation of the apoptotic cell death pathway during the cells latent period. After the cell is depleted of energy at 4-8 hrs it converted to the necrotic pathway of cell death. However, cells exposed to L demonstrated no latent period and appeared to enter into the necrotic cell death pathway within the first hr after L exposure.

1736 THIODIGLYCOL METABOLISM BY ALCOHOL DEHYDROGENASE USING NMR: SYNTHETIC ROUTE TO THE METABOLIC INTERMEDIATE HYDROXYETHYLTHIOACETALDEHYDE.

A. A. Brimfield[1] and M. J. Novak[2]. *[1]Biochemical Pharmacology, USAMRICD, Aberdeen Proving Ground, MD and [2]Chemistry, Florida Institute of Technology, Melbourne, FL.*

In aqueous solution sulfur mustard (2, 2'-bis-chloroethyl sulfide) hydrolyzes to thiodiglycol (2, 2'-bis-hydroxyethyl sulfide, TDG). We demonstrated the metabolism of TDG in homogenates of human skin and by pure horse liver alcohol dehydrogenase (ADH) [J.Biochem. Molec. Toxicol.1998. 12:361] *in vitro* by monitoring NAD reduction at 340 nm. Following the enzymatic reaction by NMR we identified 2-hydroxyethylthioacetic acid as the end prodict of the two-step TDG oxidation [for conditions see The Toxicologist. 2002.66(1S):230 abst. 1128]. Based on the absence of a down field aldehyde signal, we concluded the intermediate aldehyde remained in the active site while a second molecule of NAD was reduced. Subsequent NMR studies indicat a transient peak at 3.42 ppm, suggesting the presence of the hydrated aldehyde intermediate. Here we report a synthetic route to this labile intermediate by diisobutyl aluminum hydride reduction of the monoprotected methyl ester of 2-hydroxyethylthioacetic acid (methyl 2-[*t*-butyldimethylsilyloxy]ethylthioacetate) at -60 C° in toluene. The protective silyl group, used to prevent potential cyclization during reduction, was removed using tetrabutylammonium fluoride in tetrahydrofuran. NMR in duterochloroform exhibited signals at 9.4 ppm (*t*, J=3.3 Hz, CHO) and 2.3 ppm (*d*, J=3.3 Hz, $RSCH_2CHO$).This compound will be used to confirm the identity of the peaks in the ADH NMR spectra.

1737 REAL-TIME CONCENTRATION AND RESPIRATORY MONITORING TO CONTROL PRESENTED DOSE IN AN AUTOMATED AEROSOL EXPOSURE SYSTEM.

J. M. Hartings[1, 2], B. R. Goodenow[2] and C. J. Roy[2]. *[1]Biaera Technologies, Inc., Frederick, MD and [2]Department of Aerobiology and Product Evaluation, USAMRIID, Fort Detrick, MD.*

Well-characterized aerosol systems are critical for toxicological and pharmacological studies involving inhaled materials. Accurate and precise delivery and subsequent measurement of an a priori determined inhaled dose is a determinative factor in identifying biological responses to inhaled materials. An automated bioaerosol exposure system (ABES) has been developed that utilizes real-time respiratory and aerosol concentration monitoring to control exposure duration and presented dose. Pressure fluctuations in the head-only exposure chamber, caused by the nonhuman primate pulmonary function, were used to determine respiratory rate, tidal volume, and minute volume. For each breath detected, the ABES software multiplied the measured tidal volume by the aerosol concentration as determined by Thermo Anderson MIE personalData RAM air sampling device to calculate a presented dose. ABES summed the doses from each breath and automatically terminated the exposure when the requisite presented dose was achieved. To validate this method, rhesus macaques (n = 9) were exposed to aerosolized staphylococcal enterotoxin B with a target presented dose of 220 μg/kg as part of an ongoing vaccine efficacy study. Aerosol samples were taken by using an all glass impinger (AGI) to compare the ABES determined presented dose to that from the in-use dose measurement technique. Animal weights ranged from 4.3 to 9.8 kg, respiratory minute volumes ranged from 1059 to 2800 ml, and average aerosol concentration ranged from 35.7 to 55.7 μg/L. AGI sample analysis indicated a presented dose of 210 ± 30 μg/kg for all of the test subjects. These results indicate that this feedback-based, dose-metering algorithm of the ABES can successfully accommodate a wide range of experimental conditions and physiological parameters. The resulting precision and accuracy of delivering an a priori defined presented dose to non-human primates will isolate dose response as the primary determinant of biological outcomes.

1738 REAL-TIME DOSIMETRY INCORPORATES UNANTICIPATED RESPIRATORY CHANGES DURING SEB AEROSOL EXPOSURES WITH RHESUS MACAQUES.

C. J. Roy, B. R. Goodenow and J. M. Hartings. *Aerobiology & Product Evaluation, USAMRIID, Fort Detrick, MD.*

Staphyloccocal enterotoxin B (SEB) is a protein toxin derived from *Staphyloccocus aureus* and is considered a biological threat agent. When disseminated as an aerosol, SEB can cause a shock-like disease, inducing vascular leak and formation of pulmonary lesions as little as 24-48 hours after inhalation. Nonhuman primates are the animal model of choice in pathogenesis and vaccine efficacy studies because of the sensitivity of the species to the toxin. In an ongoing efficacy study, the use of real-time dosimetry function of the Automated Biaerosol Exposure System (ABES) was used to control the delivery of the SEB challenge dose. Six weeks after vaccination, Rhesus macaques (n=10) were challenged with aerosolized SEB (MMAD=1.0 μm, σ_g = 1.4) at a target dose of 220 μg/kg (≈10 LD_{50}s). A head-only configuration of the ABES in a class III safety cabinet was used in the exposures. Anesthetized (Telazol, 5-6 mg/kg) primates were allowed to breathe the requisite volume of atmosphere based on particle concentration to attain the predetermined dose rather than relying on a timed exposure based on predictive measures such as plethysmography. Time to reach the requisite dose was highly variable (mean ± S.D. = 22:07 ± 8:04 mm:ss) and marked fluctuations in the respiratory parameters (tidal volume, TV; frequency, F) were observed in many of the animals. Linear regression analysis of the pulmonary data showed significant de*via*tions ($p<0.05$) in the TV and F in 40 and 60% of the primates under study, respectively. Changes in respiratory function may have been a result of unanticipated acute pulmonary reactions to the aerosolized SEB or changes in the plane of anesthesia during the course of the procedure. In conclusion, the real-time dosimetry function supplied by the ABES platform during inhalation exposure proved useful in adapting to an unanticipated acute-phase changes in the respiratory patterns of the primates and improved accuracy (and overall precision) of the group target dose congruence in the challenge portion of this efficacy study.

1739 IMMUNE RESPONSE INDUCED BY AN EXPERIMENTAL MUCOSAL ADJUVANT ADMINISTERED WITH RECOMBINANT PROTECTIVE ANTIGEN (RPA) IN A GUINEA PIG CHALLENGE MODEL.

E. K. Leffel, B. R. Goodenow and C. J. Roy. *Toxinology & Aerobiology, USAMRIID, Frederick, MD.*

Bacillus anthracis, a spore-forming aerobic bacterium, is considered a biological threat agent when disseminated as an aerosol. The organism possesses three primary virulence factors, edema toxin (protective antigen + edema factor), lethal toxin (protective antigen + lethal factor) and a d-glutamyl polypeptide capsule. A recombinant protective antigen (rPA) vaccine was efficacious against aerosol challenge when administered by intramuscular injection (IM). This form of vaccination induces circulation of systemic IgG antibody, but has not been shown to induce mucosal immunity. In order to invoke mucosal immunity and reduce time to protection, target delivery of the vaccine to a mucosal surface, such as the nasal lining, is proposed. To test the alternative delivery of rPA, guinea pigs were vaccinated either intranasal (IN) or IM with 50 μg of rPA combined with the adjuvant CpG (CpG-containing oligonucleotides). This vaccine preparation was compared to the standard adjuvant Alhydrogel™ (ALH). The vaccination was administered in a three-dose schedule 28 days apart. Analysis of sera indicated higher circulating anti-rPA IgG in both the IM and IN groups. Analysis of bronchoaveolar lavage (BAL) fluid was used to detect an inflammatory response and production of s-IgA. Animals vaccinated with rPA-ALH or ALH alone were then challenged by aerosol to 200 LD_{50} of *B. anthracis* (Ames strain). Results showed partial survival in the IM vaccinees (30%) compared to ALH controls (17%) and naïve controls (0%). Aerosol challenge is scheduled for the rPA-CpG groups. The preliminary results of this study suggest that the immune response observed in the IN-vaccinees indicative of protection when compared to the antibody levels present in the survivors vaccinated by injection.

1740 A NOVEL VACCINE DELIVERY SYSTEM THAT MINIMIZES ADVERSE EVENTS WHILE IMPROVING IMMUNE RESPONSE.

J. M. Blonder[1], C. Coeshott[1], E. Verderber[1], A. Samaniego[1], C. Tate[1], K. Stone[1], S. Smithson[2], M. Westerink[2] and G. J. Rosenthal[1]. *[1]Drug Development, RxKinetix, Inc., Louisville, CO and [2]Department of Medicine, Medical College of Ohio, Toledo, OH.*

Given the rapid advances in vaccine engineering in the last decade, it is important that delivery systems for such agents are equally efficacious and amenable to widespread use. We recently developed a vaccine delivery system (F127/CpG) using Pluronic® F127 as a sustained release polymeric vehicle and a CpG motif as an adjuvant. F127/CpG has generated rapid, potent and durable antibody responses to protein antigens after a single injection in rodents. Presently, the safety of F127/CpG was examined to further establish its utility. Recombinant anthrax protective antigen (rPA) was formulated in F127/CpG and injected sc into mice. Potential adverse effects by rPA/F127/CpG were measured by following body weight gain, appearance of palpable nodules at the injection site, clinical chemistries, and blood cell differentials. Immune response was determined by measuring biologically active antibodies to rPA *via* a toxin neutralization assay. Mice treated with rPA/F127/CpG were compared to mice treated with either rPA in CpG, rPA in aluminum hydroxide (alum) as a traditional adjuvant/delivery system, or vehicles. Injection of rPA/F127/CpG caused no overt adverse effects when compared to the other rPA groups or vehicles. Over 10 weeks, the average body weight gain ranged from 3.2±1.4 g to 4.7±1.7 g. Reaction at the injection site was less pronounced in rPA/F127/CpG. Specifically, palpable nodules were observed in 1, 3, or 4 mice (out of 8) at 8 weeks and 0, 0, and 2 mice (out of 8) at 10 weeks for rPA/F127/CpG, rPA/CpG, or rPA/alum. Clinical chemistries and blood cell differentials did not vary among rPA or vehicle groups and were within normal ranges. Relative levels of neutralizing antibodies to anthrax toxin were 134, 104, and 42 for rPA/F127/CpG, rPA/CpG, or rPA/alum. These data demonstrate that injection of rPA in F127/CpG is well tolerated in mice and induces an improved immune response when compared to a more traditional vaccine system in which alum is utilized.

1741 DIFFERENTIAL SUSCEPTIBILITY OF MACROPHAGE CELL LINES TO BACILLUS ANTHRACIS (VOLLUM 1B).

B. W. Gutting, K. Gaske, R. Mackie, A. Slaterbeck, L. Sobota, A. Schilling and T. Buhr. *Naval Surface Warfare Center, Dahlgren, VA.*

Bacillus anthracis (BA) is the causative agent of anthrax disease. Macrophages (Mϕ) play critical roles in bacterial infections. During infection, BA secretes several toxins that act on Mϕs. One, lethal factor (LF), demonstrates specific cytotoxicity for Mϕs and another, protective antigen (PA), aids in delivering LF to the cytosol. The objective of the present work was to examine the susceptibility of Mϕs for the Vollum 1B (LF+PA+) strain of BA. This work used RAW264, IC-21 and J774 Mϕs. Cytotoxicity was determined using trypan blue (TB) exclusion, neutral red (NR) uptake, and lactate dehydrogenase (LDH) activity in the culture supernatant. With all Mϕs, outgrowth of vegetative BA was observed 8 hours after the addition of BA spores. RAW264 cells were the most sensitive to the toxic effects of BA; at 8 hours, a significant increase in LDH was detected (115 percent ↑ compared to control) and at 24 hours all RAW264 cells were dead (346 percent ↑ in LDH activity). Cytotoxicity of J774 Mϕs could not be detected until 20 hours post infection using LDH activity (109 percent ↑ vs. control) and a significant decrease in NR uptake was detected at 21 hours ($p<0.001$). With RAW264 and J774 Mϕs, TB staining was detected at 12 and 15 hours, respectively. In studies using IC-21 Mϕs, no significant cell death using TB, NR or LDH could be detected up to 26 after the addition of BA spores, despite vegetative growth of BA in the culture for >18 hours. Neutralization studies were conducted using anti-LF and anti-PA antibody. Here, the addition of anti-PA, but not anti-LF, prevented RAW264 cell death. These data suggest that (i) Mϕ cell lines demonstrate differential susceptibility towards virulent BA, (ii) IC-21 cells do not undergo necrosis when co-cultured >18 hours with vegetative Vollum 1B, and (iii) the mechanism of RAW264 cell death is mediated, in part, by PA. A better understanding the mechanism of Mϕ resistance or susceptibility to BA may lead to novel therapies for anthrax disease. (Supported by ONR)

1742 METHYLMERCURY CONTAMINATION IN FISH: HUMAN EXPOSURES AND RISK COMMUNICATION.

D. D. Petersen. *NRMRL, USEPA, Cincinnati, OH.*

Many people in America are at risk for methylmercury toxicity because of their consuption of contaminated fish. Often the health risks of various persistant bioaccumulating toxicants (PBTs) are underestimated because amplification in food chains results in toxic levels of contamination even though ambient levels in water bodies may be near detection limits. Two concerns have developed form this situation. First: many of the susceptible individuals are unaware of the risk, and second; there are few effective means of communicating the risk information to these individuals. Recent data shows that approximately 8% of American women bear methylmercury body burdens that are above thresholds for toxic symptoms in the developing fetus. Given the EPA reference dose of .1 mg/kg/day and can tuna with 17ug/can, the safe dose of tuna for a small child would be once every two weeks. A handbook for communicating this information has been prepared and will be diplayed.

1743 LINKING COMMUNITY OUTREACH AND EDUCATION WITH RESEARCH TO IMPROVE THE HEALTH OF A POPULATION.

C. G. Sumaya[1], G. Carrillo[2, 1], K. C. Donnelly[2, 1] and J. A. Parrish[2, 1]. *[1]Center for Community Outreach and Education , Texas A& M University Center for Environmental and Rural Health, College Station, TX and [2]School of Rural Public Health, Texas A&M University System Health Science Center, College Station, TX.*

The Community Outreach and Education Program (COEP) primarily focuses on translating the research findings of the Center for Environmental and Rural Health (CERH) and educating the rural communities of the Texas-Mexico border. A research project, "Estudio Por Ninos Saludables en Rio Bravo" was established through funds from a USEPA STAR grant and NIEHS Centers at Texas A& M and Rutgers Universities to investigate childhood exposure to pesticides. This research showed that children ages 6 to 48 months had a median exposure level to pesticides comparable to the 90th percentile from the CDC's NHANES study and that simple behavioral changes could drastically reduce potential exposures. The COEP's approach to reducing childhood exposures to pesticides was to conduct town meetings in Rio Bravo, TX to identify means of preventing children's exposure to pesticides. Local promotoras (community health workers) will be trained in pesticide and other environmental health problems affecting this population, in collaboration with the Laredo Health Department. The promotoras and colonia residents will receive a pre-intervention assessment prior to training and a post-intervention assessment after completion of the first training. This pilot program will include a bilingual environmental health curriculum to evaluate the effectiveness of the type of intervention used and an appropriate manner of delivery for this target population. The COEP is an important complement to research to expand communication in the target population and to evaluate the utility of preventive measures. Future plans include: expanding our bilingual curriculum to other environmentally related health issues and other geographic areas along the Texas-Mexico border and to link this program with Mexico health departments to reach communities on both sides of the Texas border.

1744 TRANSLATING CHILDREN'S ENVIRONMENTAL HEALTH RISK RESEARCH FOR COMMUNITIES.

C. H. Drew and E. M. Faustman. *Institute for Risk Assessment and Risk Evaluation, University of Washington, Seattle, WA.*

The Center for Child Environmental Health Risks Research at the University of Washington (CEHR) is working to understand the mechanisms defining children's susceptibility to pesticides and the potential for neurodevelopmental impacts. Researchers at CEHR focus on environmental, genetic, and developmental factors that determine susceptibility. Understanding where children spend their time is one emphasis for the Center. Researchers use environmental modeling and sampling, child activity patterns, and biological monitoring to better characterize the environmental and occupational "takehome exposure pathway" for children living on or near treated farmland. CEHR translates this research into a community-based research intervention project aimed at breaking the takehome pathway. A diverse community advisory board advises project staff about developing materials and intervention methods. Individual, group-based and community wide intervention activities have focused on overall child health risks. Community-wide events such as health fairs (n = 1200 individuals) were popular among the mostly Latino intervention community. They provided educational materials and items such as laundry detergent. Volunteers also teamed with professional health care workers to conduct smaller scale "home health parties" with families, friends and neighbors (n = 1000 events). Additionally, the project has developed a wide range of pesticide education materials for preschool and elementary students and for adults in citizenship and English as a second language classes. These include a Head Start curriculum for 3-4 year olds that teach pesticide safety with activities such as songs and puzzles. To date, over 18, 000 people have participated in more than 1800 events. (Supported by grants from the USEPA (R82688601), NIEHS (5PO1ES09601) and the Center for the Study and Improvement of Regulation).

1745 ENVIROHEALTH CONNECTIONS: A COLLABORATIVE EXPLORATION OF THE ENVIRONMENT & HUMAN HEALTH.

C. Mutryn[1], M. A. Trush[2] and G. P. Long[1]. *[1]Maryland Public Television, Owings Mills, MD and [2]JHU Bloomberg School of Public Health, Baltimore, MD.*

A 7-year NIEHS-funded project to develop, implement, evaluate, & disseminate an innovative model for using environmental health science (EHS) as an integrative context for learning (EHSIC), focusing on enhancing Maryland middle & high school teacher & student awareness and knowledge of EHS and on improving overall student academic performance by developing high-quality, cutting edge interdisciplinary instructional materials/resources, based on Maryland State Standards, to be used by teachers to deliver EHS curricular content; enhancing their ability to develop & deliver interdisciplinary instruction based on EHS concepts, issues, & career opportunities and to develop skills students need to perform successfully in all academic subjects & on state-mandated standardized testing; and providing teachers & students with opportunities to interact with and learn from environmental health scientists. The project is unfolding in 3 phases: Development of Needed Curricular Materials & Enhancement of Teacher Capacity: Fall 2000 - Summer 2003; Implementation in Schools: Fall 2003 - Spring 2006; Evaluation & Dissemination of EHSIC Model: Fall 2005 - Spring 2007. Current studies & results at start of project Phase II: Completion of instructional video & interactive website: *EnviroMysteries: Breaking the Mold* at: http://enviromysteries.thinkport.org/ re: indoor air pollution & asthma; ongoing development of EHS topic lesson plans & Tech Tours for the MPT *Thinkport* education super site at www.thinkport.org; selection of 9 Collaborative Teaching Teams developing & implementing EHS/technology infused lessons & activities; establishment of an environmental health scientist Speakers Bureau with whom teachers & students can interact & learn; implementation of an annual 2-day Winter Colloquium, an annual statewide 4-day Summer Teacher Training Institute, and an EHSIC Electronic Learning Community at: http://www.cte.jhu.edu/ep/, providing dynamic professional development communications & resources for all educators participating in the project.

1746 DEVELOPMENT OF K-12 ENVIRONMENTAL HEALTH & SCIENCE (EH&S) CURRICULA.

J. A. Gorenstein, D. Cook, J. Rodriguez and R. Fuchs-Young. *Center for Research on Environmental Disease, UT M. D. Anderson Cancer Ctr., Smithville, TX.* Sponsor: C. Walker.

K-12 education increasingly employs the Internet as a source of scientific information, classroom activities and lessons. Accordingly, our Community Outreach & Education Program (COEP) develops web-based K-12 resources and curricula to inform students about mechanisms of environmental disease. The goal is to provide accurate information that promotes a better understanding of the impact of environmental factors and toxicants on human health, and encourages healthier lifestyle choices. Materials employ EH&S as an integrating context to stimulate comprehensive, inquiry-based and integrated learning across subjects, including science, language arts, history and math. Curricular materials are produced through the cooperative contributions of faculty scientists and K-12 teachers, who, respectively, provide scientific information and translate it into age-appropriate content. Curricula are piloted and evaluated in TX schools, revised as needed and distributed free-of-charge. These easily accessible curricula provide needed resources to under-funded school systems and over-extended teachers. Veggie-mon (veggie-mon.org) is an EH&S website for 4-8 graders that presents lessons about causes and prevention of environmental disease through colorful and interactive characters and activities. The website includes sections on tobacco avoidance, UV exposure, diet and nutrition. Impact assessments (IRB approved) demonstrated that after viewing the website there was a significant increase in the number of students who could correctly identify environmental hazards that cause disease. SCREAM, a curriculum for high school students, investigates the interactions between environmental factors and genetics in determining cancer risk. Our most recent effort is a module for high school seniors and young adults that explores the Ethical, Legal and Social Implications (ELSI) of advancing genomic technologies that will generate data on disease susceptibility. All curricula are accompanied with teaching manuals and aids to facilitate their implementation into classroom lessons.

1747 BUILDING CREDIBILITY IN K-12 EDUCATION.

H. Goeden[1], P. Shubat[1], L. Solem[2] and R. Skoglund[3]. *[1]Minnesota Department of Health, St. Paul, MN, [2]Minnesota Pollution Control Agency, Duluth, MN and [3]3M, St. Paul, MN.*

The Northland Chapter of the Society of Toxicology (NLSOT) has had an active education program for elementary and secondary schools (K-12) since 1999. The committee recently evaluated past experiences and expectations, resulting in a shift of focus. NLSOT now focuses on teachers and their needs instead of toxicologists, in particular bringing toxicologists to where teachers are already prepared to learn new concepts. In the first years of programming, NLSOT planned and implemented a training session for toxicologists to equip them as guest lecturers in classrooms. NLSOT inventoried resources by collecting and evaluating toxicology curriculum materials and surveying interest and skills of chapter members. In subsequent years NLSOT trained members to use Toxicology-in-a-Box™ and ToxRAP™. The subcommittee researched teacher training and career development, and the barriers to introducing new materials into science curriculum. Over the course of three years, NLSOT found little sustained interest amongst NLSOT members in developing expertise in the K-12 classroom. NLSOT was also unable to attract teachers to attend time-intensive training in toxicology curricula. Based on successful interactions with 80 teachers at a multi-state science teachers association conference, NLSOT found that teachers who came to the conference were ready to discuss new curricula, accepted toxicology kits, attended lectures offered by toxicologists, and were interested in discussing the curricula which they developed on their own. Teaching tools developed and distributed by NLSOT included two types of seed germination and growth experiments that illustrate principles of dose-response, a lecture on toxicological principles, and a lecture on classroom resources for teaching toxicology principles. As a result of this evaluation, NLSOT has set a goal of increasing science teachers' familiarity with SOT and toxicology concepts by participating in venues where teachers are seeking curriculum enrichment, such as annual meetings of science teachers associations.

1748 THE AMBIENT PROJECT: HIGH SCHOOL ENVIRONMENTAL HEALTH SCIENCES CURRICULUM.

L. Pitman[1, 2], L. E. Fleming[1], T. Pitman[1, 4], W. Stephan[1], H. Davis[1] and K. Goodman[1]. *[1]NIEHS MFBS Center, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, Miami, FL, [2]Educational Specialists, Miami Dade County Public Schools, Miami, FL, [3]Epidemiology, Miami Dade County Public Health Department, Miami, FL and [4]Chemistry, Florida International University (FIU), Miami, FL.* Sponsor: L. O'Fallon.

The AMBIENT Project (Atmospheric and Marine-Based Interdisciplinary Environmental Health Training) is environmental health curriculum targeted at high school teachers. AMBIENT facilitates involvement of teachers and students with research scientists and community members in an interdisciplinary approach to learning about their local and global environmental health science issues. Funded by National Institute of Environmental Health Sciences (NIEHS), the AMBIENT curriculum is based on four environmental themes with associated environmental health issues of: air (asthma), water (microbes), soil (lead), and food (foodborne illness & nutrition), as well as modules in toxicology and ethics. Each module integrates language arts (creative writing and critical reading) with math, science and mapping, and ends in a culminating experience (ie. debate or role play). The problem-based learning approach uses real-life scenarios and data. All the modules (including individual stand-alone activities) can be downloaded from the AMBIENT

Website at www.rsmas.miami.edu/groups/niehs/ambient/. At summer workshops, high school teachers work in interdisciplinary groups to enhance their own understanding of environmental health sciences and ethical issues. An outside evaluator has documented the creation and implementation of the AMBIENT curriculum with the teachers and high school students in their classrooms. Evaluation has shown significant increases in environmental health knowledge among AMBIENT teachers and students after using the curriculum, as well as actual curriculum implementation in Miami-Dade County Public Schools often crowded and highly diverse inner city high school classrooms. Future work involves the dissemination of AMBIENT, as well as the creation of additional modules with educator, community and scientific partners.

1749 AN INTERDISCIPLINARY, ENVIRONMENTAL HEALTH-BASED APPROACH TO IMPROVING SCIENCE LEARNING BY ELEMENTARY TEACHERS AND STUDENTS.

N. Moreno, B. Tharp and P. Cutler. *Center for Educational Outreach, Baylor College of Medicine, Houston, TX.* Sponsor: L. O'Fallon.

The National Science Education Standards emphasize the need for improved science teaching and learning throughout grades K-12. Teacher content knowledge, appropriate teaching approaches and the use of standards-aligned curricula all have been identified as essential for effective science instruction. At the same time, the Standards emphasize the importance of connecting student learning to the real world. The Environment as a Context for Opportunities in Schools (ECOS) project in Houston, Texas is piloting an integrated approach to science teaching in elementary schools built around environmental health themes. The ECOS project uses the interdisciplinary My Health My World educational program (developed with funding from NIEHS and NCRR) as the backbone of a integrated instructional program that weaves together science, health, mathematics and reading/language arts. At the same time, the project aims to improve teacher practice through intensive summer and year-round professional development on content and teaching strategies. The ECOS project has developed and evaluated supplementary mathematics and reading materials, Spanish language components, a teacher professional development program on environmental health and new teaching units for students in grades K-2. Field tests of new materials indicated high levels of teacher satisfaction with the content and instructional approaches. In addition, teachers reported development of language arts and science skills by students. Teacher professional development provided by the project led to statistically significant increases in teacher content knowledge on topics related to environmental health and in teacher science teaching efficacy beliefs. Initial classroom observations of teachers suggest that the project is promoting interactive, inquiry teaching behaviors by teachers. The ECOS project is funded by grant number R25 ES010698 from NIEHS.

1750 PROBLEM-BASED LEARNING FOR ENVIRONMENTAL HEALTH.

E. Henry[1], D. G. Markowitz[1], P. Braus[2], P. Debes[1], K. Hart[2], D. Hursh[3] and C. Martina[3]. [1]*Environmental Medicine, University of Rochester, Rochester, NY,* [2]*Community and Preventive Medicine, University of Rochester, Rochester, NY and* [3]*Warner Graduate School of Eudcation and Human Development, University of Rochester, Rochester, NY.*

The My Environment, My Health, My Choices project offers teachers the opportunity to develop curricula to address environmental health topics in science and non-science subjects. This project involves 3-member teams, composed of science and non-science teachers, from public and private urban, rural, and suburban middle and high schools from the Rochester, New York area. Each team develops curriculum units using a problem-based learning (PBL) format to focus on a local environmental health issue. Evaluation of 4 teams participating in Phase I of this 7-year project allowed us to identify program successes and issues of concern, and enabled us to re-structure certain aspects of our program design. The evaluation included surveys, focus groups, interviews and classroom observation. Our evaluation indicated the need to overcome the difficulty in finding common curriculum planning time, meet challenges of interdisciplinary teaching within the current school structure, and solve teacher concerns about using a PBL curriculum in courses with standardized testing and state-required curricula. We also noted the need for clarity of goals and outcomes for the teachers. As a result of the evaluation, we modified certain aspects of the program, including implementing a more rigorous process to select teachers who are open to changing their classroom routine and who have strong administrative support for their participation in this project. The initial week-long training for teachers was altered from a predominantly lecture format to a hands-on workshop in which teachers participated in PBL exercises and used a curriculum development template to write initial drafts of their units. We also provided teachers will examples of how to use environmental health lessons to meet educational standards. We are currently evaluating 7 teams in Phase II of the project to assess the success of the changes that we made.

1751 DEVELOPMENT AND ASSESSMENT OF AN ONLINE, UNDERGRADUATE INTRODUCTION TO TOXICOLOGY COURSE.

K. L. Willett[1] and A. S. Bouldin[2]. [1]*Pharmacology and Environmental Toxicology, University of Mississippi, University, MS and* [2]*Pharmacy Administration, The University of Mississippi, University, MS.*

Introduction to Toxicology has been an elective course for honors pharmacy students and a required course for forensic chemistry majors at The University of Mississippi for a number of years. In order to increase the options available to students, this course was developed and delivered *via* an online format for two semesters. The course was administered through the existing Ole Miss Online structure (using Blackboard) and was divided into two blocks, each utilizing a different textbook. The first half of the course (Lectures 1-14) introduced the basic concepts and principles of toxicology while the second half was more focused on aspects of forensic toxicology including the signs, symptoms, toxicities and analytical detection methods associated with drugs of abuse (Lectures 15-28). Each week the students were responsible for viewing two or three PowerPoint presentations that supplemented their assigned online and text reading. "Lecture" was provided by voice audio that was prerecorded to explain the material on each slide. An expanded course evaluation was developed to assess student attitudes and perceptions of the online version of the course, along with perceived comparisons between online and traditional environments. A primary advantage of the online format as perceived by the students was working at a time of their own choosing; yet managing that time and "keeping up" was reported as being difficult. Most students (72%) did believe that they saved time by taking this course online (responding with a 1 or a 2 on a 5-point Likert-type scale). Additionally, no student perceived that he/she missed any content by taking the course online. Current and potential challenges associated with this teaching format along with changes that may be implemented in future versions of the course will be presented.

1752 CHARACTERIZING THE UNCERTAINTIES IN SCREENING AND ASSESSING RISKS TO CHEMICALS THAT DECREASE THYROID HORMONE CONCENTRATIONS.

M. J. DeVito and K. M. Crofton. *ORD/NHEERL/ETD, USEPA, Research Triangle Park, NC.*

A variety of chemicals alter thyroid hormones (THs) in rodents. The importance of THs during neurodevelopment, suggest that these chemicals would likely be developmental neurotoxicants. Several levels of uncertainty impact our understanding of the point of departure, mode-of-action and mechanism-of-action of xenobiotics that alter THs. Humans appear more sensitive than rodents to alterations in THs during development. In rodents, neurodevelopment toxicity may require large decreases in thyroxine (T4) concentrations (>50%). In humans, decreased IQs were observed in offspring of women subclinically hypothyroid during the first trimester with serum T4 levels decreased only 25 - 30%. The potentially greater human sensitivity to TH decreases could impact an assessment of the point of departure and the development of regulatory exposure limits. A second uncertainty is the impact of species differences in the pharmacokinetics of THs. In rodents, the serum half-life of T4 is on the order of hours whereas in humans it is 4-7 days. In humans, serum is a major storage pool for thyroid hormones. In rodents, the major storage pool is the thyroid gland. In rats, glucuronidation is a major deactivation pathway while in humans, deiodination and sulfation is more important. These differences may result in altered sensitivity to environmental chemicals. Another uncertainty is species differences due to altered sensitivity to the mechanisms of action of xenobiotics; e.g., induction of UDPGT, which increases elimination of THs, is mediated in part by CAR and PXR pathways. There are species differences in the structure activity relationship for activation of these pathways that could lead to altered species sensitivity to xenobiotics. Present mammalian screens for chemicals that thyrotoxic xenobiotics do not consider these potential species differences. Development of alternative screens that could also assess these species differences could aid in our ability to screen and assess thyrotoxic chemicals. (This abstract does not represent USEPA policy.)

1753 ESTABLISHING A SAFE DOSE FOR PERCHLORATE BASED ON HUMAN EVIDENCE OF A NO EFFECT LEVEL.

R. C. Pleus[1]. [1]*Intertox, Seattle, WA and* [2]*Pharmacology, UNMC, Omaha, NE.*

The pharmacology of perchlorate is well understood. Perchlorate acts by competitively inhibiting iodide uptake at the sodium iodide symporter in the thyroid, which is the basis of its use as a medication. The medical and toxicological aspects

of perchlorate are well known and have been extensively reviewed (Wolff 1998; Goodman 2003). Traditionally toxicologists test chemicals by looking for clear indications of adverse effects in laboratory animals. Such indications might include tumors, hyperplasia, microscopic lesions, and structural abnormalities. Once adverse effects have been characterized, toxicological testing evolves by lowering the exposure dose in order to define a no-observable-adverse-effect level (NOAEL) or failing that, a lowest-observable-adverse-effect level (LOAEL). When these doses are established, uncertainty factors are applied to establish safe values for human exposure. However, when the pharmacology of an agent is known, this knowledge can be used to establish with virtual certainty a lower bound other than zero for a safe exposure level. Identifying the threshold of a precursor to a precursor to a possible adverse effect in humans logically increases both precision and *certainty*, and thus overcomes the limitations of the traditional uncertainty factor approach. For perchlorate, iodide uptake is the initial step (precursor) of the biochemical pathway that could lead to a clinically significant reduction in circulating thyroid hormones (TH). Such a change in TH could lead to developmental deficits in the fetus, the accepted population of concern. Greer et al. (2002) measured a no-observed-effect level (NOEL) of 0.007 mg/kg-day and estimated a true no-effect level (NEL) of 0.005 to 0.006 mg/kg-day for inhibition of radioiodine uptake (RAIU) by perchlorate in men and women. Apart from being an inherently safe dose, the estimated NEL provides a plausible lower bound for a reference dose derived from traditional animal toxicology studies. Further, the NEL has inherently greater precision than a NOAEL or LOAEL and this precision should be reflected when choosing values for uncertainty factors.

1754 EXPOSURE ASSESSMENT FOR PERCHLORATE IN DRINKING WATER.

D. Proctor[1], E. Cohen[1], H. Leung[1], S. Hays[1], L. Barraj[1] and A. Madl[2].
[1]Exponent, Irvine, CA and [2]ChemRisk, San Francisco, CA.

Perchlorate is a public health issue of recent interest because it has been found at low levels in the drinking water used by millions of Americans. High level exposure to perchlorate inhibits thyroidal iodide uptake, and disruption of thyroid function can result in neurological effects. Monitoring conducted in California indicates that perchlorate has been measured in approximately 8% of the drinking water supplies, and 60% of those sources contain less than 10 ppb. Preliminary data has suggested that perchlorate can accumulate in leafy vegetables. Further, it has been found in cows milk and human breast milk. Assuming 10 ppb of perchlorate in drinking and irrigation water supplies, we evaluated the potential perchlorate exposure from a variety of scenarios: 1) ingestion of water and water based drinks (e.g., coffee) for the total US population, children age 1-6 years, and women of childbearing age, 2) mothers milk ingestion for a nursing neonate, 3) lettuce ingestion among childbearing age women, which considered recent data regarding perchlorate accumulation in lettuce, and 4) total water intake from all food and drink sources (e.g., moisture in produce, soda, dairy, etc). For the neonate evaluation, we used a simple pharmacokinetic model to estimate steady-state perchlorate levels in the milk of lactating mothers. For all other scenarios, consumption data from the USDA was used to estimate ingestion at the 50th and 90th percentiles. Nutrient recipes were used to translate the foods as consumed into various nutrients, including moisture (water). The bodyweight-adjusted doses at the 50th percentile ranged from 0.00003 mg/kg-day (nursing neonate and lettuce ingestion) to 0.001 mg/kg-day for the total water intake (direct, indirect and foodbased) for women age 18 to 40 years. Exposures at the 90th percentile were approximately 2.5-times higher. These estimates are preliminary and based on conservative simplistic exposure assumptions. The estimated doses are all lower than those that have been shown to affect thyroidal iodine uptake among humans, a precursor to know adverse effects.

1755 DERIVATION OF A RFD FOR PERCHLORATE: IDENTIFING A CRITICAL HEALTH ENDPOINT AND THE MOST SENSITIVE SUBPOPULATION.

A. Madl[2], D. Proctor[1], H. Leung[1], E. Goswami[1], S. Hays[1] and E. Cohen[1].
[1]Exponent, Irvine, CA and [2]ChemRisk, San Francisco, CA.

Perchlorate (ClO4-) is a drinking water contaminant and is known to inhibit of thyroidal iodide uptake. While environmental agencies have proposed a RfD for ClO4-, their approaches and reliance on human versus animal experimental data differ. A critical evaluation of the human scientific literature was conducted to identify a critical endpoint for the derivation of a RfD for ClO4- and clearly define possible sensitive subgroups for exposure and uncertainty assessments. Numerous studies have evaluated the effects of ClO4- in humans in medical, community, occupational and experimental settings. Based on our assessment, it was concluded that a decrease in thyroidal iodide uptake is the most sensitive effect of ClO4- and the Greer et al. (2002) study is the most appropriate for deriving a RfD. There are clear differences in thyroid function between the human fetus, neonate and adult. It was concluded from our evaluation that the developing neonate would be most susceptible to the effects of ClO4-. The basis for this conclusion is that during gestation, fetal development is dependent on and protected by transfer of maternal thyroid hormones and as long as maternal thyroid iodide uptake and hormone levels are maintained, normal fetal development would be expected. Following birth, the neonate is dependent on its own production of thyroid hormone with iodide supply originating from maternal breast milk. Iodide uptake in the neonatal thyroid and iodide levels in breast milk should be maintained at normal levels for normal neurodevelopment to occur in the infant. Dietary iodide intake in the US was concluded to be sufficient to prevent thyroid changes, precluding pregnant women as a sensitive subgroup. Thus, it appears that derivation of a RfD ClO4- based on protection against inhibition of thyroidal iodide uptake, the first step in the production of thyroid hormones, would prevent adverse effects during fetal and neonatal development. Exposure and uncertainty assessments will focus on the developing neonate as the most sensitive subgroup.

1756 SCIENTIFIC RATIONALE FOR THE DERIVATION OF A REFERENCE DOSE (RFD) FOR PERCHLORATE.

H. Leung[1], D. Proctor[1], A. Madl[2], S. Hays[1] and E. Cohen[1]. *[1]Exponent, Irvine, CA and [2]ChemRisk, San Francisco, CA.*

Perchlorate (ClO4-), a contaminant in drinking water, inhibits thyroidal iodide (I-) uptake and may disrupt the production of thyroid hormones (TH) leading to thyroid dysfunctions and other neurological deficits. Pregnant women, fetuses, neonates and people with I- deficiency or compromised TH status may be especially at risk. Governmental agencies have used various methods to draft risk assessments for ClO4-, producing disparate results. We have critically reviewed the rationale for the most appropriate approach to derive a RfD for ClO4-. The major conclusions are: (1) I- uptake inhibition is a precursor to TH level perturbation, which in turn is a precursor to thyroid dysfunction. A health risk assessment for ClO4- must recognize that I- uptake inhibition is not a genuine adverse effect; (2) Human dose-effect data are available, and are preferable to animal data in order to avoid the uncertainties of interspecies extrapolation; (3) Controlled human volunteer studies offer more reliable exposure information and are preferable to other epidemiological and community studies. (4) The benchmark dose methodology is recommended for dose-response assessment and the benchmark dose effect level (BMDL) corresponding to one standard de*via*tion from the mean of the control population should be used; (5) As the half-life of perchlorate in humans is moderate (8 h), allowing steady state level to be readily attained, no adjustment factor is needed for extrapolating I- uptake inhibition data from less than chronic exposure; (6) Many surveys have shown that the dietary I- intake is sufficient in the US population and very few women are I- deficient; (7) There is evidence that person-to-person variation, including those individuals at sensitive developmental life stages, is less than 3 fold. On the basis of these considerations, we have derived a BMDL of 0.0126 mg/kg-d based on the thyroidal I- uptake inhibition data in the Greer et al. (2002) study (Environment Health Perspect. 110:927). We applied an overall uncertainty factor of 3 to arrive at a tentative RfD of 0.0042 mg/kg-d.

1757 USE OF HUMAN AND ANIMAL PBPK MODELS IN RISK ASSESSMENT FOR PERCHLORATE.

D. R. Mattie[1], T. R. Sterner[2], E. A. Merrill[3], R. A. Clewell[3], Q. Zhao[4], J. E. Strawson[4] and M. L. Dourson[4]. *[1]AFRL, Wright-Patterson AFB, OH, [2]OpTech, Dayton, OH, [3]Geo-Centers, Wright-Patterson AFB, OH and [4]Toxicology Excellence for Risk Assessment (TERA), Cincinnati, OH.*

Perchlorate has been used as a solid rocket propellant and ignitable source in munitions for many years. Recently, it has been found in surface and ground waters of multiple environmental sites, especially in the western United States. The primary effect of this chemical is inhibition of iodide uptake by the thyroid. Sufficient inhibition over time results in a depression of thyroid hormone formation and subsequent compensatory increase of thyroid stimulating hormone (TSH). The most sensitive measure of thyroid function disruption is iodide uptake inhibition and the most sensitive population in humans is likely to be the fetus. However there are no quantitative dose-response toxicity data available for the fetus in either animals or humans. To assist in addressing this issue, perchlorate physiologically based pharmacokinetic (PBPK) models were developed for rats at different life stages including the normal adult, pregnant female and fetus, and lactating female and neonate. In addition, a PBPK model for adult humans was also developed. These PBPK models were verified using iodide uptake inhibition studies in rats and humans. Effective doses for iodide inhibition in rats and humans at different life stages were estimated. Results indicated that the fetus is the most sensitive life stage for iodide uptake inhibition. By applying the ratio of predicted effective doses between adult rats and humans to those predicted for rats of different life stages, an effective dose of 0.006 mg/kg/day can be estimated for 5% iodide uptake inhibition in the human fetus. By using this effective dose in human fetus as the point-of-departure, a risk estimate for perchlorate of 0.002 mg/kg/day can be developed by applying an uncertainty factor of 3 fold to account for intraspecies variation. This new risk level confirms the previously proposed value of 0.002 mg/kg/day determined by other methods.

1758 CAPTURING AND MODELING HUMAN INTERINDIVIDUAL DIFFERENCES FOR HEALTH RISK ASSESSMENT: HEPATIC BIOACTIVATION OF CHLOROFORM.

J. C. Lipscomb[1], J. T. Du[2], J. C. Swartout[1], D. A. Mahle[3], J. E. Snawder[4] and G. L. Kedderis[5]. *[1]ORD/NCEA, USEPA, Cincinnati, OH, [2]OW/HECD, USEPA, Washington, DC, [3]Man Tech Environmental Tech Inc., Wright-Patterson AFB, OH, [4]Taft Lab., CDC/NIOSH, Cincinnati, OH and [5]Independent Consultant, Chapel Hill, NC.*

Chloroform (CF) is a volatile drinking water contaminant. Risks *via* the oral, but not inhalation route have been estimated. This study was conducted to adjust inhalation dosimetry between animals and humans and assess the impact of variability in some factors which may predispose humans to toxicity. CF partition coefficients were determined in tissues from young and mature rats, and in blood from adult and pediatric patients. CF metabolic rate constants and hepatic CYP2E1 protein concentrations were determined in liver tissue from adult rats and human adult and child organ donors. Vmax was extrapolated to intact tissue and scaled to body weight. The distribution of hepatic blood flow (HBF) as percent of cardiac output was determined from a total of 59 published individual values in human adults. These data were incorporated into physiologically based pharmacokinetic (PBPK) models constructed for mice; rats; and human adults, preadolescents and infants. Because of the relevance of the inhalation reference concentration to chronic, lifetime exposures, constant exposure to CF was simulated until steady state was reached rather than simulating intermittent exposure scenarios. Toxicity studies with mice demonstrate a duration-adjusted NOAEL (liver effects) of 0.9 ppm CF. Simulations indicated that the human equivalent exposure concentration, based on hepatic CF metabolism, was approximately 6 ppm. Among adults, increases in CYP2E1 content and activity, blood:air partitioning, and HBF produced approximately 1, 4 and 17% increases in CF metabolism, respectively. Children converted as much as 34% more CF to metabolites than equivalently exposed adults. These findings will be useful in determining the magnitude of PK variability in the risk relevant PK outcomes between species and among humans.

1759 THE CONTRIBUTION OF PHARMACOKINETIC VARIABILITY TO VARIABILITY IN HEPATIC LABELING INDEX DATA FROM B6C3F1 MICE EXPOSED TO CHLOROFORM.

C. Tan and R. Conolly. *CIIT Centers for Health Research, Research Triangle Park, NC.*

We have extended a PBPK model for chloroform to describe a plausible mechanism of hepatic cell killing and regenerative proliferation (Toxicol. Sciences. 75, 192-200, 2003). The resulting PBPK/PD model simulates labeling index (LI) data. A Monte Carlo implementation of the PBPK model (Regul. Toxicol. Pharmacology 2, 144-155, 2000) provides an opportunity to examine the role of PK variability in the LI data. We used this approach with a hepatic LI data set where female B6C3F1 mice inhaled chloroform for 7 consecutive days at various combinations of duration and concentration (Toxicol. Sciences. 66, 201-208, 2002). Our analysis is showed that variability in the parameters of the PBPK model accounted for most of the variability in the LI data. LI variability was slightly under predicted when the LI was close to its control level (e.g., measured: control, 0.55 ± 0.26, 30 ppm for 2 h/day, 1.5 ± 1.23; simulated: 1.10 ± 1.12). For exposures that led to larger increases in LI (e.g., LIs from 2.82 to 19.92), Monte Carlo-predicted PK variability accounted for all of the variability in measured LI. Under prediction of LI variability for small changes in LI may reflect the implementation of the Monte Carlo model or uncertainties associated with experimental measurement of LI. While contribution from variability in the PD cannot be ruled out, this result suggests that PK variability is a major determinant of variability in LI data from chloroform-exposed female B6C3F1 mice. We plan to extend these analyses to male mice and to male and female rats with the goal of better understanding the role of PK variability in variability of LI data. This understanding will guide the eventual scale-up of the PBPK/PD model to humans.

1760 A TRIAL OF TOXICOGENOMIC ANALYSIS OF HUMAN UMBILICAL CORDS FOR DEVELOPING A NEW RISK ASSESSMENT METHOD OF FETAL EXPOSURE TO MULTIPLE CHEMICALS.

M. Komiyama[1, 2] and C. Mori[1, 3]. *[1]Bioenvironmental Medicine, Chiba University, Chiba, Japan, [2]Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Japan and [3]Core Research for Evolutional Sciences. and Technol. (CREST), Japan Sciences. and Technol. Corporation (JST), Kawaguchi, Japan.*

It has been shown that human fetuses are exposed to multiple chemicals including endocrine disruptors. Since there is anxiety that these multiple chemical exposures may cause delayed long-term effects, it is necessary to establish a new evaluation method of health risk derived from exposure to multiple chemicals during fetal period. We are attempting to apply toxicogenomic analysis of human umbilical cords to the future risk assessment. In this study, we analyzed relationship between concentrations of persistent chemicals and gene expression patterns in umbilical cords of 9 Japanese newborns. In the concentration of the chemicals, significant correlations were observed between total polychlorinated biphenyls and many of other chemicals. Gene expression in each umbilical cord was examined by cDNA microarrays, using cultured human umbilical vein endothelial cells as reference. Expression profiles of the umbilical cords were analyzed by principal components analysis and hierarchical cluster analysis, and compared with chemical concentration profiles. The expression profiles of highly exposed umbilical cords were different from those of umbilical cords with lower exposure levels. However, there was an exceptional case. In the exceptional umbilical cord, total concentration of the chemicals was lowest, but its gene expression profile was quite similar to those of the umbilical cords with higher total chemical concentration levels. These results suggest that gene expression profile of umbilical cord can be used for evaluation of exposure levels at fetal period. Moreover, it might be useful to detect potential high risk group, because both of actually higher exposure level and genetically higher susceptibility of an individual to multiple chemicals might be regarded as higher health risk to the individual.

1761 APPLICATION OF A PBPK MODEL TO AID IN UNDERSTANDING THE RELATIVE POTENCIES (REPS) OF DIOXIN-LIKE CHEMICALS.

L. S. Birnbaum[1], C. Emond[2] and M. J. DeVito[1]. *[1]ORD/NHEERL/ETD, USEPA, Research Triangle Park, NC and [2]NRC, NAS, Washington, DC.*

The TEF methodology is a relative potency scheme used in risk assessments for dioxin-like chemicals to derive the TCDD equivalents of a mixture. TEF values were assigned to chemicals by an expert panel based on a review of all available data. A criticism of this method is the large range, over 1000-fold, in the REPs for some dioxin-like congeners. One reason for this variability could be experimental design. The aim of this study is to apply a PBPK model to examine the impact of study designs used to estimate REPs. A PBPK model developed for TCDD in rats was used to simulate experimental studies of model dioxin-like chemicals. CYP1A2 induction is by described an Ah receptor-mediated mechanism. Model outputs for CYP1A2 induction were used to estimate REPs. Acute and subchronic exposure conditions were simulated. In the acute simulations, the model was run for up to 14 days post exposure. Subchronic exposures were simulated for 90 days. In order to simulate a variety of dioxin-like chemicals, the AhR affinity was varied (10-1 to 10-4 nmol/ml) and the elimination rate (Kel) was increased by 10 and 20 fold compared to the TCDD. Dose response simulations were run and ED50s for predicted CYP1A2 induction were estimated for each simulation. The REP was calculated as the ratio of the ED50s for model chemical vs. TCDD simulations. When only the AhR affinity is varied, study design had no impact on the estimate of the REP. Study design significantly affected the estimate of the REP when Kel was varied. The REP varied by 100 fold when Kel was increased 20-fold depending on the study design. The present study demonstrates the utility of PBPK models when applied to the TEF methodology. If sufficient data on AhR binding and pharmacokinetics in humans were available for a particular congener, it would be possible to use these models to estimate human TEF values. (This abstract does not represent USEPA policy. Funded by in part by a cooperative agreement with NRC and EPA (CR 828790)).

1762 DERIVATION OF A RANGE OF INTERIM INHALATION CANCER SLOPE FACTORS FOR TCE USING PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING.

L. Yost[1, 3], J. F. Greene[1, 2], S. M. Hays[1, 5], M. Kelsh[1, 4] and P. Sheehan[1, 2]. *[1]Exponent, Menlo Park, CA, [2]Health Risk, Exponent, Oakland, CA, [3]Health Risk, Exponent, Bellevue, WA, [4]Health, Exponent, Menlo Park, CA and [5]Health Risk, Exponent, Boulder, CO.*

Widespread use of trichloroethene (TCE) as a solvent and its relative stability has resulted in it being one of the most frequently detected groundwater contaminants in the United States. Although extensively studied, derivation of a cancer slope factor (CSF) representative of potential human health risk remains controversial and the recent EPA re-assessment of TCE did not identify an inhalation CSF. Both animal and human data are suggestive of a threshold for cancer. However, the absence of a consensus on the mechanism(s) of action complicates implementation of a threshold approach. Animal models demonstrate high species, strain, sex, and route of exposure specificity, with carcinogenicity, if it occurs at all, typically seen following cellular damage. The epidemiological studies of TCE inhalation and carcinogenicity in workers are largely negative. For those few studies reporting significant increases in cancer, there are substantial study design problems and a lack of quan-

titative information on worker exposures. This paper performs a weight-of-evidence analysis of the epidemiological and animal studies to identify the most appropriate studies, endpoints, and approaches to dose-response modeling. The most appropriate inhalation data for derivation of a CSF were the mouse liver tumor data reported by Maltoni (1988). A range of inhalation CSFs was derived based on inhaled doses of TCE, as well as several internal dose measures, derived using a physiologically based pharmacokinetic model (Clewell et al. 2000). The upper end of the resulting estimates is similar to the interim value of 0.007 (mg/kg-day)-1 established by the California Office of Environmental Health Hazard Assessment and the range provides a reasonable basis for risk management decisions. Future studies will help to refine these estimates.

1763 ISSUES IN THE VALIDATION OF PBPK MODELS FOR RISK ASSESSMENT: AN EXAMPLE WITH PERCHLOROETHYLENE.

J. Kester[2], R. Gentry[1] and H. Clewell[1]. [1]*ENVIRON Health Sciences Institute, Ruston, LA and* [2]*ENVIRON Health Sciences Institute, St. Louis, MO.*

There has been growing use of physiologically based pharmacokinetic (PBPK) models to perform the dosimetry for human health toxicity and risk assessments. One of the more problematic aspects of the application of a particular PBPK model in such cases is the determination of whether the model has been adequately validated to provide confidence in the extrapolations performed with it. A number of PBPK models have been developed for perchloroethylene (PCE), differing primarily in the parameters estimated for metabolism. All of the models provide reasonably accurate simulations of some kinetic data for PCE in mice and humans, and could therefore be considered, to some extent, validated. However, extrapolation of animal-based cancer potency estimates for PCE to humans is critically dependent on the prediction of human metabolism at low environmental exposures. Recent data (Volkel et al. 1998) on the urinary excretion of TCA, the major metabolite of PCE, at relatively low exposure concentrations (10 to 40 ppm), made it possible to compare the high- to low-dose extrapolation capability of the various published models. We found that the model of Gearhart et al. (1993), which is the only model to include a description of TCA kinetics, gave the closest predictions of the urinary excretion observed in the low-concentration exposures (within a factor of 2). Other models overestimated metabolite excretion in this study by 5- to 15-fold. Cancer risk estimates for PCE based on liver tumors in mice in the NTP study were estimated with the Gearhart et al. (1993) model, using lifetime average daily amount metabolized per volume liver as the dose metric. Assuming pharmacodynamic equivalence of mice and humans, the resulting inhalation risk estimate for lifetime exposure to PERC at one microgram per cubic meter was 0.38 per million. The corresponding risk for oral exposure at one milligram per kilogram per day was 0.00293.

1764 PREDICTION OF BIOLOGIC PARTITION COEFFICIENTS AND BINDING AFFINITIES USING STRUCTURE ACTIVITY RELATIONSHIPS (SAR) MODELS.

M. Mumtaz[1], H. A. El-Masri[1], D. Hawkins[2], D. Mills[3] and S. Basak[3]. [1]*Computational Toxicology Laboratory/Division of Toxicology, ATSDR, Atlanta, GA,* [2]*School of Statistics, University of Minnesota, Minneapolis, MN and* [3]*Natural Resources Research Institute, University of Minnesota Duluth, Duluth, MN.*

For some contaminants, toxicological data are usually not sufficient to evaluate toxic effects and conduct health risk assessments. In such cases, until appropriate data become available, ATSDR and other federal agencies often recommend the use of surrogate values obtained from computational tools such as structure activity relationship (SAR) techniques and physiologically based pharmacokinetic (PBPK) modeling. In an ongoing effort to develop alternative toxicity assessment methods, we have applied SAR to compute: 1) tissue:air partition coefficients, including fat:air, liver:air, and muscle:air, for a group of 46 low molecular weight volatile organic compounds (VOCs); 2) blood:air partition coefficients for a set of 39 VOCs; and 3) aryl hydrocarbon (*Ah*) receptor binding affinity for a set of 34 dibenzofurans using SAR calculations based on theoretical molecular descriptors. These descriptors consisted of four classes based on increased level of complexity and computational demand such as topostructural (TS), topochemical (TC), geometrical (3D) and quantum chemical (QC). Experimental vs. predicted blood:air partition coefficients utilizing only the TC descriptors for some common contaminants were as follows: benzene, 2.88 vs. 2.50; chloroform, 3.04 vs. 3.32; n-hexane, 0.83 vs. 1.28; and toluene, 2.89 vs. 3.38. The results indicate that the cross-validated variance coefficients (R^2_{cv}) values when using TS, TC, and 3D descriptors are -0.05, 0.94, and 0.07, respectively. The R^2_{cv} for TS+TC+3D combined is 0.94 which shows no improvement over the TC descriptors alone. In conclusion, (1) structure based models using simple descriptors adequately predict toxicological characteristics of some environmental contaminants, and (2) development of extensive or complex descriptors and models might not enhance this capability.

1765 ANALYSIS OF AN INTERACTION THRESHOLD IN DRUG/CHEMICAL MIXTURES ALONG A FIXED-RATIO RAY.

A. Hamm[1], C. Gennings[1, 2], H. Carter[1, 2] and R. Carchman[2]. [1]*Biostatistics, Virginia Commonwealth University, Richmond, VA and* [2]*Solveritas, LLC, Richmond, VA.*

Human exposure to drug/chemical mixtures can result from therapeutic interventions or environmental sources. Of interest is the interaction that may occur among the components of these mixtures. Since interaction can be dose-dependent, it is important to determine exposure levels to either exploit the benefits of the interaction in a therapeutic application or to avoid the effect of the interaction in the case of an environmental risk assessment. As a result of the dose-dependent nature of interactions, it follows that there are regions of additivity and regions of interaction with an unknown boundary separating the two regions. As the number of mixture components increases, the experimental effort required to support the estimation of the boundary becomes impractical. Fixed-ratio ray designs can be used to detect interactions among the components of the mixture. Along each fixed-ratio ray, the interaction threshold boundary is a point on the ray. We developed dose-response models for fixed-ratio ray designs that allow the estimation of the location of the interaction threshold for each mixture ray. Methods were developed to analyze the effect of selected subsets of mixture components on interactions and the location of the interaction threshold for each ray. These methods are illustrated with a neurotoxicity study (endpoint: motor activity) of a mixture of five pesticides (acephate, diazinon, chlorpyrifos, malathion, and dimethoate). Results indicate that on the 'full' five-pesticide ray, there was evidence of the existence of an interaction across the ray. However, when malathion was removed and the remaining four pesticides occurred at the same relative proportions on a 'reduced' ray, an interaction threshold of about 8 mg/kg was estimated, indicating additivity below and synergy above this dose. (The project described was partially supported by a grant (#T32-ES07334-01A1) from the NIEHS, NIH)

1766 D-OPTIMAL EXPERIMENTAL DESIGNS TO TEST FOR DEPARTURE FROM ADDITIVITY IN A FIXED-RATIO RAY MIXTURE.

T. Coffey[1], L. Stork[1], C. Gennings[1], W. H. Carter[1], J. E. Simmons[2] and D. W. Herr[2]. [1]*Biostatistics, Virginia Commonwealth University, Richmond, VA and* [2]*ORD/NHEERL, USEPA, Research Triangle Park, NC.*

Risk assessors are becoming increasingly aware of the importance of assessing interactions between chemicals in a mixture. Most traditional designs for evaluating interactions are prohibitive when the number of chemicals in the mixture is large. However, evaluation of interactions with many chemicals is becoming easier through recent advances in statistically-based experimental design. Using a fixed-ratio mixture ray with chemicals in relevant proportions results in an economical design that allows estimation of additivity and interaction for a mixture of interest. This methodology is easily extended to a mixture with a large number of chemicals. Using this methodology, optimal experimental conditions can be chosen that increase the power of detecting a departure from additivity. Although these designs are well known for linear models, optimal designs for nonlinear threshold models have had few applications in toxicology. A D-optimal criterion that minimizes the variances of the parameters of a nonlinear threshold model was used to create an optimal design for a fixed-ratio mixture ray. For a fixed sample size, this design selects the experimental doses and number of observations per dose level that result in minimum variance of the model parameters and increased power to detect departures from additivity. A related design that utilizes a D-optimality criterion is also presented that results in minimum variance when some mixture data are available but additional doses are desired. An optimal design for both scenarios is illustrated using data collected on a 2:1 ratio (chlorpyrifos:carbaryl) mixture experiment. For this example, the optimal designs for the nonlinear threshold model depend on prior specification of a dose threshold. *Supported by T32 ES07334-01A1 (NIEHS, NIH) and CR-828-11401 (USEPA). This is an abstract of a proposed presentation and does not necessarily reflect EPA policy or the official views of the NIEHS, NIH.*

1767 ANALYSIS OF AN INTERACTION THRESHOLD IN A MIXTURE OF DRUGS AND/OR CHEMICALS.

H. Carter[1, 2], A. Hamm[1], C. Gennings[1, 2] and R. Carchman[2]. [1]*Biostatistics, Virginia Commonwealth University, Richmond, VA and* [2]*Solveritas, LLC, Richmond, VA.*

In mixtures toxicology, risk assessors often make a default assumption that additivity is present at lower concentrations of mixture exposures and that interactions occur at higher mixture concentrations. This assumption is consistent with the concept of an interaction threshold, i.e., the boundary between the region of additivity and the region of interaction. We have developed methodology that permits the estimation of the boundary that defines these regions. The methodology is illustrated

with a two-component mixture of ethanol and chloral hydrate in a study of righting reflex in mice. Using our methods, a region of synergy is found in the low ethanol and high chloral hydrate dose combination region; departure from additivity was not found in the low dose region of chloral hydrate over all levels of ethanol. Results obtained from the application of our methodology to a smaller, independent dataset were confirmed by the results obtained in the much larger study conducted by Gessner and Cabana (1970; Journal of Pharmacology and Experimental Therapeutics 156, 602-5) for the ED50 contour of the mixture. Our methodology allows for the interaction threshold boundary to range over response levels. In the analysis of the chloral hydrate/ethanol data, our approach suggests evidence of an interaction at response rates as low as the 5% in the high chloral hydrate and low ethanol dose combination region. At and below the 1% level of response there was no indication of departure from additivity. Theoretically, our approach is not limited by the number of components in the mixture. The project described was partially supported by a grant (#T32 ES07334-01A1) from the NIEHS, NIH.

1768 TESTING FOR DEPARTURES FROM ADDITIVITY FOR A 2:1 MIXTURE OF CHLORPYRIFOS AND CARBARYL ON CHOLINESTERASE ACTIVITY IN BRAIN, PLASMA, AND RED BLOOD CELLS OF LONG EVANS RATS.

L. Stork[1], T. Coffey[1], C. Gennings[1], W. Carter[1], J. Simmons[2] and D. Herr[2]. *[1]Biostatistics, Virginia Commonwealth University, Richmond, VA and [2]ORD/NHEERL, USEPA, Research Triangle Park, NC.*

Detecting and characterizing interactions among chemicals is an important environmental issue. This study was conducted to test for the existence of a significant departure from additivity for a mixture of two cholinesterase (ChE)-inhibiting pesticides: chlorpyrifos (CPF), an organophosphate; and carbaryl (CARB), a carbamate. The effects of CPF and CARB on ChE activity in the brain, plasma, and red blood cells of male Long Evans rats were evaluated using single chemical dose-response curves (CPF: 0, 3, 10, 20, 30 and 50 mg/kg; CARB: 0, 10, 30, 50, 100 and 175 mg/kg; po; 5 rats per group) and a 2:1 (CPF:CARB) mixing ratio (total dose: 0, 28 and 35 mg/kg; 6 rats per group) evaluated at the time of near maximal ChE inhibition by the mixture (4 h after dosing). Nonlinear additivity models estimated from the single chemical data were fit for each outcome. Nonlinear mixture models estimated from the mixture data were compared to that predicted using the additivity model along the 2:1 mixture ray. A significant difference between the mixture and additivity models is considered evidence of interaction. A significant dose-response effect was detected for the single chemical and mixture data. Using a likelihood ratio test, departure from additivity was not detected for any of the three endpoints. While the additivity model was estimated from data in the active range of the dose-response curves, the mixture data included only two doses above control, which were generally near optimal effect. Hence, generalizing these conclusions to the low-dose region of the mixture ray should be made with caution. For further investigation, the existing data are useful in determining an optimal design associated with maximum power for detecting interactions. Supported by T32 ES07334-01A1 (NIEHS, NIH) and CR-828-11401 (USEPA). This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1769 RISK ASSESSMENT FOR MALE REPRODUCTIVE TOXICANTS.

I. Mangelsdorf[1], J. Buschmann[1] and B. Orthen[2]. *[1]Chemical Risk Assessment, Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany and [2]Federal Institute for Occupational Safety and Health, Dortmund, Germany.* Sponsor: H. Muhle.

In recent years, publications related to an apparent decline in male sperm quality and concerns with the potential effects of environmental estrogens have led to an increased awareness of potential effects on male fertility. The majority of the information concerning reproductive toxicants comes from animal reproduction studies. However, there is concern that mating trials are too insensitive to assess effects on spermatogenesis and epididymal functioning because of the vast excess of spermatozoa ejaculated in rodents but not in humans. A literature review was conducted, to assess the sensitivity of different endpoints of male reproductive toxicity in laboratory animals and their predictivity for the human situation. Of the endpoints indicating reproductive toxicity in animal studies, fertility was indeed a very insensitive parameter. The most sensitive endpoint was histopathology of the testes. Using refined histopathology, effects could be detected as early as after four weeks treatment. Other sensitive endpoints were the weights of reproductive organs, including accessory glands, i.e., testis, epididymis, prostate, and of the seminal vesicle, as well as sperm count, sperm morphology and sperm motility. Sperm motility was found to be in some cases more sensitive than histopathology. In most cases, not only one but several endpoints were affected at one dose level. Humans were generally not more susceptible to reproductive toxicants than laboratory animals. In conclusion, with respect to hazard identification, it is possible to detect adverse effects on male reproduction in a standard subacute study with concentrations that produce significant general toxicity. If effects indicating male reproductive toxicity are found, for the risk assessment the NOAEL has to be determined by testing specific sensitive parameters within a subacute or preferably subchronic toxicity study. Mating of the animals would then be only be needed to detect influences on mating behavior.

1770 APPROACHES FOR CONVERTING ADULT DOSE TO CHILDREN OF VARIOUS AGE GROUPS: RELEVANCE FOR THE RISK ASSESSMENT OF ENVIRONMENTAL CHEMICALS.

D. Gohore Bi and K. Krishnan. *Occupational and Environmental Health, Universite de Montreal, Montreal, QC, Canada.*

Several approaches for converting the adult dose of pharmaceutical products for children of different ages are found in the clinical and biomedical literature. These are potentially useful in the context of health risk assessment, for conducting adult to child extrapolation of external dose of chemicals in the environment. The aim of this work was to analyse and compare various approaches available in the biomedical field for converting oral dose (mg/kg/d) of non-volatile chemicals from adults (A) to children of various age groups (newborn (N), infant (I), preschool (P) and highschool (H)). After a systematic literature review, the following methods were identified: Young's rule (I), Clark's rule (II), Fried's rule (III), body surface adjustment (IV) and pharmacokinetic approaches (V). Method I, based on age of children, yielded an adult/child dose conversion factor (A/C) of 8.73, 2.75, 1.3, 0.99 and 1.06, respectively, for the N, I, P and H groups. Method II, based on body weight, yielded an A/C of 1 for all age groups, since the dose in terms of mg/kg/d was identical in all groups. Method III, recommended only for application to N and I groups based on an empirical formula, yielded A/C of 9 and 3, respectively. Method IV, facilitating dose calculations based on body surface differences, yielded A/C of 0.41, 0.49, 0.55, 0.75 respectively for N, I, P and H groups. Method V, which represents either steady-state algorithms or compartmental models, yielded varying A/C factors as a function of the age-dependent clearance and volume of distrbution, and these factors appear to be, in several cases, lower than those given by the above empirical approaches (particularly Methods I-III). This work represents an initial attempt to evaluate the usefulness of existing methods for conducting adult-children conversion of exposure doses of environmental chemicals for risk assessment purposes. Overall, the use of pharmacokinetic models should be sought instead of the empirical formulae for conducting adult-children dose extrapolations.

1771 BENCHMARK DOSE MODELING OF MERCURY-INDUCED ACUTE RENAL FAILURE IN SPRAGUE-DAWLEY RATS WITH RENAL INSUFFICIENCY COMPARED TO HEALTHY CONTROLS.

R. Brown, E. F. Madden, M. E. Stratmeyer and P. L. Goering. *CDRH, USFDA, Rockville, MD.*

Experimentally induced renal insufficiency (RI) increases the sensitivity of rodents to the adverse effects of a subsequently administered nephrotoxic agent. Since patients with renal insufficiency are at increased risk of developing acute renal failure (ARF) following exposure to nephrotoxic compounds, compared to healthy individuals, the use of animal models of renal insufficiency improves the clinical relevance of toxicity test results used for the risk assessment of nephrotoxic compounds released from medical device materials. Benchmark dose modeling was used to quantify the magnitude of the increased sensitivity in male Sprague-Dawley rats, compared to healthy controls, following iv injection of mercuric chloride (HgCl2). RI was induced by 3 daily sc injections of gentamicin (250 mg/kg). HgCl2 (0.025 to 0.5 mg/kg) was administered on Day 4. Healthy control animals received sc saline x 3 days followed by HgCl2 on Day 4. Blood was collected for analysis in anesthetized rats 24 hours after HgCl2 injection. The dose-response relationship is based on the number of animals in ARF, defined as 2x the mean value of BUN in healthy, non-mercury exposed animals. BMD50[control]/BMDL50[RI] ratios for mercury-induced ARF were in the range of 3-14, with only one dose ratio > 10 (for the BMD50[control]/BMDL50[RI] ratio derived from results obtained with the log-logistic dose-response model). Assuming a similar increased sensitivity exists for patients with RI exposed to mercury or other nephrotoxic agents, compared to healthy persons, these results suggest that the default uncertainty factor of 10 used in noncancer risk assessment to account for interindividual variability in a population response to a given dose of a compound is adequately protective in most cases.

1772 ESTIMATION OF THE MAGNITUDE OF THE PHARMACOKINETIC COMPONENT OF THE INTERINDIVIDUAL VARIABILITY FACTOR USING A PROBABILITY BOUNDS APPROACH.

A. Nong and K. Krishnan. *Occupational and Environmental Health, Universite de Montreal, Montreal, QC, Canada.*

The pharmacokinetic (PK) component of the interindividual variability factor (IVF) used in the derivation of reference concentrations (RfCs) of systemically-acting volatile organic chemicals (VOCs) has been estimated using Monte Carlo approaches and PBPK models. Since the RfC derivation considers continuous lifetime human exposure to VOCs in the environment, algorithms to compute steady-state blood concentrations (SS-BC) can be used to derive the IVF-PK. In this context, probability bounds (P-bounds) approach is also potentially useful for computing an interval of a probability distribution value of SS-BC from knowledge of population distribution of input parameters. The objective of this study was therefore to estimate IVF-PK using the P-bounds approach along with an algorithm for calculating SS-BC of VOCs. The existing steady-state algorithm, derived from physiological pharmacokinetic models, was re-written such that SS-BC could be related, without any interdependence, to the following input parameters: pulmonary ventilation scaled to body weight (Qpc), ventilation/perfusion ratio (VP), hepatic blood flow as a fraction of cardiac output (Qlc), intrinsic metabolic clearance (Cl) and blood:air partition coefficient (Pb). The IVF was calculated from the P-bounds of SS-BC corresponding to the 50th and 95th percentiles. Following the specification of population distributions of parameters (Qpc: normal, VP: lognormal, Qlc: normal, Cl: lognormal, Pb: normal) in RAMAS Risk Calc® software version 3.0 (Applied Biomathematics, Setauket, NY), the P-bounds estimates of SS-BC of toluene (TOL), styrene (STY), 1-4-dioxane (DIO), and chloroform (CHM) were obtained for low level exposures (1 ppm). The IVF intervals based on these results for TOL, STY, DIO and CHM were 1.6-1.9, 2.0-2.6, 1.8-2.3, and 1.4-1.6, respectively. The use of P-bounds method along with steady-state algorithms, as done in this study for the first time, is a scientifically-sound and practical way of computing IVF for systemically-acting VOCs.

1773 LACK OF SUBCHRONIC TOXICITY OF TRICHLOROETHYLENE ADMINISTERED VIA AQUEOUS GAVAGE TO MICE.

P. S. Palkar[1], S. S. Anand[1], M. M. Mumtaz[2] and H. M. Mehendale[1]. *[1]Department of Toxicology, University of Louisiana, Monroe, Monroe, LA and [2]ATSDR, Atlanta, GA.*

The present work is a part of an ongoing project to study potential subchronic interactive toxicity of common water contaminants such as trichloroethylene (TCE), perchloroethylene, chloroform and trichloroacetic acid. The specific objective of this work was to examine the effects of TCE alone. Male Swiss Webster mice received TCE (200, 400, 500 mg/kg/d chosen from preliminary acute and subchronic lethality studies) in an aqueous emulsion of Alkamul EL-620 (0.3 ml/mouse) by oral gavage for 30 days (d). A single administration of 2500 and 1500 mg TCE/kg resulted in 100 and 80% mortality, respectively. Repeated administration of 750 mg TCE/kg led to 100% lethality between 3 to 5 d whereas, repeated administration of 600 mg TCE/kg led to 20% mortality between 5 to 7 d in the absence of any significant liver or kidney injury, suggesting that deaths might be due to extrahepatic and extrarenal effects, possibly by CNS toxicity. The dose range of 200 to 500 mg TCE/kg for 30 d did not cause any mortality. Liver injury was measured by ALT on 7, 15, 21, 30 d, and at 24, 48, 72 h after the last exposure (30 d) and by histopathology (H&E - stained liver sections) on 30 d. Liver tissue repair was assessed by [^{3}H]-thymidine incorporation into hepatonuclear DNA and by PCNA at 0 and 30 d. BUN was measured to assess kidney injury on 7, 15, 21, 30 d, and at 24, 48, 72 h after the last exposure (30 d). ALT or BUN levels were not significantly increased over the time course except for marginal increase in ALT on 21 d and at 48 h after the last exposure. Histopathology and PCNA findings were consistent with the biochemical end points. Hepatic microsomal CYP2E1 protein measured by Western blotting on 0 and 30 d was unaltered by TCE in the present study. Hepatic glutathione measured on 0 and 30 d was unchanged by TCE. These biochemical findings are consistent with lack of hepatic damage after subchronic exposure to TCE. The effects of TCE in combination with other water contaminants remain to be explored (supported by ATSDR U61/ATD 681482).

1774 OCCUPATIONAL EXPOSURE TO AIRBORNE QUATERNARY AMMONIUM CHLORIDE DISINFECTANTS: MARGIN OF EXPOSURE ANALYSIS.

J. D. Hamilton, S. S. Willems and D. A. Daggett. *Global Product Safety, JohnsonDiversey, Inc., Sturtevant, WI.*

Institutional and Industrial (I&I) disinfectants are generally sold as concentrated liquids that require controlled dilution with water prior to use. The USEPA has traditionally required respiratory protective equipment for quaternary ammonium chloride (quat) disinfectant concentrates and use solutions based on the outcome of acute inhalation toxicity studies. To determine whether respiratory protection is warranted, an inhalation exposure assessment was conducted by JohnsonDiversey based on an established Unit Exposure (UE) method to characterize exposure to the quats n-alkyl dimethyl benzyl ammonium chloride and didecyl dimethyl ammonium chloride. The UE method was derived from analysis of inhalation exposure monitoring data collected from biocide application studies. The UE method calculates a dose in mg/kg/day based on the concentration of active ingredient in the product and the application method. Using a worse-case scenario assumption that the disinfectant is applied as a concentrate (i.e., not diluted 1:256), inhalation exposure to quat according to the UE method was estimated to be 3.4 x 10^{-6} mg/kg/day. A Margin of Exposure (MOE) approach was used to investigate the potential risk associated with the UE estimate. MOE values of 100 or above are typically associated with no unreasonable risk for adverse effects. Using the lowest EPA-accepted NOAEL of 3 mg/kg/day for the quat active ingredient, the MOE value calculated here was 8 x 10^5. To further explore potential exposures to quat during use of an I&I disinfectant, a chamber exposure assessment was conducted by spraying 389.7 grams of undiluted disinfectant into a 10.8 m^3 test chamber with the ventilation turned off. Airborne quat was not detected in the study (with a limit of detection of 2 mg/m^3). It was concluded that exposure to quat concentrates presents no known unreasonable risk for adverse effects by inhalation, and respiratory protection during use is not warranted.

1775 ASSESSMENT OF RESPIRATORY ENDPOINTS RESULTING FROM NICKEL EXPOSURE: DERIVATION OF MINIMAL RISK LEVELS.

H. Abadin[1], L. Ingerman[2], S. Wilbur[1] and M. Fay[1]. *[1]Agency for Toxic Substances and Disease Registry, Atlanta, GA and [2]Environmental Science Center, Syracuse Research Corp, Syracuse, NY.* Sponsor: P. McGinnis.

The respiratory tract is the most sensitive target of nickel toxicity following inhalation exposure. This is supported by a number of short- and longer-term studies in rats and mice. A series of National Toxicology Program (NTP) reports identified no-adverse-effect levels (NOAELs) in rats exposed to nickel sulfate, nickel subsulfide, and nickel oxide. Of the three compounds, nickel sulfate was the most toxic, likely due to its solubility. The Agency for Toxic Substances and Disease Registry (ATSDR) released a toxicological profile for nickel in 1997 that included minimal risk levels (MRLs) derived using the rat data from the NTP study. An update to the 1997 profile was developed and released in October 2003. As a result, the database for nickel was updated and the MRLs for nickel were re-examined. The re-assessment utilized the same NTP study that identified NOAELs of 0.06 mg/m^3 and 0.03 mg/m^3 in rats exposed for 13 weeks and 2 years, respectively. The key findings at higher doses were chronic lung inflammation, atrophy of the olfactory epithelium, and fibrosis. Minimal alveolar macrophage hyperplasia was observed at all doses, but was not considered adverse at the lower doses. When lung effects consisted only of the alveolar macrophage hyperplasia, the incidence and severity was not significantly different from controls. Thus, this effect was not believed to compromise lung function at those exposure levels, but was considered part of the normal physiologic response to inhaled particles. The resulting intermediate- and chronic-duration MRLs were 0.2 ug/m^3 and 0.09 ug/m^3, respectively.

1776 DOSE-RESPONSE ANALYSIS OF COMBINED DATA FROM CLINICAL TRIALS: A CASE STUDY OF DATA ANALYSIS FOR SYSTEMIC CONTACT DERMATITIS IN A SENSITIZED POPULATION.

Q. Zhao[1], L. Haber[1] and A. Bathija[2]. *[1]TERA, Cincinnati, OH and [2]USEPA, Washington, DC.*

Human data are always of the greatest value in risk assessment; however, we often do not have the luxury of a rich human data set suitable for dose response analysis. For data from experimental clinical studies, it is often difficult to establish a clear NOAEL based on the individual studies, due to the small size of the groups tested, the wide variability among individuals, and limited doses tested in each experiment. Here, we present a dose-response analysis of data from multiple clinical studies on systemic contact dermatitis in sensitized subjects who were challenged with oral soluble nickel doses, by using a meta analysis technique to control these shortcomings. The data from a total of 12 studies with a total sample size of 300 subjects were used in the meta analysis, and the combined data provide responses over a wide dose range (0.45 ~ 5.6 mg). Our analysis estimated that the ED05 (the dose estimated to cause an extra risk of 5%) and the ED10 in the sensitized population are 0.559 mg and 0.914 mg, respectively; the corresponding daily doses are 8.0 x 10-3 and 1.3 x 10-2 mg/kg-day, respectively, assuming a 70 kg body weight. By using this technique, we are able to provide a dose-response analysis for data which normally would not have been considered suitable for dose-response assessment. The opinions expressed are those of the authors and do not reflect USEPA policy.

1777 THE ATSDR CHRONIC ORAL MINIMAL RISK LEVEL (MRL) FOR FLUORIDE.

L. Ingerman[1], C. Tylenda[2] and D. Jones[2]. *[1]Environmental Science Center, Syracuse Research Corp, Saratoga Springs, NY and [2]ATSDR, Atlanta, GA.*

ATSDR is currently updating its Toxicological Profile for Fluorine, Hydrogen Fluoride, and Fluorides. Numerous municipalities have implemented fluoridation programs to decrease the prevalence of dental caries in children. The US Department of Health and Human Services has recommended a drinking water fluoride concentration of 0.7-1.2 ppm. A number of community-based studies have examined potential health effects associated with this fluoride concentration, but study results have been variable. The primary target of fluoride toxicity following chronic oral exposure is the skeletal system. ATSDR has derived a chronic-duration oral MRL for fluorides based on adverse bone effects. A chronic-duration MRL is an estimate of daily exposure to a substance that is likely to be without appreciable risk of adverse effects over a period of 1 year or more. A number of epidemiology studies of populations exposed to 1 ppm fluoride (0.03 mg/kg/day) have found increases, decreases, or no effect on hip fracture rates among older women. Studies evaluating exposure to higher doses have consistently found significant increases in the risk of nonvertebral fractures, particularly hip fractures. The MRL derivation was based on a study of older residents of 6 communities in China exposed to differing levels of fluoride in drinking water (Li et al. 2001). This study considered other potential sources of fluoride and used communities with similar calcium intakes. A significant increase in the prevalence of hip fractures was observed in the community exposed to 4.32-7.97 ppm (average total fluoride intake of 0.25 mg/kg/day), but not in the community exposed to 2.62-3.56 ppm (0.15 mg/kg/day); the community with a fluoride level of 1.0-1.06 ppm (0.055 mg/kg/day) was used as the comparison group. In accordance with established procedure, the NOAEL of 0.15 mg/kg/day was divided by an uncertainty factor of 3 to account for human variability to derive the MRL of 0.05 mg/kg/day.

1778 UPDATING THE FREE CYANIDE RFD.

L. T. Haber[1], B. R. Stern[2], Q. Zhao[1], J. Strawson[1] and N. Chiu[3]. *[1]TERA, Cincinnati, OH, [2]B.R. Stern Associates, Alexandria, VA and [3]USEPA, Washington, DC.*

Cyanide is used in a number of industrial processes, including mining, metallurgy, manufacturing, and photography, because of its ability to form stable complexes with a range of metals. These multiple forms of cyanide have significant differences in toxicity due to their different ability to liberate the CN- moiety. Some cyanide complexes have little or no bioavailability. Cyanide or cyanogenic compounds (ones that are metabolized to cyanide) are found in many foods; smoking can also be a significant source of cyanide. The previous free cyanide RfD was developed in 1985, based on thyroid and nervous system effects at a LOAEL of 30 mg CN/kg-day, and a NOAEL in a separate study of 10.9 mg/kg-day. This RfD has been updated in light of new studies. In particular, a subchronic study conducted with sodium cyanide in drinking water evaluated a range of endpoints at daily doses up to 12.5 mg/kg-day in rats and 28.8 mg CN/kg-day in mice (NTP, 1993). This study found no histopathological evidence of damage to the thyroid or nervous system, although thyroid hormone levels were not evaluated. However, there were several dose-related decreases in male reproductive parameters. Decreased caudal epididymis weight was not supported by a decrease in spermatozoa count, but decreased testis weight at the high dose in rats was supported by decreased spermatid count. BMDLs based on different toxic effect endpoints were derived. The RfD was derived including uncertainty factors for interspecies differences and to protect sensitive populations. In addition, an endpoint-by-endpoint analysis was used for determination of the appropriate composite uncertainty factor for subchronic to chronic extrapolation and for database uncertainties, taking into account the lack of progression seen with various endpoints and for related compounds, and the potential for neurodevelopmental effects secondary to thyroid effects. Disclaimer: The opinions and conclusions expressed in this abstract are those of the authors and do not necessarily reflect those of the USEPA.

1779 DEVELOPMENT OF PROVISIONAL TOXICITY VALUES FOR COBALT. H CHOUDHURY. NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT, OFFICE OF RESEARCH AND DEVELOPMENT, USEPA, CINCINNATI, OH.

H. Choudhury. *ORD, NCEA, USEPA, Cincinnati, OH.*

USEPA, in the absence of toxicity values on the IRIS, develops peer reviewed provisional toxicity values in support of superfund site assessments. Cobalt is known to have a nutritional function as a vital component of vitamin B12. However, there is no evidence that the intake of cobalt is limiting in the human diet which ranges from 1E-2 to 1E-4mg/kg-day, and therefore no Recommended Daily Allowance has been set by the National Research Council. Human data have been suggestive of a possible association between exposure to cobalt and respiratory tumors. Chronic studies have demonstrated the carcinogenic potential of inhaled cobalt in rats and mice, with alveolar and bronchial tumors being the most prevalent. The precise mechanism of cobalt-induced carcinogenicity has not been fully determined. Evidence indicates that cobalt is capable of eliciting genotoxic effects. The most likely mechanism for the carcinogenic effects of cobalt involves the generation of cobalt-induced oxidative stress. In accordance with the proposed Guidelines for Carcinogenic Risk Assessment (USEPA, January 2001), cobalt is likely to be carcinogenic to humans, and under the 1986 Guidelines, cobalt is classified as group B1. Using the default linear dose-response model, a provisional inhalation unit risk of 2.8E-3 (ug Co/cu.m)-1 was derived for cobalt, based on increased incidence of respiratory tumors in B6C3F1 mice. Data were inadequate to derive an oral slope factor for cobalt. Using polycythemia in humans as a sensitive indicator of cobalt deficiency, an RfD of 2E-2 mg/kg-day has been derived by applying an uncertainty factor of 10 to the LOAEL, for hematological effects in anemic patients (factor of 3- for LOAEL and 3- for database deficiency). (This abstract does not necessarily reflect USEPA Policy.)

1780 THE IMPORTANCE OF CONSIDERATION OF MODE OF ACTION DATA IN NON-CANCER RISK ASSESSMENT: THE CASE OF ETHYLENE CYANOHYDRIN.

M. Osier and M. Odin. *Syracuse Research Corporation, Syracuse, NY.*

Consideration of mode of action is of critical importance in the evaluation of the toxic effects of a chemical. While an emphasis on mode of action is evident in recent thinking regarding cancer risk assessment, it is also important when considering noncancer effects. Derivation of a provisional RfD for ethylene cyanohydrin provides an excellent practical example of the role that understanding of mode of action can have in noncancer risk assessment. No chronic oral studies of ethylene cyanohydrin toxicity have been reported. Of the three subchronic studies, one examined only a single endpoint and another reported only group and failed to report statistical analysis of the data; as such, neither study provides a sufficiently detailed evaluation of ethylene cyanohydrin toxicity to allow for the derivation of an RfD. The third study evaluated a broad spectrum of endpoints in a single species. The study only reported slight, statistically significant changes in the absolute weights of the heart and brain. A lack of more clearly toxic effects would generally be of concern when attempting to derive an RfD, perhaps even resulting in no number being derived. However, consideration of a proposed mechanism of action for ethylene cyanohydrin, in situ generation of cyanide, identified the brain and the heart, both of which are very sensitive to changes in cellular energy status, as potentially sensitive targets of ethylene cyanohydrin toxicity. With this consideration in mind, subtle, statistically significant changes in organ weights of these sensitive targets were considered to be toxicologically relevant, and NOAEL and LOAEL values were identified from the study and used in the derivation of an RfD. (This abstract does not necessarily reflect EPA policy).

1781 LACK OF EFFECTS OF 1439 MHZ ELECTROMAGNETIC NEAR FIELD EXPOSURE ON THE BLOOD-BRAIN BARRIER IN IMMATURE AND YOUNG RATS.

M. Kuribayashi[1,4], J. Wang[2], O. Fujiwara[2], Y. Doi[3], K. Nabae[3], S. Tamano[3], T. Ogiso[1], M. Asamoto[1] and T. Shirai[1]. *[1]Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan, [2]Electrical and Computer Engineering, Nagoya Institute of Technology, Nagoya, Japan, [3]Daiyu-kai Institute of Medical Science, Nagoya, Japan and [4]Ono Pharmaceutical Co. Ltd., Safety Research, Fukui, Japan.*

The use of cellular phones has been spreading rapidly all over the world and the potential risk of electromagnetic near-fields (EMFs) from their antennae to human health, especially with respect to disturbance of the blood-brain barrier (BBB), is a source of concern. In the present study, we performed experiments to explore whether EMF exposure causes any damage to the BBB in rats with ages equivalent to the time of BBB genesis (4-weeks-old) and the teenage (10-weeks-old) period. For this purpose, alteration of BBB-related genes, such as those encoding p-glycoprotein, aquaporin-4 and claudin-5, was assessed by immunohistochemistry and quantitative RT-PCR in the brain. First, we verified that expression of the 3 genes is influenced by 1, 3-dinitrobenzene (DNB), a chemical reported to destroy the BBB, and then we performed an EMF exposure experiment with 0, 2.0 and 6.0 W/kg brain-average specific absorption rates at 1.5 GHz for 90 min/day for 1 or 2 weeks. Vascular permeability was also investigated using dye-transfer technique by FITC-

dextran and albumin immunohistochemistry. As a result, expression of the 3 genes was found to be clearly decreased after administration of DNB as a positive control, when compared with the control values. However, there were no biologically meaningful differences with the EMF at any exposure level at either age. Vascular permeability, monitored with reference to transfer of FITC-dextran, was not affected by EMF exposure. Furthermore, albumin leakage from capillary blood vessels to brain parenchymal tissue was detected in the brains of rats receiving DNB, although there was no change with EMF exposure. Thus, these findings suggest that local exposure to 1.5 GHz EMF exerts no adverse effects on the BBB in immature and young rats.

1782 RESULTS OF SCREENING TESTS ON RE-REFINED MINERAL OILS DERIVED FROM USED OILS.

W. Dalbey, R. McKee, S. Hong, M. Amoruso and J. Freeman. *ExxonMobil Biomedical Sciences, Inc., Annandale, NJ.*

Used or waste lubricating oils (such as used engine oils) are sometimes re-refined to produce base stocks for use in finished lubricant products or other applications. Engine oils, particularly from passenger cars, accumulate potentially carcinogenic polycyclic aromatic compounds (PACs) during use. The PACs may not be completely removed during re-refining. The presence of biologically active PACs was assessed in re-refined oils using method ASTM E 1687-95, a modification of the Ames test. A mutagenicity index (MI, or the slope of a linear dose-response) less than 1.0 in this assay indicates a high probability that the oil would be noncarcinogenic in skin-painting bioassays. Among 130 samples of re-refined oils tested in this assay since 1985, >20% had MI >1.0, compared to no MI >0.8 among 53 currently marketed virgin base oils as recently reported by major producers. Directionally, used oils re-refined by hydrotreating had lower MI values than the non-hydrotreated samples, but both had MI values higher than virgin base oils. Data from analysis for PACs are limited. Analyses of specific PACs have typically not been useful as a screening method, but directionally PACs among re-refined oils with MI <1.0 were higher than in virgin base oils. Skin-painting tests in mice were performed on 5 re-refined oils, all having MI values <1.0 and relatively low PAC content. None caused skin tumors in the skin-painting assay. Contamination with PCBs and metals also continues to be an area of concern based on historical and recent data. Due in part to potential health effects, the use in finished lubricants of re-refined oils without severe processing and appropriate feed quality controls should generally be discouraged.

1783 AN EMPIRICAL EVALUATION OF THE CANCER POTENCY OF DIOXIN TOXIC EQUIVALENTS (TEQs) IN FOUR PCB MIXTURES.

R. E. Keenan[1], J. M. Hamblen[1], J. B. Silkworth[2], M. N. Gray[1], P. O. Gwinn[1] and S. B. Hamilton[3]. *[1]AMEC Earth & Environmental, Portland, ME, [2]Global Research Center, General Electric Co., Niskayuna, NY and [3]Environmental Programs, General Electric Co., Fairfield, CT.*

In 1996, the USEPA evaluated PCB cancer risks by reviewing both human epidemiology and animal bioassay data on PCB mixtures and developed a range of cancer slope factors (CSFs), based on animal models, for use in risk assessments of PCBs. In 2000, USEPA released its revised draft Dioxin Reassessment, which uses a toxic equivalency method to relate the cancer potency of the designated *dioxin-like* chemicals, including *dioxin-like* PCB congeners, to the cancer potency of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and to estimate the potency of mixtures containing those congeners. The current study was conducted to evaluate the application of the toxic equivalency approach in estimating the cancer potency of PCBs. The dose-response assessment was based on the results of 2-year cancer bioassays in which female Sprague-Dawley (SD) rats were fed TCDD or one of four PCB mixtures (Aroclors 1016, 1242, 1254, and 1260) with varying degrees of chlorination. Using EPA benchmark dose software, a rodent cancer slope factor (CSF) was determined for TCDD and for the dioxin toxic equivalent (TEQ) component of each PCB mixture. A basic precept of the toxic equivalency method is that a given dose of TEQ has equal biological potency irrespective of the chemical mixture from whence it came. If the tenet is correct, then every CSF determined in this way should be approximately equal to each other and to that of TCDD. CSFs of 9600, 220000, 16000, 8900, and 50000 per mg/kg-d were generated for TCDD and for TEQ components in Aroclors 1016, 1242, 1254, and 1260, respectively. The current study revealed that the TEQ CSFs varied by a factor of 24 across the range of PCB mixtures tested and, therefore, were not equal to the potency of TCDD. These results indicate that there is considerable uncertainty associated with the use of the dioxin toxic equivalency method for characterizing the cancer potency of PCB mixtures.

1784 ESTIMATION OF A NO OBSERVED EFFECT LEVEL FOR 2, 4-DIANIMOTOLUENE, A GENOTOXIC LIVER CARCINOGEN, IN A 16-WEEK FEEDING STUDY USING MALE F344 RATS.

N. IAMI[1], T. ICHIHARA[1], H. YOSHINO[1], H. WANIBUCHI[2], K. MORIMURA[2], A. HAGIWARA[1] and S. FUKUSHIMA[2]. *[1]Daiyu-kai Institute of Medical Science, Ichinomiya, Japan and [2]Department of Pathology, Osaka City University Medical School, Osaka, Japan.*

The carcinogenic potential of 2, 4-diaminotoluene(2, 4-DAT) at very low doses was evaluated in male F344 rats. Groups of 90 or 30 animals (21-day old at commencement) were given 0 (as control), 0.001, 0.01, 0.1, 1, 10, 100 and 300 ppm 2, 4-DAT in their diet for 16 weeks. Marked retardation of body weight increase was found in 300 ppm 2, 4-DAT group, but not in animals fed a diet containing less than 100 ppm. No adverse effects were found in terms of survival and clinical sign. Administration of a dietary concentration of 300 ppm to rats caused prominent elevation of liver weight, serum glucose (GLU), total cholesterol (T-CHO), phospholipid (PL) and triglyceride(TG), and quantitative values of gluthathioneS-transferase placental from (GST-P) positive hepatocytic foci, which are preneoplastic lesion. Significant increased liver weight was also noted in rats fed 100 ppm 2, 4-DAT, but not with doses of 10 ppm 2, 4-DAT or less. Quantitative values for GST-P positive foci were significantly increased in 100 ppm groups, but at10 ppm and lower there were no differences from controls. Thus, a no-observed effect level of 2, 4-DAT was estimated to be 10 ppm (0.654 mg/kg/day) under the present experimental conditions.

1785 BENCHMARK DOSE ANALYSIS OF PAPILLOMA INDUCTION IN THE SKIN OF TG.AC MICE FOLLOWING ORAL OR DERMAL EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN.

M. E. Wyde[1], A. Braen[2], M. R. Hejtmancik[3], J. D. Johnson[3], A. F. Fuciarelli[4], M. K. Vallant[1], J. R. Bucher[1] and N. J. Walker[1]. *[1]NIEHS, Research Triangle Park, NC, [2]Hoffman-La Roche, Nutley, NJ, [3]Battelle, Columbus, OH and [4]Battelle, Richland, WA.*

The Tg.AC transgenic mouse develops epidermal papillomas in response to some non-genotoxic and complete carcinogens. 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD), a multi-site rodent carcinogen, induces the formation of papillomas in the skin of Tg.AC mice. To evaluate the dose-response relationship for TCDD-induced papilloma formation by the dermal and oral routes of administration, female Tg.AC mice were exposed dermally to TCDD at 0, 5, 17, 36, 76, 121, 166, 355 or 760 ng/kg or by gavage to 0, 105, 450, or 1250 ng/kg for 26 weeks. The number of mice with papillomas increased in a dose-dependent manner by both routes. The skin concentration of TCDD was higher with increased doses of TCDD, demonstrating a linear relationship between administered dose and internal dose at the target site. Papilloma incidence was used to calculate Benchmark Dose for an effective dose resulting in a 10% increase over background (ED10). Papilloma response was lower in mice receiving TCDD by gavage than by dermal exposure. The estimated ED10 for increased papilloma incidence by TCDD using average daily doses and an empirical log-logistic model was 333 ng/kg by gavage and 3.90 ng/kg by dermal exposure. The slope of the dose-response curve for the incidence of papillomas with respect to skin concentrations of TCDD was steeper and the incidence of papillomas higher in mice receiving TCDD by dermal exposure than by gavage. The estimated ED10 for papilloma incidence with respect to mean skin TCDD concentrations at the end of the study was 320 pg/g by dermal exposure and 2710 pg/g by gavage using log-probit and log-logistic models, respectively. These studies demonstrate that papilloma formation in Tg.AC mice is more responsive to TCDD by dermal exposure than by gavage. These differences likely reflect pharmacokinetic and pharmacodynamic differences in the delivery of TCDD to the skin *via* the two routes of exposure.

1786 COMPARISON OF RISK ASSESSMENT METHODS FOR POLYCYCLIC AROMATIC HYDROCARBON (PAH) MIXTURES IN AIR.

H. I. Williams[1], A. Wiman[1], C. A. Williams[1], C. Stineman[2] and T. Husain[3]. *[1]Ecology and Environment, Inc., Tallahassee, FL, [2]Ecology and Environment, Inc., Chicago, IL and [3]Faculty of Engineering, Memorial University of Newfoundland, St. Johns, NF, Canada.*

Polycyclic aromatic hydrocarbons (PAH) are common environmental contaminants formed by incomplete combustion of organic material. Generally, when PAH mixtures in the environment are analyzed, the concentrations of 16 individual PAH are measured, 7 of which are considered probable human carcinogens by the USEPA (EPA). PAH mixtures usually include hundreds of compounds, not just the 16 that are typically measured. EPA currently uses the relative potency approach to

assess the carcinogenic risk posed by PAH mixtures. The concentrations of the 7 carcinogenic PAH are measured, their cancer potencies are estimated relative to benzo(a)pyrene (BaP), and then risk is estimated by summing the risks from individual PAH. However, the World Health Organization (WHO) and other regulatory agencies recommend a different approach, termed the surrogate method, which is intended to estimate risk posed by an entire PAH mixture. The surrogate method assumes that the potency of a PAH mixture is proportional to its BaP content. The cancer potency of a mixture is expressed in terms of its BaP content, using BaP as a surrogate for the entire mixture. The risk posed by an unknown PAH mixture is estimated by comparing its BaP content to that of well studied PAH mixtures with established cancer potencies. This study compares the risk estimates obtained by applying these two methods to real world data. Concentrations of 16 individual PAH were measured in respirable particulate matter (PM 10) samples collected at two locations downwind from the 1991 Kuwaiti oil fires over the duration of the fires. For this mixture, the surrogate method gave risk estimates 6 to 7 times higher than the relative potency method currently used by the EPA. Reasons for the differences in results using the two methods are discussed.

1787 UPDATED CANCER RISK ASSESSMENT OF ACRYLONITRILE.

C. R. Kirman[1], M. Gargas[2], J. Klaunig[3], J. Collins[4], T. Starr[5], D. Strother[6] and R. Deskin[7]. *[1]The Sapphire Group, Beachwood, OH, [2]The Sapphire Group, Dayton, OH, [3]Indiana University, Indianapolis, IN, [4]Dow Chemical Company, Midland, MI, [5]TBS Associates, Raleigh, NC, [6]BP Chemical, Arlington, VA and [7]Cytec Industries, West Paterson, NJ.*

The database to support cancer risk assessment for acrylonitrile (AN) has been greatly improved over the past 20 years and includes robust epidemiological data, bioassay data from two rodent species, animal and human PBPK models, and extensive mechanistic data. A cancer dose-response assessment was conducted for AN using this information. Consideration of the cancer weight of evidence for AN is complicated by the fact that the toxicological and epidemiological databases provide conflicting evidence. Animal studies indicate that AN is carcinogenic in multiple tissues in rats and mice. In workers, however, a number of large epidemiology studies, including those with well-documented exposure information, indicate no evidence of a causal association between AN exposure and cancer mortality of any type. The epidemiology data cover the full range of past worker exposures, including early industry exposures (1940s-1978) that are far higher than occur today and that approach levels found to be tumorigenic in animals. A dose-response assessment was prepared based upon the most sensitive animal tumor site and species (rat brain). Recent mechanistic studies for AN-induced brain tumors in rats strongly implicate a role for oxidative stress due to an oxidative metabolite of AN (2-cyanoethylene oxide or CEO, cyanide), but do not rule out a potential role for direct genotoxicity for CEO. A PBPK model was used to support the dose-response assessment. Internal doses were predicted for 12 data sets which were pooled together to provide a characterization of the dose-response relationship for brain tumors in the rat. Based upon the weight of evidence provided by the mechanistic studies the cancer dose-response assessment for AN-induced brain tumors in the rat was performed using nonlinear methods. The range of well studied human exposures was employed to add further perspective about appropriate margins of exposure.

1788 NEW DATA AND GUIDELINES SUPPORT A REVISED CANCER RISK ASSESSMENT FOR ACRYLAMIDE.

P. R. McClure[1], D. W. Wohlers[1] and R. S. DeWoskin[2]. *[1]Environmental Science Center, Syracuse Research Corporation, Syracuse, NY and [2]National Center for Environmental Assessment, USEPA, Research Triangle Park, NC.*

Since EPA last assessed acrylamide carcinogenicity in 1988, new cancer and mode-of-action (MOA) data and new EPA guidelines (1999) for cancer risk assessment have been released. The 1988 assessment classified acrylamide as a probable human carcinogen, and derived an oral cancer slope factor based on an analysis of the results from a 2-year drinking water study in F344 rats with pooled incidence data for tumors in CNS, oral cavity, and thyroid tumors (both sexes); mammary gland and uterine tumors (females); and tunica vaginalis mesotheliomas (TVMs; males). This study had abnormally high CNS and oral cavity tumors in control males, and possible complications from a viral infection. A new cancer bioassay was subsequently conducted with a larger number of rats (increased statistical power), and dose spacings that improved the characterization of the dose-response relationship. The newer study results corroborated the acrylamide-induced thyroid tumors, mammary gland tumors, and TVMs, but not the previously observed CNS, uterine, and oral tumors, indicating that these tumors that were observed in the earlier study may not have been directly related to acrylamide exposure. Based on the more recent tumor data, and in accordance with the 1999 cancer guidelines, benchmark doses were calculated as points of departure for the low-dose cancer risk assessment (e.g., LED10, the lower bound on the 10 percent effect level), and a linear extrapolation to the origin was derived based on a genotoxic MOA. Although a nongenotoxic MOA (and potential nonlinear dose-response relationships) has been hypothesized for acrylamide-induced thyroid tumors, mammary gland tumors, and TVMs in rats, the supporting evidence is weak for some of the tumor types, and there is good evidence for acrylamide's genotoxic potential. In summary, application of the revised cancer guidelines and incorporation of new data provide a more scientifically defensible cancer slope factor. [This abstract does not necessarily reflect EPA policy.]

1789 AVAILABLE TOXICITY DATA ON ALTERNATIVE DRY CLEANING CHEMICALS.

J. F. Collins, A. G. Salmon, J. D. Budroe, M. A. Marty and G. V. Alexeeff. *CalEPA/OEHHA, Oakland, CA.*

Concerns about the carcinogenicity of perchloroethylene (perc; tetrachloroethylene) led to the introduction of alternatives in dry cleaning. The South Coast Air Quality Management District has proposed its elimination in dry cleaning by 2020; the State legislature is considering elimination by 2013. Available toxicity data on four proposed alternatives, already in use in some areas, have been reviewed. (1) Cyclic siloxanes. Preliminary results with decamethylcyclopentasiloxane (D5) indicated that inhalation of 160 ppm for 12 or 24 months by female rats led to endometrial adenocarcinomas. Octamethylcyclotetrasiloxane (D4) is estrogenic. D5 (log Kow = 5.2) and D4 are bioconcentrated. (2) In a two-year inhalation study by NTP, propylene glycol tert-butyl ether resulted in neoplastic and non-neoplastic lesions of the liver in male and female mice. Rats showed hyaline degeneration of olfactory epithelium in both sexes, and basophilic liver foci and kidney lesions in males. (3) 1-bromopropane (BP), a non-ozone depleting chemical, is a neurotoxicant and a reproductive toxicant. The NTP is currently conducting a two year inhalation bioassay of BP. (4) Other alternatives are based on mineral spirits, which can cause neurotoxicity. One formulation also contains 5% fluorocarbons, mainly ethyl perfluoroisobutyl ether and ethyl perfluorobutyl ether. Additional information is needed on the environmental persistence of these chemicals. All these alternatives have some toxicity, which, in addition to environmental fate data, must be considered before they are widely substituted for perc. Health protective values for chronic inhalation exposure have been estimated as 700 $\mu g/m^3$ for D5, 200 $\mu g/m^3$ for propylene glycol tert-butyl ether, 1.1 mg/m^3 for 1-propyl bromide, 1.2 mg/m^3 for the C10 and C11 isoparaffins in mineral spirits, and 19 mg/m^3 for HFE-7200. In addition an inhalation unit risk for cancer of 5.2×10^{-7} $(\mu g/m^3)^{-1}$ was estimated for propylene glycol tert-butyl ether.

1790 RFD AND CANCER ASSESSMENT FOR DICHLOROACETIC ACID (DCA).

J. M. Donohue[1], H. Galal-Gorchev[1], K. B. Altschuler[2] and W. Brattin[3]. *[1]Health and Ecological Criteria Department, USEPA, Washington DC, DC, [2]ICF Consulting Inc., Fairfax, VA and [3]Syracuse Research Corp., Denver, CO.* Sponsor: E. Ohanian.

The Environmental Protection Agency released a Reference Dose (RfD) and quantitative cancer assessment on the Integrated Risk Information System (IRIS) for the disinfection by-product, DCA in September, 2003. The reference dose was derived from a study in male and female dogs given doses of 0, 12.5, 39.5, or 72 mg/kg/day in gelatin capsules for a 90-day period (Cicmanec et al., 1991). Changes in testicular histopathology, mild vacuolization of the cerebral and cerebellar myelinated nerves, and increases in liver weight were observed at the lowest dose tested and increased in severity with increasing doses. An uncertainty factor of 3, 000 was applied to the LOAEL to derive an RFD of 0.004 mg/kg/day. IRIS (1996) had previously categorized DCA as a probable human (B2) carcinogen but had not quantified the dose-response. The DCA IRIS summary was updated to provide the quantitative data for cancer and a weight-of-evidence determination that the data are sufficient to conclude that DCA is a likely human carcinogen that lacks a cohesive mode of action. Support for this conclusion is provided by: a number of independent studies reporting consistently positive results at roughly comparable doses; site concordance for tumor formation between two species; clear evidence of a dose-response relationship; and apparent development of tumors from more than one hepatic cell line. A recent drinking water study of the tumorigenicity of DCA in mice (De Angelo et al., 1999) provided the dose response information which was used to derive an oral cancer slope factor of 0.05 per mg/kg/day and a drinking water unit risk of 1.4 E-3/mg/L. [The opinions expressed represent those of the authors and do not necessarily reflect the opinions of the USEPA.]

1791 CHILDRENS HEALTH BENEFITS FROM REDUCTIONS IN CRITERIA AIR POLLUTION CONCENTRATIONS.

E. Wong[1], J. Gohlke[1], W. Griffith[1], S. Farrow[2] and E. M. Faustman[1,2]. *[1]Institute for Risk Analysis and Risk Communication, Department Environ Occup Health Sciences, University of Washington, Seattle, WA and [2]Engr Public Policy, Carnegie Mellon University, Pittsburgh, PA.*

Benefit-cost analyses of environmental legislations are increasingly required, and evaluations of controlling criteria air pollutants have used health risk assessment methodologies. Children are significantly impacted by improved air quality, yet key

environmental health policy analyses have not focused upon effects in children. A meta-analysis approach examined the hypothesis that child-specific health impacts, both in number of cases and economic benefits, due to the US Clean Air Act would be similar to health benefits for adults. For only five endpoints were estimates of both reductions in cases and economic benefit able to be calculated using risk assessment methodologies. For concentration reductions predicted to occur by 2010, we estimate 200 (90% CI: 50, 300) fewer expected cases of postneonatal mortality, 10, 000 (4000, 20 000) fewer asthma hospitalizations with estimated benefits of $20-46 million ($1990) and 40, 000 (10 000, 70 000) less emergency room visits with estimated benefits of $1.3-5.8 million in children 1-16 years, 20 (10, 20) million school absences avoided by children 6-11 with estimated benefits of $0.7-1.8 billion, and 10, 000 (-20 000, 70 000) fewer low birth weight infants with estimated benefits of $230 million. Inclusion of this limited child-specific data resulted in an additional $1-2 billion ($1990) to the $8 billion in health benefits estimated from decreased adult morbidity, and an additional $600 million compared to the $100 billion from decreased mortality. These estimates highlight the need for increased consideration of health effects in children including improved mechanistic information relating environment to health effects in children, additional life-stage information for susceptible populations, and improved health economics information for children. Funded by Center for the Study and Improvement of Regulation at Carnegie Mellon University/University. WA and EPA /NIEHS (EPA-R826886, NIEHS 1P01 ES09601).

1792 DRINKING WATER ECONOMIC ANALYSIS (EA): THE ARSENIC CESSATION LAG MODEL.

A. E. Schulman[1], M. Manibusan[3], C. O. Abernathy[2], M. Messner[3], S. Qian[4] and D. Gaylor[4]. *[1]OECA/OC, USEPA, Washington, DC, [2]OW/OST, USEPA, Washington, DC, [3]OW/OGWDW, USEPA, Washington, DC and [4]Sciences International, Alexandria, VA.*

The 1996 Safe Drinking Water Act Amendments require an EA for all regulations. The EA examines treatment and analytical costs and public health benefits. Included in the public health benefits section is a cancer latency analysis to account for decreased future cancer risk after current exposure reduction. If public health benefits are discounted due to disease developmental latency, then credit should also be applied to acknowledge the proportional health benefits accrued from immediate exposure reduction. A mathematical model is required to describe the cessation lag, or in other words, how the attributable cancer case rate moves from its current assumed steady state toward its new lower limit. To that end, we used the available Arsenic data in an attempt to model a chemical-specific cessation lag. Due to the lack of sufficient data for Arsenic, we decided to use data on smoking from Hrubec and McLaughlin (1997) to model the rate at which the new limit is approached. Key assumptions in this comparison include: 1. the mode(s) of action is/are similar, and 2. the specific cessation lag for lung cancer due to smoking approximates that for all cancers associated with ingested arsenic exposure. This model approximates that at 2, 10 and 20 years post-exposure reduction of As exposures in drinking water from 50 ppb to 10 ppb, there is a corresponding 41%, 83% and 90% maximum reduction in lung cancer risk, respectively. This model can be applied to other exposure reduction situations when relative risks are known for individuals at both high (pre-regulation) and low (post-regulation) exposure conditions. [The opinions expressed in this abstract are those of the authors and not necessarily those of USEPA.]

1793 INFLUENCE OF THE NATIONAL RESEARCH COUNCIL (NRC) REPORT ARSENIC IN DRINKING WATER: 2001 UPDATE ON THE DERIVATION OF THE CANCER SLOPE FACTOR FOR INORGANIC ARSENIC.

T. McMahon[1], C. Abernathy[2], J. Chen[1] and I. S. Dooley[2]. *[1]OPP, USEPA, Washington D.C., DC and [2]OW, USEPA, Washington D.C., DC.*

Several EPA program offices are involved in regulation of inorganic arsenic due to its presence in the environment, e.g., water, and in various pesticides, including CCA (chromated copper arsenate), a widely used wood preservative. In 1996, USEPA charged the NRC with reviewing EPA's characterization of potential human risks from ingestion of inorganic arsenic in drinking water, the available data on carcinogenic and non-carcinogenic effects of ingested inorganic arsenic, the data on metabolism, kinetics, and mode(s) of action of inorganic arsenic, and research needs to fill data gaps. EPA applied many of the recommendations from the resulting 1999 NRC report in the January 2001 regulation for arsenic in drinking water. In March 2001, USEPA charged the NRC to review the EPA risk analysis with respect to model choice, exposure estimates, and population differences in light of new information from studies published since the 1999 NRC report The resulting 2001 report by the NRC made specific recommendations to EPA with respect to the assumptions and models used by EPA supporting the risk analysis for inorganic arsenic. As a result of the 2001 NRC report, the Office of Pesticide Programs (OPP) formed an intra-Agency workgroup charged with examining the NRC recommendations and their potential influence on regulation of inorganic arsenic by the various program offices. For OPP as well as for future updates to the EPA's Integrated Risk Information System (IRIS) for inorganic arsenic, the current recommendations of the NRC as published would increase the cancer potency factor for inorganic arsenic by a minimum of 3-fold from the current value of 1.5 E +0. The workgroup is examining the NRC recommendations for any further potential regulatory influence.

1794 EVIDENCE FROM EPIDEMIOLOGICAL AND MODE OF ACTION STUDIES SUPPORT A NONLINEAR DOSE-RESPONSE RELATIONSHIP FOR ARSENIC-INDUCED CARCINOGENESIS.

A. Schoen[1], B. Beck[1], R. Sharma[2] and E. Dube'[1]. *[1]Gradient Corporation, Cambridge, MA and [2]Arch Chemicals, Inc., Norwalk, CT.*

Recent risk assessments for arsenic conducted by both the USEPA (USEPA) and the National Research Council (NRC) have assumed a linear dose response for arsenic-induced carcinogenesis. In this presentation, we evaluate both epidemiological and mechanistic evidence and conclude that the dose-response for arsenic is likely to be nonlinear at low doses and unlikely to be of toxicological concern at levels commonly found in the US. While epidemiological studies of populations outside the US demonstrate that arsenic concentrations greater than several hundred mg/L are associated with cancer, studies in the US of populations exposed to elevated concentrations of arsenic in drinking water do not provide evidence of a dose-response relationship between arsenic and increased cancer. We reviewed several proposed mechanisms of arsenic-induced carcinogenesis, including generation of oxidative stress, perturbation of DNA methylation patterns, inhibition of DNA repair, and modulation of signal transduction pathway. All of these mechanisms are consistent with a nonlinear (specifically sublinear) dose response relationship for arsenic. It is probable that these mechanisms do not act in isolation, but overlap, and contribute to the complex nature of arsenic-related cancers. Arsenic's toxicity can also be modulated by nutritional factors. Diets adequate in methyl donor groups (i.e., choline or methionine), selenium, and antioxidants (as is typical of the US diet) can mitigate arsenic's toxicity. Based on a consideration of arsenic's plausible mechanisms, modulation by nutritional factors, and of evidence from epidemiological studies, we recommend the use of non-linear methods, either *via* biologically based modeling or use of a margin-of-exposure analysis, to characterize arsenic cancer risks.

1795 HORMESIS DATABASE.

R. R. Blain and E. J. Calabrese. *Environmental Health Sciences, University of Massachusetts, Amherst, MA.*

Until recent years, the concept of hormesis (i.e., low dose stimulation, high dose inhibition) has generally been overlooked by the scientific community. In an attempt to examine the occurrence and generalizability of the hormetic curve, two separate databases have been developed. The first is a relational retrieval database (i.e., Retrieval Hormesis Database). This database permits an evaluation of studies over numerous parameters, including the type and quality of the studies as well as the physical/chemical properties of the agents. The information obtained from the approximately 5500 dose-responses entered indicates the generalizability of the hormetic response across models (e.g., plant, animal, bacteria), *in vitro* and *in vivo* study designs, with many different chemicals, and across many different endpoints (e.g., growth, metabolism, immune response). While the database was originally designed to assess the hypothesis concerning whether hormesis was a demonstrable phenomenon that was reproducible, the database has not only provided a means to affirmatively address this question but to also permit significant insight into the nature of the dose response in the sub-NOAEL zone and its broad-based generalizability. The second database was designed specifically to address the frequency of hormesis in the toxicological literature (i.e., Hormesis-Frequency Database). This database demonstrated that 40% of the dose-responses that fit the rigorous entry criteria followed a hormetic response. Pro and cons of each database and the information they can provide will be part of the poster session

1796 AN EXPANDING WEB RESOURCE ON COMPARATIVE RISK INFORMATION: THE INTERNATIONAL TOXICITY ESTIMATES FOR RISK (ITER) DATABASE JOINS THE NATIONAL LIBRARY OF MEDICINE'S (NLM) TOXNET SYSTEM.

A. Wullenweber[1], P. Wexler[2] and M. Dourson[1]. *[1]TERA, Cincinnati, OH and [2]NLM, Bethesda, MD.*

Among the many limitations of using risk values to assess overall chemical risks is the over-reliance on data from one or another particular organization. Since 1996, Toxicology Excellence for Risk Assessment (TERA), through its ITER database, has

extended the horizon for risk assessors by presenting risk data from a variety of authoritative groups worldwide. Presented in tabular format for easy comparison, and enhanced with a synopsis explaining differences in data, ITER provides key risk data and/or cancer classifications from the Agency for Toxic Substances and Disease Registry, Health Canada, International Agency for Research on Cancer, National Institute of Public Health and the Environment (RIVM, The Netherlands), USEPA (EPA), and independent parties. Operational for nearly 20 years, the TOXNET system, a product of NLM, consolidates a variety of bibliographic, factual, nomenclature and other information related to potentially toxic chemicals and other agents. Among its files are the scientifically peer reviewed Hazardous Substances Data Bank (HSDB), TOXLINE, USEPA's Integrated Risk Information System (IRIS), the National Cancer Institute's Chemical Carcinogenesis Research Information System (CCRIS), and the ChemIDplus database. NLM supplements these with a variety of other online resources and tools in toxicology and environmental health, for the professional and public. The recent addition of ITER to TOXNET has benefited both and resulted in an enhanced risk resource. For example, as part of TOXNET, ITER can now be searched by chemical synonym and *via* free text. Multi-file searching allows a single query to be run against multiple toxicological databases, including ITER, and links to TOXLINE allow users to obtain current literature references and abstracts on ITER chemicals. Meanwhile, ITER has strengthened and broadened TOXNET's data in support of risk assessment by its incorporation of sound risk values from highly regarded national and international organizations.

1797 NATO WORKSHOP ON COMPARATIVE RISK ASSESSMENT AND ENVIRONMENTAL MANAGEMENT: SUMMARY AND CURRENT TRENDS.

I. Linkov. *ICF Consulting, Lexington, MA.*

Site contamination and disturbance by industrial development operations requires management decisions that weigh the benefits of remedial alternatives against the risks and disruptions associated with its implementation. In particular, a framework is needed that integrates risk assessment and engineering options, generates performance standards, compares options for risk reduction, communicates uncertainty, and effectively allows reiteration of the decision-making process. Comparative Risk Assessment (CRA) is emerging as a methodology with much promise in these areas; it can be applied to facilitate decision-making when various possible activities compete for limited resources. The CRA framework may be an especially valuable tool for prioritization of remediation efforts and for making choices among various environmental policies specific to oil industry operations. The goal of the North Atlantic Treaty Organization (NATO) workshop on CRA (October, 2002) was to review recently developed concepts and mechanics of CRA, assign them to a quantitative analytical framework, and use CRA to help decision-makers choose among various environmental policies. This talk summarizes presentations and discussions on the use of CRA to provide the scientific basis for selection environmentally sound and cost-efficient policies, strategies, and solutions to environmental challenges. This workshop was supported by the Society of Toxicology.

1798 SCREENING TOOLS FOR CONTEXT-BASED DECISION-MAKING.

I. Walls and . ILSI RSI Expert Panel. *Risk Science Institute, International Life Sciences Institute, Washington, DC.* Sponsor: P. Fenner-Crisp.

When predicting toxicity, ideally all relevant information is available on (a) the potentially toxic agent; (b) the host species of interest; (c) the exposure scenario; (d) the dose at which exposure occurs. Typically, complete information is not available, so a decision must be made using less-than-ideal data. Screening tools are developed for predicting toxicity of chemicals in the absence of a comprehensive dataset on the toxicology of a chemical. The ILSI Risk Science Institute established an Expert Panel to develop a framework for evaluating the utility and application of screening tools for decision-making. The framework includes: 1. Problem formulation. 2. Identify tools to address specific questions (e.g., literature, databases, research studies) 3. Develop a plan on how to proceed 4. List assumptions and caveats 5. Assess degree of confidence in plan to address questions 6. Make "Context-Based" decision 7. Evaluate Decision In problem formulation, an important concept is recognition that a very specific question should be asked, including the context (e.g., health issues, exposure and host factors) in which the question is posed. For example, one might ask, "We have found agent X in drinking water. Is it safe for the population to drink this water or should we recommend that they drink bottle water?" Once a specific question has been posed, the user can assess the tools available to address the question. The Expert Panel is identifying criteria for determining the appropriate screening tool. The choice of tool will depend on the question and the level of uncertainty that may be appropriate for the context. For example, it may be important to have a rapid result, or it may be important to have a high degree of confidence in your answer. The framework is iterative, in that users may refine the question as they proceed. Case studies are being developed to illustrate how the framework can be used effectively to address specific questions for cancer and non-cancer endpoints.

1799 COMPARATIVE APPROACHES TO PRIORITY SELECTION OF CHEMICALS FOR TOXICITY ASSESSMENT/REASSESSMENT: IMPLICATIONS FOR PUBLIC HEALTH PROTECTION.

B. R. Stern[1] and P. McGinnis[2]. [1]*BR Stern and Associates, Annandale, VA and* [2]*Syracuse Research Corporation, Philadelphia, PA.*

Only a small subset of the hundreds of thousands of chemicals in commerce, and their byproducts, have been evaluted for potential toxicity to humans and ecological organisms, raising concerns that public health is not adequately protected by regulatory agencies. This study compares and contrasts the approaches taken by state, federal, and international agencies to nominating and selecting chemicals for priority assessment/reassessment. Five US state agencies, four US federal agencies, three countries (Canada, Netherlands, Germany) and five international organizations (EU/ECB, IARC, IPCS, WHO, JEFCA) are compared with regard to (1)criteria utilized for chemical priority selection; (2)methodologies/processes used to perform the selection; (3)throughput; (4)transparency and stakeholder input; and (5)resource utilization. The results show that there is a continuum of prioritization schemes, from those that are health-based and incorporate only direct and indirect (surrogate) measures of public health impacts to those that include mainly surrogate measures of public health impact (eg, legislative mandates) and simple indicators of economic impact (eg, production volume). Methodologies range from those that are qualitative and subjective with minimal stratification ranking schemes, to those that are highly quantitative, with alogrithms for scoring and ranking. The degree of transparency and stakeholder input is also highly variable; some processes are informal and less transparent whereas others involve numerous levels of formal review and public comment. The more comprehensive the process, the greater the cost and time impacts. Interestingly, throughput is not greatly altered by the complexity of the process and thus public health is likely to be underprotected. Several recommendations are made to improve the process in the US and include harmonization of approaches among state and federal agencies, and use of external advisory panels to review data and and prioritize chemicals for review.

1800 STATUS OF HEALTH ENDPOINTS SUBMISSIONS IN THE USEPA HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM.

J. Tao, R. Hefter, R. Northrop, L. Scarano, M. Sonawane, A. Benson and D. Sawhney. *USEPA, Washington DC, DC.*

The USEPA HPV Challenge Program was initiated in 1998 to encourage the chemical industry to voluntarily submit basic hazard information on all US High Production Volume chemicals. In response, 334 chemical companies committed to provide the public with health and environmental effects information on 2, 165 HPVs. EPA's goal for the HPV program is to have at least one adequate study for each identified health effect endpoint in order to perform a screening level hazard assessment. To minimize the testing burden, USEPA encouraged manufacturers to group chemicals as a category where appropriate based on similar structures and/or toxicological and other properties. EPA had received approximately 245 submissions for 1, 125 chemicals (95 categories and 150 single chemicals) and 213 of EPA's responses have been posted on the EPA website. Of the reviewed submissions, 59 have at least one adequate study on all endpoints, and 102 have adequate data for one or more endpoints, but not for all. For sixty-two out of the 102 submissions, manufacturers proposed new testing for one or more endpoints, and proposed testing on all endpoints for 4 submission. For 5 submissions, USEPA agreed with manufacturers not to do any new testing because of the physicochemical properties of the chemicals, and in 11 other submissions, USEPA agreed that reduced testing was appropriate. EPA was unable to evaluate completely 31 other submissions, and in those cases either requested additional information or returned the submission to the submitter(s). Finally, no action was required on one submission because production has ceased. Once screening level data have been obtained, USEPA will prioritize the chemicals to determine whether more in-depth information is needed to adequately address potential hazards. The views expressed are those of the authors and do not represent EPA policy.

1801 USING ANIMAL AND HUMAN MODE OF ACTION INFORMATION IN ASSESSING HUMAN RISK: A FRAMEWORK FOR ANALYSIS.

P. A. Fenner-Crisp and . ILSI RSI Human Relevance Work Group. *Risk Science Institute, International Life Sciences Institute, Washington, DC.*

In late 2003, the International Life Sciences Institute's Risk Science Institute (ILSI RSI) published the product of a two-year effort on using animal and human mode of action (MOA) information to generate a framework for evaluating the human

relevance of non-genotoxic carcinogens. That report presents a case study analysis of six MOAs that led to development of a four-part Human Relevance Framework (HRF) for systematic and transparent analysis of MOA data and information. The HRF features a "concordance" analysis of MOA information from both animal and human sources, with a focus on determining the appropriate role for the animal MOA information in human risk assessment. With MOA information increasingly available for risk assessment purposes, the sponsors of this project, the USEPA and Health Canada, asked ILSI RSI to evaluate the utility and applicability of the new HRF for a broader set of endpoints, including mutagenicity as it relates to carcinogenicity and endpoints other than cancer. The RSI Working Group, including some participants from the original project, is testing the HRF with ten new case studies representing developmental effects, male reproductive effects, neurotoxic effects, and renal toxicity. The Workgroup also is examining the HRF's applicability to mutagenic carcinogens. The MOAs analyzed include chemically-induced alterations in thyroid homeostasis associated with hearing loss, disruption in brain cell replication associated with behavioral effects, and decreased fetal Leydig cell testosterone production associated with abnormal Wolffian duct system development.

1802 CATEGORY APPROACH IN THE USEPA HIGH PRODUCTION VOLUME CHALLENGE PROGRAM.

L. Scarano, R. Hefter, R. Northrop, M. Sonawane, J. Tao, A. Benson and D. Sawhney. *USEPA, Washington DC, DC.*

In 1998, the USEPA High Production Volume (HPV) Chemical Challenge Program began to develop a new approach to acquire basic hazard data on HPV chemicals. The Challenge Program encouraged the US chemical industry to voluntarily provide basic hazard data and make it publicly available. In response, 334 chemical companies committed to provide the public with health and environmental effects information on 2, 165 HPV chemicals. Traditionally, testing is done on individual chemicals. In the Challenge Program, USEPA encouraged manufacturers to consider testing groups of related chemicals using a category approach. Categories of chemicals have similar structures and/or properties. In general, principles of structure-activity relationships are considered by EPA to be appropriate for developing a category. In an ideal category, tests performed on one or more members can be used to read across to other, untested category members. This approach saves time, money and animals because complete testing of every chemical in an appropriate category may not be necessary. EPA received the first submission in the Challenge Program in April 2000. To date, about 90% of the chemicals (975 out of 1125) submitted in the Challenge Program are category members. The number of chemicals in a given category submission has ranged from 3 to over 150. Upon review of the submissions, USEPA believes some categories are more appropriate than others. The outcome of the proposed testing of category members is the ultimate test for whether a category proposal is reasonable. EPA and others' comments are posted on the EPA website and is now beginning to receive industry responses to comments and/or the results of testing for proposed category submissions. This presentation will discuss (1) the adequacy of completed categories to date; (2) using the test results for possible future risk assessment purposes; and (3) the future use of categories in other EPA programs. The views expressed are those of the authors and do not represent EPA policy.

1803 ESTIMATING SEVERITY FOR DEVELOPMENT OF THE CONTAMINANT CANDIDATE LIST (CCL).

A. M. Mahfouz[1], J. Donohue[1], N. Chiu[1], J. Du[1], O. Conerly[1], C. O. Abernathy[1], B. Ambika[1], S. Kueberuwa[1] and W. Mendez[2]. *[1]Office of Water, USEPA, Washington, DC and [2]ICF Consulting, Fairfax, VA.*

In compliance with the 1996 Amendment to the SDWA, OW developed a process to select contaminants in drinking water for possible future regulations. In March 1998, USEPA published the first Contaminant Candidates List (CCL) and asked the National Academy of Sciences (NAS) to peer review the process used in developing this list. The NAS's National Research Council (NRC), provided its recommendation in its 2001 publication entitled "Classifying Drinking Water Contaminants for Regulatory Consideration". At the present time EPA is testing the use of the process recommended by the NRC to develop future CCLs. Five attributes (potency, severity, prevalence, magnitude, persistence/mobility) were recommended by the NRC to select the CCL from a group of initially screened contaminants referred to as the Pre-CCL. The NRC report included a scoring scale for severity which served as a starting point for the EPA work. The NRC scoring protocol was refined through several cycles of testing against data on chemicals with potential to be on the Pre-CCL and then refining the severity scale based on the test results. The metric selected to provide the severity data was the critical effect associated with the parameter selected for scoring potency (RfD, LOAEL, etc). For one phase of testing the severity scoring protocol, the first 100 chemicals on IRIS were used as a data source for critical effects. The critical effects were scored against a nine point severity scale and binned by score. This permitted a detailed evaluation of the scoring scale. For some chemicals, the critical effect given in the IRIS database was found to be ambiguous, others had several critical effects listed and others had none. This required further refinement of the severity scoring protocol. Some issues are still under consideration in scoring severity; these include concerns about sensitive population, concerns about chemicals with extensive data gaps, and testing the severity scores in conjunction with the other attribute scores in several CCL prototype decision algorithms.

1804 ESTIMATING POTENCY FOR DEVELOPMENT OF THE CONTAMINANT CANDIDATE LIST (CCL).

G. Blumenthal[2], J. M. Donohue[1], O. Conerly[1], K. Sullivan[2], A. Mahfouz[1], J. Du[1], S. Kueberuwa[1], C. Abernathy[1], N. Chiu[1] and A. Bathija[1]. *[1]Health and Ecological Criteria Department, USEPA, Washington, DC and [2]ICF Consulting Inc., Fairfax, VA.*

The 1996 Safe Drinking Water Act requires EPA to develop a list of contaminants with potential for regulation on a five-year cycle, and to make regulatory determinations from each list three years after its publication. EPA requested assistance from the National Research Council (NRC) of the National Academy of Sciences in providing suggestions for a comprehensive, transparent process of selecting contaminants for inclusion on the CCL. The NRC recommendations were published in 2001. They provided a framework for the process in which a Pre-CCL is initially selected from the universe of contaminants of concern. The CCL is chosen from the Pre-CCL using a computerized decision algorithm and scored attributes for health effects (potency, severity) and occurrence (prevalence, magnitude, persistence and mobility). EPA has developed and tested an approach for scoring the non-cancer potencies of Pre-CCL chemicals using as input parameters several QSAR-generated or measured toxicological values [reference dose (RfD), No-Observed-Adverse-Effect-Level (NOAEL), Lowest-Observed-Adverse-Effect-Level (LOAEL), LD50]. The scoring for each parameter was calibrated based on data from a learning set of known drinking water contaminants. The distribution of the RfD, NOAEL, LOAEL, and LD50 values for the learning set chemicals was examined and found to be roughly log-normal. The log-based distribution was mapped onto a ten point scoring scale by equating a value of 5 on the scale to the modal value in the distribution resulting in an set of equations that can be used to score each type of potency input. Scores were fairly consistent across related inputs for individual chemicals. Agreement was weakest for the weakly toxic chemicals in the example data sets. [The opinions expressed represent those of the authors and do not necessarily reflect the opinions of the USEPA.]

1805 THE USEPA CONTAMINANT CANDIDATE LIST(CCL) DEVELOPMENT PROCESS.

O. D. Conerly[1], C. Abernathy[1], A. Bathija[1], N. Chiu[1], J. Donohue[1], J. Du[1], S. Kueberuwa[1], A. Mahfouz[1] and W. Mendez[2]. *[1]Office of Water, USEPA, Washington, DC and [2]ICF Consulting, Fairfax, VA.*

The Safe Drinking Water Act Amendment of 1996 requires the United States Environmental Protection Agency (USEPA) to develop a list of contaminants(CCL) every 5 years to assist in priority-setting efforts for the drinking water program. From each list, the Agency is required to make regulatory determinations for at least 5 contaminants 3 years after its publication. The first CCL (1998) was criticized by some stakeholders for not considering a wider range of chemical and microbial contaminants. Accordingly, USEPA requested assistance from the National Research Council (NRC) to develop a prioritization process that would consider all potential drinking water contaminants. In response to USEPA, NRC published a series of reports. The most recent and final report, Classifying Drinking Water Contaminants for Regulatory Consideration, recommends that the Agency develop the CCL using two steps. The process would begin with a universe of potential and known drinking water contaminants. Using screening and expert judgment, this universe would be reduced to a preliminary CCL (PCCL). The CCL would be selected from the PCCL using a prototype classification algorithm in conjunction with expert judgment. To evaluate the feasibility of the NRC suggestions, USEPA formed and has been actively working with an advisory group under the National Drinking Water Advisory Council (NDWAC). The workgroup consists of a diverse group of representatives from academia, water utilities, State governments and environmental interest groups. Recommendations from the working group are expected in early 2004. [The opinions expressed in this abstract are those of the authors and not necessarily those of USEPA.]

1806 A CRITICAL LOOK AT THE EU DRINKING WATER PARAMETRIC VALUE AND ITS USE AS AN INDICATOR OF HEALTH RISK.

E. R. Nestmann, L. A. Haighton, E. Cheng and R. A. Halford. *CANTOX HEALTH SCIENCE INTERNATIONAL, Mississauga, ON, Canada.*

The EU drinking water parametric value for pesticides of 0.1 ppb is based largely on the precautionary principle and technological considerations rather than toxicity or risk. Problems can arise when any pesticide present at levels above 0.1 ppb is misinterpreted as being a definitive health risk. Clauses in the EU directive stating: "in

the event of non-compliance..member states shall consider whether that non-compliance poses any risk to human health" and "consumers are notified except where the competent authorities consider the non-compliance with the parametric value to be tri*vial*" are often overlooked. For this risk assessment, WHO health based GVs were evaluated and compared to the arbitrary EU value for over 40 chemicals. The WHO values were higher than the EU value, by as much as 3000 times. The pesticides were prioritized on the basis of margin of difference between the WHO and EU value. Exposures from drinking water with pesticides at a level of 0.1 ppb (about 0.003 microgram/kg/day) were also compared to ADIs; exposures of 0.003 microgram/kg/day represent 0.003% of the ADI for bentazone. This assessment provides much needed perspective as to which pesticides would constitute a real health concern if the EU value is exceeded, and which would not. Another problem with the EU parametric value has been its misuse for beverages. Recently, a localized populace became outraged by claims from an NGO that their beverages contained pesticides. Although tests commissioned by their government indicated that the NGO results were inaccurate, the controversy continued. At the root of the conflict was the use of the EU parametric value as an indicator of danger. It is inappropriate to use the EU limit for beverages for a number of reasons such as technological difficulties in measuring pesticides at such a low level, differences in consumption, and use of ingredients, other than water, that may have individual pesticide tolerances. The recent controversy indicates that activists can initiate social unrest by such misapplication of the EU value.

1807 PRINCIPLES AND PRACTICES FOR DIRECT DOSING OF PRE-WEANING MAMMALS IN TOXICITY TESTING AND RESEARCH.

T. Zoctis and . ILSI RSI Working Group. *Risk Science Institute, International Life Sciences Institute, Washington, DC.*

Most standardized toxicology testing protocols recommend the use of adult animals to characterize hazards. However, children represent a unique segment of the population, for which adult animal models may not be predictive. Indirect exposure of a developing animal *via* maternal milk is assumed to occur in developmental toxicology studies that include a lactation phase, but it is seldom measured directly. Direct dosing is defined as the administration of a test substance directly to a pre-weaning mammal. The ILSI RSI working group developed a handbook on how to undertake direct dosing laboratory studies on pre-weaning mammals. When designing the protocol, several issues must be considered, including selection of the test species, the route of administration, dose levels and the timing of doses. Test species discussed included rodents, rabbits, canines, swine and non-human primates. Knowledge of the maturational status of the test species and information on critical windows of development are important in creating a valid study design. Most common routes of administration (e.g., oral, inhalation, injection) for direct dosing are possible with typical laboratory species; however, some adjustments may be necessary due to practical considerations. Care must be taken in the selection of dose levels for the pre-weaning mammal; it may be necessary to conduct a preliminary dose range finding study to determine appropriate dose levels. Information on pharmacokinetics in young animals versus adults and in the test species versus humans can be useful. The conduct of the study and the interpretation of the data might also be influenced by stress on the pre-weaning mammal that can result from interruption of normal maternal care, or from handling and dosing procedures. Ultimately, the success of the study will depend upon careful preparation, including thorough training of the technical staff.

1808 VALIDATION OF THE HUMAN EPIDERMIS MODEL SKINETHIC FOR SKIN CORROSION TESTING ACCORDING TO NEW OECD TEST GUIDELINE 431.

M. Liebsch[1], H. Kandarova[1], H. Spielmann[1], R. Guest[2], A. Whittingham[2], N. Warren[2], A. O. Gamer[3], T. Kaufmann[3], M. Remmele[3] and B. De Wever[4]. *[1]ZEBET, Federal Institute for Risk Assessment (BfR), Berlin, Germany, [2]Safepharm Laboratories, Derby, United Kingdom, [3]Toxikologie Z470, BASF AG, Ludwigshafen, Germany and [4]Skinethic Laboratories, Nice, France.* Sponsor: A. Goldberg.

Reconstituted human skin models are increasingly investigated for their usability in toxicological hazard identification/safety testing, because primary cells in an organotypic structure with a functional stratum corneum allow for *in vitro* test designs modeling bioavailability. Moreover, variability of results is lower than observed with human skin. However, skin models will only be accepted for industrial and regulatory use if they are at the same time predictive, reproducible and available. While promising predictivity and reproducibility have been shown in validation studies, availability has been a problem: commercially produced skin models went off the market, and with them the validated tests disappeared. The availability issue can be solved if studies prove that robust test protocols applied to various skin models reveal comparable results. When the EPISKIN skin corrosion test validated by ECVAM became unavailable (Fentem et al. Toxicol. *In Vitro* 12, 483-524, 1998) a "catch-up-validation" with the model EpiDerm revealed almost identical results (Liebsch et al. ATLA 28, 371-401, 2000). Therefore, the new OECD Test Guideline 431 "*In Vitro* Skin Corrosion: Human Skin Model Test" defines general and functional skin model conditions, including reference chemicals that need to be correctly classified before a new skin model is used in the context of TG 431. In the summer of 2003, ZEBET tested several chemicals from the ECVAM validation trial, applying the validated EpiDerm skin corrosion test protocol to the SkinEthic model and obtained identical results. In November/December 2003 a blind trial will be performed in which ZEBET, Safepharm and BASF will re-test the reference chemicals specified in OECD TG 431. Data on reproducibility between laboratories and over time, as well as predictions obtained will be presented.

1809 WELFARE-ENHANCED CAGE DESIGN FOR PRIMATE TOXICOLOGY STUDIES.

J. Hedley and S. Grainger. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: D. Everett.

Non-human primates have been utilised in the performance of regulatory toxicology studies for many years with one of the more common species of choice being the Cynomolgus Macaque (Macaca fascicularis). The current standards of care and welfare afforded to these animals reflect the increasing awareness of the necessity to address their social and environmental needs. It is now widely recognised that the provision of social and environmental stimuli in a caging environment, that supports and encourages the animals in demonstrating their natural behaviour patterns, has a positive effect on their health and well being. It can therefore be argued that such benefits to the animal will enhance the scientific integrity of any experimental work in which they are involved. In 2001 plans were drawn up for the design and construction of a purpose built primate facility, the principal function of which was to support the conduct of regulatory toxicology studies utilising the Cynomolgus Macaque. The facility would house primates in bespoke caging designed to satisfy the social and environmental needs of the animals, so far as reasonably practicable, whilst maintaining sufficient restrictions to facilitate the performance of routine daily experimental procedures. It was necessary that any caging would exceed guidance for the housing of non-human primates as specified within Home Office Code of Practice (Animals (Scientific Procedures) Act 1986), and satisfy draft recommendations for the revision of Appendix A of the European Convention (ETS 123). Over the following 18 months cage designs and prototypes were assessed and refined. Modifications were made where necessary and long-term trials were undertaken with large groups of animals. The result was a caging system that supports the social requirements of the animal, affords environmental stimuli in various forms and enhances the conduct of the experimental work for which the animals are intended.

1810 DO PRECLINICAL STUDIES IN PREADOLESCENT ANIMALS PREDICT CLINICAL TOXICITY?- LANSOPRAZOLE (A PROTON PUMP INHIBITOR) AS A CASE EXAMPLE-.

A. Youssef. *TAP Pharmaceutical Products Inc., Lake Forest, IL.*

The reference standard in treating adults with acid-related disorders, are increasingly being used in children despite limited pediatric safety and pharmacokinetics data. Proton pump inhibitors, similar to more than 60% of all pharmaceutical products, are metabolized by CYP3A4. The new FDA guidance on "Non Clinical Safety Evaluation of Pediatric Drug Products" expects the conduct of juvenile preclinical studies as a method to predict/evaluate the toxicity and pharmacokinetics prior to clinical studies. However, this guidance does not address clearly the difference between humans and animal models in the growth and development of the stomach as a target organ. Furthermore, the impact of the difference in ontogeny of CYP enzymes and their subtypes, between animals and humans, lakes emphasis. A case study using preadolescent animal models will be discussed. Discussion of some differences in the ontogeny and the subtypes of CYP between rats and humans which impacts the validity of the rat as a model was published (Youssef et al., 2003). The ontogeny of the enterochromaffin-like cells (target cells for proton pump inhibitors) in the stomach between rodents and humans will be discussed. Additional information from a 13 week study in the preadolescent dog model using lansorprazole will be provided. The learned lessons and recommendations based on using the dog and rat preadolescent models to predict the toxicity and pharmacokinetics in pediatrics, will be presented.

1811 ICCVAM PROCESS FOR NOMINATION AND SUBMISSION OF NEW, REVISED, AND ALTERNATIVE TEST METHODS.

L. M. Schechtman[1], W. S. Stokes[2], M. L. Wind[3], B. C. Blackard[2, 4] and R. R. Tice[2, 4]. *[1]NCTR, USFDA, Rockville, MD, [2]NICEATM, NIEHS, Research Triangle Park, NC, [3]DHS, CPSC, Bethesda, MD and [4]NICEATM, ILS. Inc., Research Triangle Park, NC.*

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) coordinates (a) the technical evaluation of new, revised and alternative test methods of Federal agency interest, and (b) cross-agency issues relat-

ing to the validation, acceptance and national and international harmonization of toxicological test methods. ICCVAM recently developed and adopted a process by which test method nominations and submissions are considered and prioritized for review and evaluation. Prioritization of proposed test methods is based on several criteria, including: the applicability of the method to regulatory testing needs; the extent of anticipated use by one or more agencies; the level of multi-agency interest; the potential for the method to reduce, refine or replace animal use; the prospect of the test method to provide improved prediction of adverse health or environmental effects compared to current test methods accepted by regulatory agencies; and the extent to which the test method affords other advantages, such as reduced time or cost. The newly revised ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods describe: 1) the ICCVAM nomination and submission process, 2) the information that should be provided in a test method submission or nomination and an outline for organizing the necessary information; and 3) the ICCVAM process for developing performance standards, which communicate the basis on which a validated and accepted test method has been determined to have sufficient accuracy and reliability for a specific testing purpose. Test method submitters/ nominators are encouraged to utilize these Guidelines and communicate with NICEATM and ICCVAM during the preparation of test method submissions and nominations. These Guidelines are expected to facilitate the preparation of test method submissions and nominations and their consideration by ICCVAM. ILS staff supported by NIEHS Contract NO1-ES-35504.

1812 THE ICCVAM/NICEATM PROCESS FOR DEVELOPING TEST METHOD PERFORMANCE STANDARDS.

W. S. Stokes[1], L. M. Schechtman[2], A. Rispin[3], R. N. Hill[3], K. Hamernik[3], B. C. Blackard[1, 4] and R. R. Tice[1, 4]. *[1]NICEATM, NIEHS, Research Triangle Park, NC, [2]NCTR, USFDA, Rockville, MD, [3]OPPTS, USEPA, Washington, DC and [4]ILS, Inc., Research Triangle Park, NC.*

Test methods proposed for regulatory testing should routinely undergo validation studies to assess their reliability and relevance for specific applications. Regulatory agencies can then determine if the test method is sufficiently accurate and reliable to be accepted for a proposed specific use. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) has established a process for developing and using performance standards (PS) to evaluate the acceptability of proposed test methods that are based on similar scientific principles and that measure or predict the same biological or toxic effect as an accepted test method. ICCVAM defined the three critical components of PS as: (1) essential test method components, i.e. the requisite structural, functional, and procedural elements of a validated test method that should be included in the protocol of a proposed mechanistically and functionally similar test method; (2) a minimum list of reference chemicals, which is used to assess the accuracy and reliability of the analogous test method; and (3) comparable accuracy and reliability values that should be achieved by the proposed test method when evaluated using the minimum list of reference chemicals. The ICCVAM also established a process for developing and recommending PS during future test method evaluations. NICEATM and an ICCVAM working group will develop proposed test method specific PS, which will be made available for public comment. An independent peer review panel will review the proposed PS for completeness and appropriateness as a part of the panels evaluation of the proposed test method. ICCVAM will then finalize and forward recommended PS together with test method recommendations to Federal agencies and make these available to the public. The availability of PS is expected to facilitate the development and validation of improved test methods that are similar to previously accepted methods. ILS staff supported by NIEHS contract NO1-ES-35504.

1813 RISK MANAGEMENT UNDER REACH - THE NEW EUROPEAN CHEMICALS POLICY.

T. Petry, A. Conrad and T. Golojuch. *The Weinberggroup LLC, Brussels, Belgium.*

In autumn 2003, the European Commission proposed a first draft of its new chemicals legislation, known as REACH (Registration, Evaluation and Authorisation of Chemicals.) It represents a major overhaul on management of risks associated with chemicals within the European Union (EU). This draft is the result of a complex series of discussions and negotiations between scientists, policy maker and politicians, individual Member States, the chemical manufacturers and downstream users of chemicals as well as non-governmental organizations. At its core, REACH will require producers and users of chemical substances to register these uses in a volume-triggered system. Submission of chemical assessment reports containing information on the hazards, exposures and risks associated with the uses of the chemical substances for review by governmentally-appointed expert committees will be required. Chemicals deemed hazardous, e.g., substances said to be Category 1 or 2 carcinogens, mutagens or toxic to the reproductive system according to the EU Dangerous Substances Directive 67/548/EEC, will trigger a complex review and authorisation procedure. As it stands, authorisation will also be required for substances considered to be *of concern* such as endocrine disrupters or substances that are persistent, bioaccumulative and toxic. In the authorisation process, an expert panel within a newly established EU chemicals agency will be expected to utilize scientific and socioeconomic data and information in their decision on the authorisation of uses of substances of concern. The results of a study to characterize the specific data requirements and the process of risk management in this emerging legislation will be reported. Specific consideration will be given to ill-defined areas under the proposed authorisation scheme. An effective approach that aims at putting risk into context to assure that politically driven risk management programs recognize the required balance between risk of use against risk of loss of the substance in achieving sustainability in the EU will be discussed.

1814 REGULATION OF VETERINARY ANTIMICROBIAL DRUG RESIDUES IN FOOD: FDA AND VICH APPROACHES.

A. H. Fernandez. *CVM, USFDA, Rockville, MD.*

The effect of antimicrobial drug residues in food on the human intestinal flora has been a FDA concern for more than a decade. Based on conclusions from international meetings and results from FDA sponsored research, the Center for Veterinary Medicine has been assessing the human food safety of antimicrobial drug residues based on adverse effects on human intestinal flora in the last 7-8 years. A guidance document (Guidance #52) for addressing this issue was published in January of 1996 and a modified guidance in October of 2003. Because of the international concern about the effect of antimicrobial residues in food on the human intestinal flora, the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) charged a Task Force to harmonize regulatory requirements for this area and recommend testing methods for assessing the effect of antimicrobial drug residues on endpoints of human health concern. The VICH guideline GL #36 is currently at the later stages of the VICH process. Both the FDA and VICH documents request that sponsors first address the need for a microbiological ADI following a pathway approach. This approach assesses the activity of the drug and/or its metabolites on relevant human intestinal bacteria and the concentration of residues that remain microbiologically active in the human colon after ingestion of food containing these residues. The no-observed effect level (NOEL) should be determined for the adverse effects that have been linked to public health concern: development of resistant bacteria and disruption of the colonization barrier of the flora. If necessary, both guidelines recommend using *in vitro* or *in vivo* test systems for determining NOELs for these endpoints. Specific test systems are discussed for each endpoint only in the VICH guideline. In addition, the VICH guideline recommends procedures for deriving an ADI using data from these methods. It also includes a glossary of terminology and appendices recommending future areas of research and statistical procedures to be used for data evaluation.

1815 VICH HUMAN FOOD SAFETY GUIDELINES: PROGRESS REPORT.

L. T. Mulligan. *Human Food Safety, USFDA/CVM, Rockville, MD.* Sponsor: A. Fernandez.

VICH (International Cooperation on Harmonization of Technical Requirements for Resgistration of Veterinary Medicinal Products) was established in 1996 and patterned after ICH, the human drug international harmonization process. VICH has nine Working Groups charged with harmonizing technical requirements for the registration of veterinary products in the participating regions: The European Union, Japan, USA, Australia/New Zealand, and Canada. The Safety Working Group (SWG) was established in 1997 and mandated to determine the complete package of toxicological studies needed to establish an Acceptable Daily Intake (ADI) for residues of veterinary drugs. The SWG had its last meeting in the USA in December of 2003. To date, they have completed eight guidelines. These consist of "General Approach to Testing Guideline" containing the overall toxicological requirements for human food safety and seven other specific guidelines. The specific guidelines include genotoxicity, reproductive toxicity, developmental toxicity, repeat-dose toxicity (90-day and chronic studies), carcinogenicity, and effects of drug residues on human intestinal flora. The guidelines are either new or based on ICH or OECD guidelines. Following implementation, the guidelines will replace the present guidelines and may require major changes in veterinary drug regulation in the regions. The purpose of this presentation is to review the VICH guidelines and emphasize significant changes that will impact the current veterinary drug regulatory process.

1816 EVALUATION OF ACUTE DRUG-INDUCED VASCULAR INJURY BIOMARKERS.

C. Louden[1], D. Brott[1], L. Foster-Brown[1], S. Gould[2], H. Jones[2], H. Prior[2], J. Valentin[2], G. Evans[2], S. Bjurstrom[3], K. Kenne[3], I. Schuppe-Koistinen[3] and G. Betton[2]. *[1]Safety Assessment - US, AstraZeneca Pharmaceutical, Wilmington, DE, [2]Safety Assessment - UK, AstraZeneca Pharmaceuticals, Alderley Park, United Kingdom and [3]Safety Assessment - Sweden, AstraZeneca Pharmaceuticals, Sodertalje, Sweden.* Sponsor: P. Ciaccio.

The unavailability of non-invasive diagnostic markers for drug-induced vascular injury is of concern to the pharmaceutical industry. Therefore, the discovery and validation of potential markers with clinical applicability would add great value to risk assessment and risk management and hence expedite the drug development process. However, validation of potential biomarkers is complicated by the fact that vascular wall biology is complex and comparing responses across species is challenging. In an attempt to identify markers of drug-induced vascular injury, we hypothesized that assessment and evaluation of endothelial cells, sub endothelial collagen, medial smooth muscle cells, adventitial fibroblasts, and/or circulating elements of the blood should yield potentially useful diagnostic markers. In a preliminary study, we evaluated markers of tissue injury, coagulation cascade, acute inflammation and cellular adhesion molecules in serum and/or plasma from dogs treated with an endothelin receptor antagonist ZD1611. In addition, protein expression in the right coronary artery, from control and treated dogs, was evaluated using immunohistochemistry and proteomics. Our results show a clear increase in circulating vWF in treated dogs as early as 3 hours post-dosing, this effect was sustained for 12 hours and returned to baseline at 24 hours post-dosing. This finding is of interest since vWF is frequently used as a diagnostic marker of vascular integrity in humans. The other serum and plasma biomarkers evaluated, including routine hematology and chemistry, were not altered post-dosing. However, immunohistochemistry showed the specific loss of Caveolin-1 expression within the lesions. These data suggest that vWF is a potential bridging biomarker of acute drug-induced vascular injury and that acute lesions have a loss of Cav-1 expression.

1817 ROLE OF CAVEOLIN-1 IN DRUG-INDUCED VASCULAR INJURY.

D. Brott[1], A. Katein[1], L. Foster-Brown[1], J. Morelli[1], G. Evans[2], H. Jones[2], S. Gould[2], G. Betton[2], S. Bjurstrom[3], H. Prior[2], J. Valentin[2] and C. Louden[1]. *[1]Safety Assessment-US, AstraZeneca Pharmaceutical, Wilmington, DE, [2]SA-UK, AstraZeneca Pharmaceuticals, Alderley Park, United Kingdom and [3]SA-Sweden, AstraZeneca Pharmaceuticals, Sodertalje, Sweden.* Sponsor: M. Dyroff.

In toxicology studies, drug-induced vascular lesions in rats and dogs is an issue of major concern because there are no obvious predictive biomarkers for pre-clinical or clinical monitoring and there is an intellectual gap in our knowledge and understanding of the pathogenesis of these lesions. There is strong evidence suggesting localized vasodilation, inability of the vasculature to maintain tone and alterations in regional myocardial blood flow are critical events in the development of drug-induced arterial lesions in dogs. While vasodilation and increased shear stress appear to play a role in drug-induced vascular injury, the exact mechanism of smooth muscle cell (SMC) and endothelial cell (EC) injury/death is unknown. In acute drug-induced vascular injury, we observed early decreases and/or loss of caveolin-1 (cav1) expression. Since cav1 is expressed on both SMC and EC, the primary targets of vascular injury, and based on location and function we hypothesized that cav1 could play an important role in the mechanism of drug-induced vascular injury as well as be a mechanistically linked diagnostic marker of vascular integrity. Therefore, we attempted to develop an *in vitro* system to study the mechanism of drug-induced vascular toxicity and determine if loss of cav1 precedes cell death. In our initial studies we used a rat, embryonic, aortic smooth muscle cell line (A10) and IBMX as our test compound. IBMX was selected because it is a broad-spectrum phosphodiesterase inhibitor (PDE) and inhibition of PDE type IV can cause mesenteric arterial lesions in the rat. Our preliminary results show that IBMX caused loss of cav1 in a dose-dependent manner in A10 cells and furthermore, the loss of cav1 preceded apoptosis. Minoxidil had similar but less pronounced effects with A10 cells. These data suggest that cav1 may be playing an important role in the initiation of drug-induced vascular injury in rats and dogs.

1818 NOVEL TECHNIQUES FOR ISOLATION AND CHARACTERIZATION OF ENDOTHELIAL CELLS AND VASCULAR SMOOTH MUSCLE CELLS FROM CANINE CORONARY ARTERIES.

X. Yu[1], M. K. Dame[2], R. Garrido[1], D. Stump[1], W. Bobrowski[1], J. E. McDuffie[1], J. Varani[2] and M. A. Albassam[1]. *[1]Worldwide Safety Sciences, Pfizer Global Research & Development, Ann Arbor, MI and [2]Pathology, University of Michigan, Ann Arbor, MI.* Sponsor: A. Brown.

Beagle dogs are commonly used in drug safety studies. The morphological changes of endothelial cells (EC) and vascular smooth muscle cells (VSMC) were observed in drug-induced vascular injury. The ability to isolate and characterize pure EC and VSMC cultures could prove useful in the investigation of compound-evoked vascular cell responses. EC and VSMC cultures from canine coronary arteries are not commercially available and the methods for isolating these cells have not been published. This work was initiated to establish novel methods for isolation of pure ECs and VSMC from Beagle dog coronary arteries. For isolation of ECs, different periods of enzyme incubation time (10-40 minutes) were performed. The ECs collected following 10-minute enzyme incubation were found to have the highest purity. The EC cultures were characterized by uptake of Dil-Ac-LDL, immunocytochemical staining for von Willibrand factor and by transmission electron microscopy (TEM). Multi-stage enzyme digestion (30 minutes to 2 hours) was performed to isolate VSMC. The VSMC collected following three consecutive 30-minute enzyme incubation/wash periods were found to have the highest purity. The VSMC cultures were characterized by immunocytochemical staining for smooth muscle a-actin and by TEM. The VSMC cultures failed to stain for von Willibrand factor (an endothelial cell marker) or basic fibroblast growth factor (a fibroblast cell marker), suggesting that the VSMC isolates were pure. Pure canine coronary EC and VSMC cultures may serve as *in vitro* models for investigating compound-evoked cell-specific signal transduction.

1819 ENDOTHELIN-1 AND VASCULAR TOXICITY OF HIV COCKTAILS.

V. Y. Hebert, B. Crenshaw, R. L. Romanoff, V. P. Ekshyyan and T. R. Dugas. *Pharmacology, LSU Health Sciences Center, Shreveport, LA.* Sponsor: K. McMartin.

Pulmonary arterial hypertension (PAH) is a progressive disease of the pulmonary vasculature involving vascular smooth muscle cell (VSMC) proliferation, vasoconstriction, right ventricular hypertrophy, and eventually, right heart failure and death. The incidence of PAH in the general population is ~1:200, 000, however, the incidence of HIV-associated PAH is 1:200. Further, while conventional HIV therapy with NRTIs (nucleoside reverse transcriptase inhibitors) leads to regression of PAH, the more aggressive Highly Active Antiretroviral Therapy (HAART, two NRTIs plus a protease inhibitor) increases the incidence of HIV-associated PAH as much as 2-fold. Though there are relatively few models for PAH, previous reports indicate the disease can be initiated by endothelial injury and release of the mitogen ET-1. ET-1, in turn, stimulates VSMC proliferation. To determine whether HAART exacerbates endothelial cell injury and release of cytokines like ET-1, we treated human umbilical vein endothelial cells with micromolar amounts of AZT, the protease inhibitor indinavir, or AZT plus indinavir, and measured cell *via*bility, respiration, ATP, and ET-1 release. Both AZT and indinavir induced marked decreases in cellular oxygen uptake and ATP production, as well as increases in nitric oxide and ET-1 release. Though the drugs had no apparent effect on proliferation in VSMC alone, in cocultures of VSMC plus endothelial cells, the drugs increased proliferation of both endothelial cells and VSMC. Finally, when cocultures of endothelial cells and VSMC were treated with selective antagonists for ET_A and ET_B receptors, HAART-induced VSMC proliferation was attenuated. We thus hypothesize that HIV drug cocktails exacerbate HIV-associated PAH by inducing endothelial mitochondrial dysfunction, in turn stimulating the release of ET-1, and ultimately, VSMC proliferation.

1820 POTENTIAL MARKERS OF ACUTE CORONARY ARTERY INJURY IN DOGS FOLLOWING ADMINISTRATION OF CI-1034, AN ENDOTHELIN A RECEPTOR ANTAGONIST.

E. McDuffie, X. Yu, Y. Song and M. Albassam. *Worldwide Safety Sciences, Pfizer Global Research & Development, Ann Arbor, MI.* Sponsor: A. Brown.

The objective of this study was to investigate potential markers for coronary vascular injury evoked by CI-1034, a selective nonpeptide endothelin A receptor antagonist. CI-1034 was administrated to male beagle dogs intravenously (iv) for 4 days. Treated groups consisting of 5 animals received CI-1034 at 120 mg/kg (iv) in bolus. Control animals (n=3) received the vehicle only. Animals were sacrificed on day 5; one treated animal was sacrificed on day 4 due to moribundity. Macroscopically, drug-related effects including hemorrhage were observed in the extramural coronary groove and atrium in the treated animals. Histologically, coronary changes were noted in the treated animals and characterized by medial hemorrhage and necrosis, and mixed inflammatory-cell infiltrates in the intima, media and adventitia. Immunohistochemistry (IHC) staining indicated increases in expression of inducible nitric oxide synthase (iNOS) and cleaved caspase 3 (a marker of apoptosis) in the coronary arteries from the treated group. Similar levels of expression of endothelial nitric oxide synthase (eNOS) in both control and treated animals were noted by IHC. Colorimetric assay showed increased levels of nitrite and nitrate (markers for accumulation of nitric oxide production) in both serum and urine from the treated animals only. Increased levels of pro-inflammatory cytokine, interleukin-6 (IL-6) and acute phase proteins, fibrinogen and serum amy-

loid A (SAA), were detected by enzyme-linked immunosorbent assay (ELISA) in the blood from CI-1034-treated animals. These findings suggest that CI-1034-evoked acute coronary artery injury involves activation of iNOS and apoptosis; increased levels of IL-6, fibrinogen and SAA may serve as markers of acute drug-induced coronary artery injury.

1821 AN EXPERIMENTAL PDE IV INHIBITOR-INDUCED VASCULAR INJURY (VI) ASSOCIATED WITH INCREASED MAST CELL DEGRANULATION AND ELEVATED SERUM LEVELS OF ACUTE-PHASE PROTEINS IN SPRAGUE-DAWLEY RATS.

J. Zhang[1], R. Honchel[1], J. L. Weaver[1], A. Knapton[1], E. H. Herman[1], F. M. Goodsaid[2], J. W. Davis II[2], I. Y. Rosenblum[2] and F. D. Sistare[1]. *[1]Division of Applied Pharmacology Research, Center For Drug Evaluation and Research, USFDA, Laurel, MD and [2]Toxicology, Schering-Plough Research Institute, Lafayette, NJ.*

Evaluation of drug-induced VI has been hampered by a lack of early clinical signs and accessible biomarkers. As part of an ongoing effort to establish a rat model of drug-induced VI, rats were gavaged with an experimental PDE IV inhibitor at doses of 3 to 160 mg/kg/day (mkd) for 1 to 9 days and sacrificed 24 hr after the last dose. Doses of 6 mkd and higher resulted in systemic toxicity. Vascular lesion development appeared to be both time- and dose-dependent. A semiquantitative scale (0 to 5+) was used to grade mesenteric vascular lesions. Lesions increased in both incidence and severity from 0.6+ to 4.7+ following three doses of 3, 6, or 40 mkd. Similar lesions and scores were observed following 9 doses of 3 mkd (1.2+) or 7 doses of 9 mkd (3.0+). In mesenteric vasculature, the compound induced arteriolar fibrinoid necrosis, arterial hemorrhage and necrosis, and perivascular inflammation, accompanied by endothelial cell (EC) activation, mast cell degranulation, increased nitrotyrosine formation on EC, and apoptosis of EC and smooth muscle cells. VI correlated well with the extent of neutrophilia, lymphopenia, and increased serum levels of haptoglobin and α1-acid glycoprotein at all time points. Increased serum IL-6 was seen only at early time points. The pathologic findings suggest that mechanisms responsible for the VI might involve peroxynitrite-mediated damage to EC and a concurrent activation of certain immune system components. Further work is required to refine the model and elucidate the pathogenesis of VI. We are evaluating additional blood-based biomarkers to identify drug-induced VI and differentiate it from inflammation. A more specific biomarker panel will be critical to expand our capabilities in safety monitoring of preclinical or clinical studies with PDE inhibitors.

1822 ESTABLISHMENT OF AN *IN VITRO* METHOD FOR ASSESSMENT OF DRUG-INDUCED VASCULITIS.

Y. Zhou[1], H. Yamada[1], I. Horii[1] and K. Suzuki[2]. *[1]Worldwide Safety Sciences, Pfizer Global Research & Development, Nagoya Laboratories, Pfizer Inc., Nagoya, Japan and [2]Department of Molecular Pathobiology,, Mie University School of Medicine, Tsu, Japan.*

[Purpose] Vasculitis has been shown to cause injury of organs such as liver, heart, kidney in both preclinical toxicity studies and clinical studies. Recently, several drugs were reported to be associated with a high incidence of vasculitis. This indicates the importance of establishing a system for assessing drug-induced vasculitis during early phase evaluation of candidate drugs. In this study, we established an *in vitro*, low bulk rapid vasculitis assessment method using cultured human umbilical-vein endothelial cells (HUVECs). We verified the correlation between data obtained from the *in vitro* method with that obtained from *in vivo* studies using animals. [Methods] Cultured HUVECs were exposed for 3, 6 and 24 h to varying concentrations of the vasculitis-inducing drugs, Siguazodan (PDE III inhibitor) and Fenoldopam (5-HT2 and dopamine DA1 agonist). The degree of HUVECs injury was evaluated by determining the concentrations of the following molecular markers of coagulation/fibrinolysis in the culture medium using ELISA: von Willebrand factor (vWF), tissue type plasminogen activator/plasminogen activator inhibitor-1 complex, and soluble intercellular adhesion molecule-1 and TNF-a. Morphological changes in HUVECs and the expression of vWF in HUVEC cytoplasm, platelet endothelial cell adhesion molecule-1 and tissue factor on the cell membrane were determined by immunostaining. [Results & Conclusion] When HUVECs were exposed to the above vasculitis-inducing drugs, vasculitis-related markers were increased in the culture medium, functional molecules were expressed on the cell membrane, and cell morphological changes were observed in a dose-dependent fashion. The dose of drugs that induced remarkable changes in HUVECs was in the same range as concentrations of animal (dog and rat) plasma from *in vivo* studies. These findings suggest that this method may be useful for evaluating and screening drug candidates induced the vascular endothelium injury.

1823 USE OF A NON-INVASIVE TELEMETRY SYSTEM (EMKA) FOR FUNCTIONAL CARDIOVASCULAR ENDPOINTS IN TOXICOLOGY STUDIES.

H. Prior[1], D. Hunter[1], J. Schofield[1], K. Gracie[1], J. Moors[1], K. Philp[1], P. Carter[2], J. Valentin[1] and T. Hammond[2]. *[1]Safety Pharmacology, AstraZeneca UK Ltd., Macclesfield, Cheshire, United Kingdom and [2]Safety Assessment UK, AstraZeneca UK Ltd., Macclesfield, Cheshire, United Kingdom.*

Introduction: Inclusion of ECGs in toxicology studies has conventionally been at snap-shot time-points, with restrained dogs in slings, which can lead to stress and heart rates above the normal resting values. This can mean that the sensitivity of the system is reduced for the detection of effects of test compounds on ECG parameters, in particular QT interval. New technological advances in non-invasive telemetry systems permit inclusion of longer-term, higher quality measurements, thus improving the detection of such effects. Objectives: To demonstrate the value of including non-invasive ECG recordings in a dog toxicology study. Methods: 30 dogs on a one month oral toxicity study had ECGs recorded for up to 22 hours, using external skin electrodes (EMKA technologies, France) on Days −5, 2, 7, 14, 21 and 27, in order to investigate suspected QT prolongation effects identified in a previous single dose and 7 day Safety Pharmacology (SP) telemetry study. All other measurements (toxicokinetics, clinical pathology, pathology) were as normal for this type of regulatory study, but are not discussed within the context of this abstract. Results: Inclusion of these recordings eliminated the need for a separate follow-up SP study, since the data from the control animals and four dose levels of compound (3M/3F per group) produced a clear dose- and time-related effect on QT interval. Conclusions: Inclusion of cardiovascular SP end-points within toxicology studies can therefore be advantageous from an animal welfare, data quality, compound requirement and resource perspective.

1824 A METHOD FOR THE LONG TERM MONITORING OF CARDIOVASCULAR AND RESPIRATORY FUNCTION IN THE WISTAR RAT.

N. McMahon[1], A. Robinson[1], E. Martel[2] and J. Valentin[1]. *[1]Safety Pharmacology, Safety Assessment, AstraZeneca R&D, Macclesfield, Cheshire, United Kingdom and [2]CERB, Baugy, France.* Sponsor: T. Hammond.

The development and utility of pharmaceutical agents may be limited by cardio- and/or respiratory toxicity. We present a method for simultaneous evaluation of functional cardiovascular and respiratory effects. Five male and five female Wistar rats, approximately 9 weeks old, weighing 294-330g were chronically implanted under anaesthesia with a DSI telemetry implant (TL11M2-C50-PXT) 2 weeks before study. While on study rats received daily oral doses of 1% carboxymethylcellulose and 0.1% Tween80. Respiratory and cardiovascular data was collected pre-dose and for 4 and 24 hours post-dose, respectively, on Days 1, 14, 28, 63, 91, 126, 154 and 182. This was achieved by placing a conscious unrestrained rat in a whole-body plethysmography chamber (EMKA, PLY/UN2P) residing on top of a telemetry receiver 30 minutes prior to dosing and for 4 hours post-dose, after which time it was returned to its home cage on top of the same receiver. In this configuration cardiovascular parameters (systolic and diastolic blood pressures and heart rate) and respiratory parameters (rate, peak inspiratory and expiratory flows, inspiration and expiration times, tidal volume, minute volume and index of airway resistance PenH) were simultaneously acquired. Respiratory data was acquired from at least 3 rats/sex to Day 182 and cardiovascular data from at least 3 rats/sex to Day 63. We have shown that using combined telemetry and plethysmography techniques, cardiovascular and respiratory function can be successfully studied for up to 2 and 6 months, respectively. This approach may be used investigate acute and chronic effects of pharmaceutical agents on the cardiovascular and respiratory systems and their interactions. This type of study can be performed independently or as part of a toxicology study using satellite groups. Furthermore, there is the potential for reuse.

1825 ASSESSMENT OF QT INTERVAL PROLONGATION USING A TELEMETRY SYSTEM IN CONSCIOUS GUINEA PIGS.

T. Harada, M. Shiotani, J. Abe, M. Nagata, Y. Hamada and I. Horii. *PGRD Nagoya Lab., Pfizer Inc., Taketoyo, Aichi, Japan.* Sponsor: M. Kurata.

Prolongation of QT interval is an important safety issue because it may be associated with life-threatening ventricular arrhythmias such as torsades de pointes. However, since distribution of cardiac ion channel in humans differs from that in animals, it is essential to use the most appropriate animal species to be able to assess the risk for a cardiac event induced by a novel agent in humans. Although isolated papillary muscles from guinea pigs are currently used to evaluate the action potential duration (APD) because of the similarity of the ionic currents in ventricular myocytes between humans and guinea pigs, the evaluation of the QT interval in conscious guinea pigs has not been established as yet. In this study, we focused on

the technical and scientific validation of *in vivo* measurement of the QT interval in conscious guinea pigs. Transmitters were implanted subcutaneously in male Hartley guinea pigs and a telemetry system was used to record and analyze ECGs. ECGs were recorded 24 hours before and after treatment with d, l-sotalol (SOT, 40 mg/kg, i.v.) and astemizole (ATZ, 30 mg/kg, p.o.) to compare the QT/RR plots between pre- and post-dosing. Thus, a well-defined T wave was continuously recorded in this study. ATZ and SOT produced the QT interval prolongation, and it was more obvious at lower heart rate. SOT also produced marked bradycardia 0-2 hours after the dosing. APDs were also prolonged in isolated guinea pig papillary muscles as assessed using a microelectrode technique. The results of the present study coincided with those of other studies on drug-induced QT prolongation, and clarified the timecourse of QT changes in guinea pigs. Therefore, it is suggested that, in the early stage of drug development, the telemetry system is a useful tool to examine cardiac electrophysiology in guinea pigs *in vivo*, especially regarding the evaluation of the QT interval. In addition, simultaneous evaluation *in vivo* and *in vitro* would increase the reliability of the risk assessment of QT prolongation.

1826 COMPARISONS OF HOLTER AND TELEMETRIC ECG MONITORING FOR EVALUATION OF QT INTERVALS IN CYNOMOLGUS MONKEYS.

S. kitani, K. Sakamoto, N. Muto, M. Nomura and W. Tierney. *Ina Research Inc., Nishiminowa, Ina, Nagano, Japan.*

Telemetric ECG monitoring is an invasive procedure, whereas Holter monitoring is not. Analysis of QT intervals has been generally thought to be difficult by Holter monitoringDWe established an improved method of Holter monitoring method using animals acclimated to experimental procedures. ECG parameters including R-R intervals, PR, QRS and QT intervals recorded by these 2 methods were compared in cynomolgus monkeys dosed orally with sotalol hydrochloride, an antiarrythmic agent. Three male cynomolgus monkeys, 4-6 years of age weighing 4-8 kg, from INA RESEARCH PHILIPPINES, INC. were used in this study. Initially, telemetric ECG recordings were conducted, followed by Holter ECG monitoring after an approximately 2-month rest and recovery period. Electrodes were inserted into the subcutis for telemetric monitoring and placed on the shaved skin surface for Holter monitoring, both at the right subcla*via*n and the left abdominal areas. Animals were dosed with sotalol hydrochloride orally at 5 mg/kg. ECGs were recorded from 1 hour pre-dosing until 24 hours post-dosing. Mean heart rates were 103 and 97 times/min in Holter and telemetric ECGs, respectively. Prolongation of QT intervals peaked 1-2 hours post-dosing in both methods and QT intervals were clearly prolonged by 141% and 149% in Holter and telemetry ECGs, respectively, compared to the pretreatment values. Comparable results were obtained by both telemetric and Holter monitoring. Telemetric ECG recordings are routinely employed to conduct QT intervals evaluations in conscious, unrestrained animals. Similarly, evaluation of drugs for prolongation of QT intervals can be detected by Holter monitoring. The results of this study indicate that simple non-invasive Holter ECG monitoring may be available and practical in routine safety evaluations of pharmaceuticals.

1827 COMPARISONS OF NON-INVASIVE VERSUS INVASIVE DOG TELEMETRY.

H. Prior[1], D. Hunter[1], S. Jason[1], K. Gracie[1], J. Moors[1], K. Philp[1], J. Valentin[1] and T. Hammond[2]. *[1]Safety Pharmacology, AstraZeneca UK Ltd., Macclesfield, Cheshire, United Kingdom and [2]Safety Assessment UK, AstraZeneca UK Ltd., Macclesfield, Cheshire, United Kingdom.*

Introduction: Conscious dog telemetry is routinely used for *in vivo* cardiovascular Safety Pharmacology (SP) studies (ICHS7A guidelines), generally involving invasive surgery to position the telemetry implants. Therefore, colonies of dogs are generally required for long-term use on these studies. Advances in non-invasive telemetry systems may allow additional methodologies to be used, without prior surgical intervention. This allows dogs to be used from stock, or within toxicology studies. Objectives: To evaluate two different telemetry systems; invasive DSI® telemetry (analysis *via* Notocord HEMv3.4), and non-invasive external ECG telemetry (EMKA Technologies, France, data capture and analysis *via* IOXv1.7.0 and ECG Autov1.5.7). Methods: Dogs were allowed at least a 2 week recovery period from implantation of TL11M2-D70-PCT transmitters (ECG electrodes in a lead II configuration). For the non-invasive methodology, dogs had external skin electrodes attached in a lead II position, which were held in place by a jacket holding the battery and transmitter. ECG recordings were made from six, male Alderley Park beagle dogs (10-14 kg) using both DSI® and EMKA technologies, simultaneously. Dogs were recorded for 1 hour pre-dose then 6 hours post-dose under control conditions, then following ascending oral doses of (±)sotalol at intervals of 1-2 days. Results: Correlation in data (HR, PR, QT) produced by the 2 systems was within ±5% expected, except for QRS duration. Similar increases in QTC (Van de Waters formula) were apparent using both systems, peaking at 10.4, 13.5 and 17.8% (DSI), or 8.9, 11.5 and 14% from control (EMKA; 4, 8, and 16 mg/kg, respectively). Conclusions: The EMKA system is complementary to DSI. This allows inclusion of quality measurements of SP endpoints in toxicology studies, thus reducing animal usage and compound requirements, by reducing the need for separate SP studies.

1828 SIMULTANEOUS CARDIOVASCULAR AND PULMONARY STUDIES IN MONKEYS AND DOGS.

C. R. Hassler. *Safety Pharmacology, Battelle, Columbus, OH.* Sponsor: M. Brooker.

S7A cardiovascular (CV) and pulmonary (PUL) safety pharmacology studies are most frequently performed in different species and/or different animal models instead of being simultaneously performed in the same animal. When simultaneous CV and PUL measurements are used, it is of interest to ascertain the cardiovascular alterations imposed upon the animal model by the restraint required for the pulmonary measurements. Cardiovascular responses in appropriately trained dogs and monkeys were compared between restraint for pulmonary function and normal cage housing conditions. Restraint slings and restraint chairs were used for dogs and monkeys respectively. Pulmonary flows were collected *via* head dome and pneumotach. Monkeys were placed in isolation boxes. Animals were instrumented *via* implanted telemetry for blood pressure, heart rate and ECG. Trained groups of dogs (N=17) and monkeys (N=21) were evaluated up to 7 hours both restrained and unrestrained. Sling restraint/PUL in dogs increased blood pressures 8% and decreased heart rate 6%. Chair restraint/PUL in monkeys increased blood pressures 14% and increased heart rate 22%. Properly conditioned dogs and monkeys may be restrained for pulmonary function determinations with only minor alteration of CV parameters. Restrained-to-home cage differences were nominally 15% or less except for monkey heart rates which were 22% higher during restraint. These CV differences for the restraint conditions used are unlikely to alter the CV conclusions that would be derived from this data. However, these differences in measured parameters require that the CV data be compared to similarly restrained animals. Dogs, especially females, are more amenable to the restraint process and can be readily trained to accept the presence of humans in the experimental room. However, monkeys must be isolated from human contact. Appropriate restraint environment and adequate conditioning are key to the successful implementation of simultaneous CV/PUL collection.

1829 ECG CHANGES DURING INHALATION OF DILUTED ENGINE EMISSIONS IN A RAT MODEL OF MYOCARDIAL INFARCTION (MI).

J. MORIN[1], S. LORIOT[1], F. ANSELME[2], A. CHAGROUI[1], J. HENRY[1] and F. DIONNET[3]. *[1]E9920, INSERM, ROUEN, France, [2]Cardiology Unit, CHRU Rouen, Rouen, France and [3]CERTAM, Saint Etienne du Rouvray, France.* Sponsor: R. FORSTER.

We developed a MI rat model for investigating the cardiac effects of inhaled diluted Diesel engine emissions as a representative surrogate for urban atmospheric pollution. Methods : Left ventricular MI was induced in 12 wistar rats by left coronary artery ligation. 2 months after surgery, rats were implanted with TA-F40 (DSI) transmitter for ECG lead II telemetry monitoring. ECG analysis was performed using ECG-Auto software (EMKA France), allowing direct automatic quantitation of arrhythmic events. 3 hour inhalation exposure periods of conscious unrestraint rats (6 rats per group) to a continuous flow of diluted Diesel engine emissions (Particulate Matter 0.5 mg/m3, mean aerodynamic diameter 90nm, CO 10 ppm, NOx 10 ppm)were performed. Results : No significant alteration of ECG but a slight sinusal tachycardia and decrease in HRV were observed in non MI rats exposed to exhausts. In non exposed MI rats, increased QT duration by 20-30 ms and a slight bradycardia (-20-30 bpm) and polymorphic ventricular extrasystoles (PVES) were observed, with preserved RR variability. In Exhaust exposed MI rats, a slight tachycardia (+ 40-50 bpm), frequent arrhythmia was observed consisting in increased PVES (x3times more), episodes of unsustained ventricular tachycardia (UVT) (3 to 6 consecutive beats), of sustained VT (SVT) (over 50 consecutive beats > 550 bpm)and of bigeminy. Bigeminy and VT episodes were not observed in non exposed MI rats. The induction of these arrhythmic events was rapid (within 20 min) for increase in PVES, by approx 2h for unsustained VT episodes, and by approx 4h for sustained VT episodes. Return to basal situation was observed within 12 to 24h after exposure termination. Conclusion : Upon exposure to combustion aerosols, MI rats exhibit marked ECG changes whereas sham controls do not. This model will facilitate the experimental study of outcomes and mechanisms associated with ambient air pollution-related increases in cardiorespiratory morbidity, as identified epidemiologically.

1830 THE CARDIOVASCULAR EFFECTS OF CLINICALLY RELEVANT DOSES OF D, L-SOTALOL AND VERAPAMIL, OBSERVED AFTER ACUTE ORAL ADMINISTRATION TO CONSCIOUS, TELEMETERED CYNOMOLGUS MONKEYS.

P. L. Munt, D. J. Beard, P. H. Davies, C. Winfield, J. J. Daniels, R. Brammer and F. Sannajust. *Huntingdon Life Sciences, Huntingdon, Cambridgeshire, United Kingdom.* Sponsor: C. Hardy.

Clinically recommended daily doses extend to 320 mg for d, l-sotalol and 480 mg for verapamil, which are respectively 6 mg/kg and 10 mg/kg, assuming a body-weight of 50 kg. The effects of acute oral administration of 5 and 10 mg/kg of each compound were examined on blood pressure (BP), heart rate (HR) and ECG in 5 conscious telemetered monkeys. BP, HR and ECG were continuously measured before and up to 24 hours after dosing, the data being collected and analysed using a DSI /Gould Po-Ne-Mah data system. The QT intervals were corrected for HR using a linear regression analysis (QTcR). In comparison with vehicle (water), d, l-sotalol at 5 and 10 mg/kg significantly ($p<0.01$) reduced HR and increased QT. A significant ($p<0.01$) prolongation in QTcR was observed at 10 mg/kg with maximal effect observed during 1.5 to 4 hours post-dose. Responses of BP to these treatments were minimal reductions. Verapamil induced a non dose-dependent reduction in QTcR over the 4 to 12 hour post-dose period, which was significant at both dose levels ($p<0.05$), 12 hours post-dose. At 10 mg/kg, verapamil produced a moderate but non-significant reduction in heart rate and slight reduction in blood pressure. We found that, in telemetered cynomolgus monkeys, oral doses of 5 and 10 mg/kg of d, l-sotalol and verapamil induced changes in QT, as indicated by the corrected QT (QTcR) that were consistent with clinically reported effects. The cynomolgus monkey is therefore considered a suitable and predictive species for detection of potential QT changes.

1831 VALIDATION FOR QT PROLONGATION IN CONSCIOUS CYNOMOLGUS MONKEYS ADMINISTERED SOTALOL VIA NASOGASTRIC ROUTE.

M. miyamoto, C. M. Kelly and S. J. Gosselin. *Huntingdon Life Sciences, East Millstone, NJ.*

The purpose of this study was to evaluate the DSI (Data Sciences, St. Paul, MN) telemetry system in our Testing Facility environment with an agent known to cause QT prolongation in cynomolgus monkeys. Under general anesthesia, telemetry transmitters were implanted in 8 adult cynomolgus monkeys (4 /sex). Pretest data were collected for a 48 hour period prior to initiation of dosing. Sotalol was administered by nasogastric gavage in a dose escalation regimen at 0, 4, 8, and 16 mg/kg, respectively. Data were collected every 15 minutes for 2 hours prior to dosing and every 30 minutes for 24 hours following each dose. The effect of sotalol on QT interval was assessed by several methods including QTca (regression analysis of QT on RR interval for each animal from the pre-treatment data), % and absolute QT and QTc change from baseline, comparison of absolute QT with similar HR, and QT:HR outlier analysis. For statistical analysis, data were averaged over the following time periods: 0 to 3 hours, 3 to 6 hours, 6 to 12 hours and 12 to 24 hours. Baseline was defined as the mean of all measurements in the 2 hours prior to dosing. Analysis of covariance for repeated measures with treatment, animal and time period as factors and baseline as a covariate was used to test whether treatment effects varied during the above time intervals. Treatments were compared to control using Williams′ test, and for time intervals, each sex was also analyzed separately. The peak effect of sotalol on QT prolongation occurred between 0-3 hours, and the degree of QT prolongation was dose-related in males only. However, the peak effect of sotalol on QTc was dose-related between 0 and 3 hour in both sexes. QT intervals were prolonged when compared with similar HR between 0 to 6 hours in both sexes. The number of outliers were dose related at 0-3 hours in both sexes. In conclusion, we have demonstrated the suitability of using DSI telemetry systems in cynomolgus monkeys to detect changes in QT interval in our Testing Facility environment.

1832 TRIANGULATION, REVERSE-USE-DEPENDENCE AND INSTABILITY DISTINGUISH THE PROARRHYTHMIC POTENTIAL OF DRUGS IN A PACED ISOLATED LANGENDORFF RABBIT HEART.

J. Valentin[1], K. Gracie[1], S. Palethorpe[1], T. Hammond[1] and L. Hondeghem[2]. *[1]Safety Pharmacology, Safety Assessment, AstraZeneca R&D, Macclesfield, Cheshire, United Kingdom and [2]Hondeghem Pharmaceutical Consulting, Oostende, Belgium.*

QT is used as a surrogate marker for a rare but potentially fatal ventricular arrhythmia known as Torsades de Pointes (TdP). However the clear link between QT prolongation and the proarrhythmic risk has not been demonstrated. To evaluate the effects of 36 clinically-relevant drugs in an automated *in vitro* proarrhythmia model using electrophysiological techniques[1]. Monophasic action potentials were recorded from paced Langendorff-perfused female adult rabbit hearts. Parameters evaluated included action potential duration (APD_{60}), **T**riangulation (T), **R**everse-use-dependence (R) and **I**nstability (I; TRIad). Thirty-six drugs covering the entire range of torsadogenic propensity[2] were evaluated at concentrations encompassing and exceeding the effective free therapeutic plasma concentration (EFTPC). Compounds were correctly identified and categorized according to their clinically recognised proarrhythmic risk. Briefly, antiarrhythmics which have Ikr block as a primary pharmacodynamic mechanism concentration-dependently increased APD_{60} and TRIad within or at low multiples of the EFTPC. Drugs for which there have been no incidences reported or only isolated reports of TdP in humans, showed no change in TRIad with or without APD_{60} prolongation at high multiples of EFTPC. Drugs for which numerous cases of TdP have been reported (including those suspended or withdrawn from markets) showed increases of elements of TRIad with complex concentration-response relationships relative to the EFTPC. This automated proarrhythmic model and the use of TRIad analysis, accurately distinguishes between drugs associated with proarrhythmia and those free from proarrhythmic risk. We conclude that this model provides unique and valuable information, which, when coupled to existing models, helps to establish an in-depth integrated risk assessment. 1. Hondeghem, LM *et al.*, 2001. Circ Res **103**: 2004-2013. 2. Redfern, WS *et al.*, 2003. Cardiovasc Res **58**: 32-45.

1833 THE INTERACTION OF SEROTONERGIC AND DOPAMINERGIC SYSTEMS IN THE DEVELOPING RAT AFTER NEONATAL COCAINE EXPOSURE.

T. B. Summavielle[1, 2], C. J. Alves[1], A. Magalhaes[1], L. de Sousa[1] and M. A. Tavares[3, 1]. *[1]Neurobehavior, Institute for Molecular and Cellular Biology, Porto, Portugal, [2]Biomedical Sciences, Allied Health Sciences School of Porto, Porto, Portugal and [3]Institute of Anatomy, Medical School, University of Porto, Porto, Portugal.* Sponsor: S. Ali.

Exposure to cocaine in the adult rat is known to increase serotonin (5-HT) release in the ventral mesencephalon (VTA-SN), nucleus accumbens (ACCB) and striatum (STR), while decreasing cell firing in the raphe nuclei (RN). It was suggested that cocaine acts on the dopaminergic system through a 5-HT-DA interaction, regulating dopamine (DA) action in the ACCB and STR. This interaction would begin in the RN and could modulate cocaine-induced behavioral dysfunctions. Neonatal cocaine is well known to affect the development of both DA and 5-HT systems. Here, we have studied the effects of prolonged neonatal cocaine in the RN, VTA-SN, STR and ACCB, throughout the development of these two systems. Male and female Wistar rats were injected s.c., from postnatal day 1 (PND1) to PND30 with cocaine hydrochloride in two daily doses at 15 mg /kg/day. Control animals were given isovolumetric saline. On PND14, 21 and 30, open-field behavioral tests were conducted to evaluate changes in motor activity. At the same time, rats from at least three different litters (N=6-9 per gender) were sacrificed and brains dissected for determination of DA and 5-HT in the RN, VTA-SN, ACCB and STR, by high performance liquid chromatography with electrochemical detection (HPLC-EC). Significantly increased DA and 5-HT levels were observed in all regions on PND14 and 21, but not on PND30. In both cocaine exposed male and female pups, there was a clear parallelism between levels of 5-HT and DA in all assessed regions, except in the ACCB. In accordance, exploratory and global locomotor actives were seen to increase in both genders on PND14, and decrease on PND30. Therefore, we suggest that, as in the mature, also in the developing rat, cocaine acts on the dopaminergic system through 5-HT-DA interaction in the neural pathways.

1834 ROLE OF ENVIRONMENT IN FETAL BASIS FOR ADULT DISEASES: DEVELOPMENT OF AN ANIMAL MODEL FOR NEURODEGENERATION (ND). AE AHMED, S. JACOB AND G. CAMPBELL, DEPARTMENT OF PATHOLOGY, THE UNIVERSITY OF TEXAS MEDICAL BRANCH, GALVESTON, TEXAS 77550-0609.

A. E. Ahmed. *Pathology, UTMB, Galveston, TX.*

Nearly every human disease has an environmental component. Fetuses are sensitive to environmental insults and developmental exposure may have a far more detrimental effects in the etio-pathology of fetal and adult diseases. Knowledge of the mechanisms of how environmental insults program fetuses for adult diseases is scanty. Our objective is to develop an animal model to examine the role of drinking water disinfectant byproducts (DBP) in fetal basis for adult ND. Pregnant mice, at day 6 of gestation (GD 6), received a daily oral dose of chloroacetonitrile (CAN, 25 mg/kg), a member of the haloacetonitrile (HAN) group of DBP. At GD 12, two groups were injected with 2-14C-CAN (333 mCi /kg, i.v.) and sacrificed at 1 and 24 h after treatment. Fetal brain uptake of CAN was examined using quantitative in situ whole-body autoradiography. The remaining animals continued treatment

until GD18. One treated group and its control were anesthetized and fetal brains were extracted for quantitation of oxidative stress (OS) markers. The other group and its control were anesthetized, perfused and fetal brains were prepared for histological examinations for ND. The results indicate a) rapid placental transfer, fetal brain uptake and retention of 2-14C-CAN/ metabolites in cerebral cortex and hippocampus, b) CAN uptake in fetal brain induced OS as indicated by decreased GSH/GSSG (4 folds), increased lipid per-oxidation (1.3 folds), and oxidative DNA damage (1.3 folds), c) Significant increase in apoptotic ND in treated fetal brains as detected by DeOlmos cupric silver staining in the cortical regions, and increased immunohistochemical labeling (TUNEL) of apoptotic nuclei in the cortex and choroid plexuses. In conclusion, CAN, following *in utero* exposure, crossed the placenta and fetal blood brain barriers and induced OS that triggered apoptotic ND in fetal brain. Future development of this model will explore the molecular biological mechanisms of these events and its impact on offspring.

1835 DELTAMETHRIN-INDUCED DELAYS IN BEHAVIORAL DEVELOPMENT.

M. A. Cheh[1], L. Michna[2], A. W. Kusnecov[3] and G. C. Wagner[3]. *[1]Neuroscience, Rutgers University, New Brunswick, NJ, [2]Toxicology, Rutgers University, New Brunswick, NJ and [3]Psychology, Rutgers University, New Brunswick, NJ.* Sponsor: K. Reuhl.

Deltamethrin (DM) is a commonly used insecticide purportedly having low acute mammalian toxicity. Here we show that prenatal exposure to DM delays the development of cerebellar-dependent skills in BALB/c mice. Pregnant mice were administered DM (10 mg/kg sc) or vehicle on embryonic day 13 (E13). Neonates were subsequently exposed to DM at the same dose or vehicle on postnatal day 14 (P14) (in a 2 X 4 design). The pups of treated dams showed no difference in their ability to surface right (P5-9). However, male E13-treated pups showed a delay in the maturation of the mid-air righting reflex and an impairment in their grip strength (P13-P19) while female pups were unaffected. Treatment with DM on P14 did not cause disruption of skill ontogeny or an exacerbation of the delay observed following E13 treatment. This is consistent with neurochemical analaysis of pups exposed to DM (10 mg/kg sc) or vehicle on P7 and P14. HPLC revealed no changes in striatal or cerebellar dopamine, serotonin, or their metabolites 2 hours after acute treatment with DM compared to age-matched vehicle-treated pups. In addition, acute exposure of adult C57/BLJ6 mice to DM (9 mg/kg) did not alter levels of dopamine, serotonin, or their metabolites in the striatum, hypothalamus, or frontal cortex at 1, 12, 24, or 72 hours following exposure. These observations indicate that prenatal exposure to low doses of DM alters behavioral development of males in a manner that appears to be independent of brain monoamines. Acknowledgements: NS 43981, ES 05022, ES 11256, NJ Governor's Council on Autism

1836 EXPLORING A FISH MODEL OF DEVELOPMENTAL ETHANOL NEUROTOXICITY.

S. Oxendine[1, 2], S. Padilla[1] and D. E. Hinton[3]. *[1]Neurotox. Division, USEPA, Research Triangle Park, NC, [2]Curr. in Toxico., UNC-CH, Chapel Hill, NC and [3]Nicholas School of the Environment, Duke University, Durham, NC.*

Rapid methods are needed for screening chemicals for potential toxicity to the developing nervous system. Methods are also needed that isolate the developing organism without confounding factors surrounding maternal detoxification or other influences. To meet these needs, we are assessing developmental neurotoxicity using Japanese medaka fish (*Oryzias latipes*) as an alternative animal model. Early life stage toxicity tests in fish may serve as developmental toxicity screens for evaluating the effects of chemical exposures. Ethanol is a well-documented mammalian neurotoxicant that produces craniofacial abnormalities, optic impairment, and cognitive deficits in individuals exposed during development. In the first study, pre-hatch medaka embryos were treated with 0.5 to 5.0% ethanol in embryo rearing medium at 26°C (n=10 per group). Treatment and observation of embryos began 24 hours after fertilization and continued for up to two weeks (day nine is the normal hatching day). Ethanol concentrations at or above 3% were lethal to embryos. A second study was designed to determine how the timing of ethanol exposure affects developmental toxicity. Medaka embryos were treated with 1% ethanol during three distinct periods of development (days1-3, 4-6, or 7-9 post fertilization) to determine potential windows of vulnerability. Whereas treatment during 7-9 days post fertilization tended to delay hatching, treatment during days 1-3 produced more abnormal embryos, suggesting that this stage represents a period of heightened sensitivity to ethanol. These results suggest that the medaka fish may serve as a useful alternative model for assessing neurotoxic potential during development. This is an abstract of a proposed presentation and does not imply Agency policy. **S. Oxendine is funded by the Minority Neuroscience Fellowship Program*

1837 DOPAMINE TRANSPORTER AND VESICULAR MONOAMINE TRANSPORTER LEVELS ARE INCREASED BY PERINATAL HEPTACHLOR EXPOSURE.

W. M. Caudle, J. R. Richardson, E. D. Dean, M. Z. Wang and G. W. Miller. *Center for Neurodegenerative Disease, Rollins School of Public Health, Emory University, Atlanta, GA.*

Heptachlor, a chlorinated cyclodiene insecticide, was a widely used pesticide for many years until it was banned in the late 1970s. In the early 1980s residents in Hawaii were exposed to high levels in the milk supply from contaminated dairy cattle, raising the concern that the offspring of women exposed to heptachlor may have been adversely affected. Previous studies have shown that gestational exposure of rats to 4.2 or 8.4 mg/kg/day from gestation day (GD)10 through GD21 results in increased levels of the dopamine transporter (DAT) in the offspring on postnatal days (PND) 10 and 22. In this study, we sought to determine the effects of longer-term lower level to exposure to heptachlor on DAT, and two other crucial components of the dopaminergic system, the vesicular monoamine transporter 2 (VMAT2) and tyrosine hydroxylase (TH). Female C57BL/6J mice received 0 or 3 mg/kg heptachlor in peanut butter every 3 days for 2 weeks prior to breeding. Treatment continued on the same schedule throughout gestation and lactation until the offspring were weaned on postnatal day (PND) 21. On PND 28, mice were sacrificed and the striatum was removed and frozen on dry ice for determination of monoamine levels by HPLC and dopaminergic markers by western blotting. Perinatal exposure to heptachlor resulted in no overt toxicity to either the dams or the offspring. Striatal levels of dopamine and its metabolites, DOPAC and HVA, were similarly unaffected by treatment. However, DAT levels were increased 2.1 fold. VMAT2 levels and TH levels were also increased by 1.7 and 1.3 fold, respectively. Since increased levels of DAT appear to predict susceptibility of brain regions to Parkinsons disease (PD) and organochlorine insecticides have been linked with increased risk for PD, these results suggest that alterations of the dopaminergic system by perinatal exposure to heptachlor may increase susceptibility to PD. (Supported by ES-012315).

1838 EFFICACY OF *IN VITRO* CARBOXYLESTERASE DETOXICATION OF ORGANOPHOSPHATES IN ADULT AND JUVENILE RAT LIVER AND SERUM.

J. T. Pittman[1], C. A. Moore[1], E. B. Shows[1], H. H. Chambers[2], J. L. Wagner[1] and J. E. Chambers[1]. *[1]College of Veterinary Medicine, Mississippi State University, Mississippi State, MS and [2]Department of Entomology, Mississippi State University, Mississippi State, MS.*

Juvenile animals are more vulnerable than adults to the acute toxicity of organophosphorus (OP) insecticides. Acute toxicity levels are strongly related to the presence and the efficiency of specific detoxication mechanisms, primarily several protective esterases such as carboxylesterases (CbxE). The *in vitro* sensitivities of liver [adult, postnatal day (PND) 12, and PND1] and serum [adult and PND 12] CbxE were determined for the active metabolites (oxons) of a group of OP insecticides (e.g. paraoxon, chlorpyrifos-oxon), to investigate age-related differences in toxicity. In addition, the effect of tissue concentration on the *in vitro* potency of the CbxE inhibitors was investigated. CbxE activity was assessed spectrophotometrically using 4-nitrophenyl valerate as the substrate. The assessment of the protective capabilities of CbxE for each oxon, in each age group, was based on IC50 values, calculated by linear regression. All oxons showed higher IC50 values for high tissue concentration compared to low concentration, reflecting the higher number of CbxE molecules present. The oxons tested showed a variable range of IC50 values for both liver and serum for each age group. Of the compounds tested, chlorpyrifos-oxon (Cpxn) had the lowest IC50 for both liver and serum in all ages, reflecting the high sensitivity of CbxE for Cpxn. IC50s were higher in serum than liver in most cases. Generally, for all of the OP compounds tested in both liver and serum, the IC50 for each age group increased with increasing age which may reflect the higher CbxE levels in adult tissue. These data indicate that the stoichiometric detoxication efficiency of CbxE is compound-specific as well as age-dependent, and it is of paramount importance to consider comparable tissue or enzyme concentrations when making comparisons of potency among compounds. (Supported by NIH R01 ES011287)

1839 EFFECTS OF EARLY POSTNATAL ORAL EXPOSURE TO CHLORPYRIFOS AND METHYL PARATHION ON CHOLINE ACETYLTRANSFERASE IN REGIONS OF THE RAT BRAIN.

J. A. Dobbs, M. B. Dail, R. L. Carr, E. C. Meek and J. E. Chambers. *Center for Environmental Health Sciences, Mississippi State University, Mississippi State, MS.*

The effects of early postnatal exposure to the commonly used organophosphorus insecticides chlorpyrifos (CPS), a diethyl phosphorothionate, and methyl parathion (MPS), a dimethyl phosphorothionate, on choline acetyltransferase (ChAT) activ-

ity in regions of the developing rat brain were investigated. Using an incremental dosing regimen, postnatal rats were orally gavaged daily with either corn oil or chlorpyrifos at a low dose of 1 mg/kg from postnatal day (PND) 1 to 21; a medium dose of 1mg/kg from PND 1-5, 2mg/kg from PND 6-13, and 4mg/kg from PND 14-21; a high dose of 1.5mg/kg from PND 1-5, 3mg/kg from PND 6-13, and 6mg/kg from PND 14-21, or methyl parathion at a low dose of 0.2mg/kg from PND 1-21; a medium dose of 0.2mg/kg from PND 1-5, 0.4mg/kg from PND 6-13, and 0.6mg/kg from PND 14-21; a high dose of 0.3mg/kg from PND 1-5, 0.6mg/kg from PND 6-13, and 0.9mg/kg from PND 14-21. Exposures to insecticides did not produce signs of overt toxicity as indicated by no adverse effects on weight gain. Cholinesterase (ChE) activity was measured spectrophotometrically. Animals displayed a range of 10-50% brain ChE inhibition on PND 21, 10-40% at PND 30 and 10-30% at PND 40. Pups were sacrificed on postnatal days 25, 30 and 40 for the radioenzymatic microdetermination of ChAT activity in the hippocampus and medulla/pons. Pups exposed to low doses of MPS exhibited an increase in hippocampal ChAT activity in all age groups. In medulla/pons of MPS treated rats, significant increases were seen in the high dose group at PND 30, and in the low and medium dose groups at PND 40. Within the CPS treatments, the medulla/pons of the high dose animals at PND 40 was significantly higher than controls. Cerebral cortex and corpus striatum are currently under investigation following the same protocol. (Supported by NIH R01 ES 10386).

1840 HUMAN LIVER CARBOXYLESTERASE DURING POSTNATAL MATURATION AND ITS SENSITIVITY TO CHLORPYRIFOS OXON.

C. Pope[1], S. Karanth[1], J. Liu[1], J. Shaikh[1] and B. Yan[2]. [1]*Physiol Sciences, Oklahoma State University, Stillwater, OK and* [2]*Biomed Sciences, University Rhode Island, Kingston, RI.*

Age-related expression of carboxylesterase activity in laboratory animals has been correlated with differential sensitivity to organophosphorus insecticides including chlorpyrifos. We compared human liver carboxylesterase activity (using p-nitrophenyl acetate as substrate) in samples from infants of 2, 3, 4, 8 and 24 months of age and adults (20 and 21 years of age). Liver S9 fractions were obtained from XenoTech (Lenexa, KS) with information on patient demographics and microsomal enzyme activities. The rank order of carboxylesterase activity in the age groups was determined (2 mo<3 mo<20 yr<24 mo<4 mo<21 yr<8 mo). Carboxylesterase activity across samples was highly correlated with microsomal cytochrome b5 (Spearman r=0.93, p=0.007) and testosterone 6β-hydroxylation (r=0.96, p=0.003) but was not correlated with other microsomal activities (total cytochrome P450, coumarin 7-hydroxylation, 7-ethoxyresorufin O-dealkylation). Proteins (3 μg) of each sample were separated and blotted using antibodies against recombinant rat hydrolase S (HS) and human carboxylesterase (HCE) types 1, 2 and 3. Relatively similar levels of HS and HCE-1 (ranges: 19.1-28.3, 17-28.2 units, respectively) were noted across age groups except for the 2 mo sample (0.8, 8.5 units). Generally lower HCE-2 levels were noted in these same samples (3.6-20.1 units), but with the lowest level (1.5 units) again noted in the 2 mo sample. Within the 3 mo-21 yr subset, no correlation between age and expression of either HS, HCE-1 or HCE-2 was noted. HCE-3 was not detected in any fractions. Aliquots were preincubated (30 min, 37°C) with a range of chlorpyrifos oxon concentrations to evaluate *in vitro* sensitivity. No significant differences in IC50 values were obtained in 3 mo-21 yr samples (range: 1.42-2.12 nM), while IC50 was significantly lower in the 2 mo sample (0.5 nM). While preliminary, these results suggest that liver carboxylesterase expression or sensitivity to chlorpyrifos oxon changes little during postnatal maturation after about 3 months of age.

1841 EFFECTS OF PERINATAL EXPOSURE TO PCB 153 ON THE BRAIN NEUROTRANSMITTERS OF OFFSPRING RATS.

T. Honma, M. Miyagawa, M. Suda, R. Wang, K. Kobayashi and S. Sekiguchi. *Department of Health Effects Research, National Institute of Industrial Health, Kawasaki, Japan.* Sponsor: M. Chiba.

Different types of toxicity of PCBs have been investigated. Some reports have suggested that exposure to PCB mixtures at perinatal period may affect the development of the central nervous system both in humans and animals, however, developmental neurotoxicity of each congener remains still unclarified. To investigate the effects of *in utero* exposure to PCB 153 (2, 2', 4, 4', 5, 5'-hexachlorobiphenyl), a representative non-planar PCB congener, on hormonal and neurobehavioral development in the offspring of rats, pregnant CD (SD) IGS rats were given PCB 153 (0, 16, 64 mg/kg/day) orally on gestation day 10 (GD 10) to day 16 (GD 16). Offspring rats were examined at different weeks of age regarding the effects of PCB 153 on (1) endocrine system (thyroid and testis), (2) brain neuroactive substances, and (3) cognitive functions (learning and memory). In this paper, we described the effects of PCB 153 on the brain contents of neuroactive monoamines of the offspring. PCB exposure did not produce any significant effects on body weights of dams during gestation and lactation. No dose-dependent changes in body weights, body length, and organ weights in offspring were observed. On post natal day (PND) 7, 21, and 42, serotonin (5HT) and its metabolites, 5-hydroxyindoleacetic acid content of the brain of male rats increased in a dose-dependent manner. Dopamine (DA) and its metabolites, 3, 4-dihydroxyphenylacetic acid and homovanillic acid, increased in the male brain on PND 7 and 42. These results indicate that 5HT and DA release from the nerve terminals was increased. The activity of 5HT and DA neurons in the male brain may be elevated in some limited situations following perinatal exposure to PCB 153. These results suggest that the exposure to relatively high dose of PCB 153 at the early stage of life alters the cerebral neurotransmission in the offspring rats.

1842 PERINATAL EXPOSURE TO DELTAMETHRIN ALTERS DOPAMINERGIC NEUROCHEMISTRY IN THE DEVELOPING MOUSE BRAIN.

J. R. Richardson, W. M. Caudle, E. D. Dean, M. Z. Wang and G. W. Miller. *Center for Neurodegenerative Disease, Rollins School of Public Health, Emory University, Atlanta, GA.*

During the last decade there has been increasing concern that children are not adequately protected from the adverse effects of pesticides. Because of this concern, many pesticides have had their uses restricted or banned. In their place, pyrethroids have become one of the pesticides of choice because they are environmentally labile and generally exhibit low mammalian toxicity. Recently, it has been shown that deltamethrin (DM), a widely used type II pyrethroid, may exert specific effects on the nigrostriatal dopaminergic system by altering striatal dopamine uptake and dopamine transporter (DAT) levels in adult mice. In this study, we sought to determine whether perinatal exposure to low levels of DM would alter dopaminergic neurochemistry in the developing mouse brain. Female C57BL/6J mice received 0 or 3 mg/kg DM in peanut butter every 3 days for 2 weeks prior to breeding. Treatment continued on the same schedule throughout gestation and lactation until the offspring were weaned on postnatal day (PND) 21. On PND 28, mice were sacrificed and the striatum was removed and frozen on dry ice for determination of monoamine levels by HPLC and dopaminergic markers by western blotting. Perinatal exposure to DM elicited no overt toxicity to either the dams or the offspring. Striatal levels of dopamine or its metabolites, DOPAC and HVA, were not affected by treatment. However, levels of the dopamine transporter (DAT) were increased by 1.9 fold, similar to that seen in adult animals exposed to higher dosages of DM. In addition, we determined the levels of the vesicular monoamine transporter 2 (VMAT2) and tyrosine hydroxylase (TH), two additional markers of dopaminergic neurons. Striatal VMAT2 levels were increased by 1.7 fold and TH levels were increased by 1.5 fold in the offspring of DM treated animals. Taken in concert, these data suggest that the developing dopaminergic system is particularly sensitive to alterations induced by low level exposure to DM during the perinatal period. (Supported by DOD-00267036).

1843 METHAMPHETAMINE-INITIATED NEURODEVELOPMENTAL DEFICITS ARE ENHANCED IN *OXOGUANINE GLYCOSYLASE 1* (*OGG1*) KNOCKOUT MICE.

A. W. Wong[1], W. Jeng[1] and P. G. Wells[1, 2]. [1]*Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada and* [2]*Department of Pharmacology, University of Toronto, Toronto, ON, Canada.*

Teratogenesis may involve oxidative DNA damage, in particular the lesion 8-oxoguanine (8-oxoG), which in mammals is repaired by oxoguanine glycosylase 1 (Ogg1). We have shown that *in utero* exposure to methamphetamine (METH) on gestational day (GD) 17 enhances DNA oxidation in CD-1 fetal mouse brain, and causes permanent postnatal motor coordination deficits [Toxicologist 72(S-1): 342 (No. 1661), 2003]. Also, *ogg1* knockout mice are more susceptible to METH-enhanced 8-oxoG levels in the fetal brain [Toxicologist 72(S-1): 342 (No. 1663), 2003]. Here we used *ogg1* knockout mice to investigate the developmental roles of 8-oxoG and Ogg1 in the respective initiation of and risk for METH-initiated neurodevelopmental anomalies following *in utero* exposure. METH (40 mg/kg) or its saline vehicle was administered on GD 17 to pregnant heterozygous (+/-) *ogg1*-deficient females (mated to *ogg1* +/- males). Beginning at 6 weeks after birth, the offspring were tested for motor coordination using the rotarod apparatus. Female ogg1 homozygous *null* (-/-) offspring exposed *in utero* to METH had enhanced coordination deficits compared to saline-exposed controls of the same genotype at 6, 8, 10 and 12 weeks (p=0.03, p<0.001, p=0.008 and p=0.04, respectively). Long-term deficits were also observed in female -/- offspring compared to wild type (+/+) littermates at 8, 10 and 12 weeks (p<0.001, p<0.01 and p=0.005, respectively), and compared to +/- littermates at 6 and 8 weeks (p=0.02 and p=0.05, respectively).

Exposure to METH in male -/- mice resulted in enhanced coordination deficits compared to saline -/- controls at 6 and 8 weeks ($p<0.05$). This is the first evidence of postnatal motor coordination impairment in drug-exposed mice fetuses lacking Ogg1, indicating that 8-oxoG constitutes an embryopathic molecular lesion, and that functional DNA repair in the fetus may be important in protecting against METH-initiated neurodevelopmental deficits. (Support: Canadian Institutes of Health Research; SOT Covance Fellowship [WJ])

1844 INCREASED METHAMPHETAMINE-ENHANCED DNA OXIDATION IN FETAL BRAIN OF *COCKAYNE SYNDROME B* (*CSB*) KNOCKOUT MICE.

T. J. Preston[1], P. G. Wells[1, 2], W. Jeng[1] and A. W. Wong[1]. *[1]Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada and [2]Department of Pharmacology, University of Toronto, Toronto, ON, Canada.*

Repair of 8-oxoguanine (8-oxoG), a form of oxidative DNA damage, on the non-transcribed strand of DNA is primarily catalyzed by oxoguanine glycosylase 1 (Ogg1). However, 8-oxoG in actively transcribed DNA is repaired *via* the transcription-coupled repair (TCR) pathway, catalyzed by the Cockayne Syndrome B (CSB) protein. We have shown that knockout mice deficient in Ogg1 have enhanced levels of DNA damage to the fetal brain following *in utero* exposure to the neurotoxic drug methamphetamine (METH) on gestational day (GD) 17 [Toxicologist 72(S-1): 342 (No. 1663), 2003]. Here we used *CSB* knockout mice to investigate the developmental role of CSB in repairing METH-initiated fetal DNA oxidation following *in utero* exposure. METH (40 mg/kg) or its saline vehicle was administered on GD 17 to pregnant heterozygous (+/-) *CSB*-deficient females (mated to *CSB* +/- males). Dams were sacrificed 4 hr later, and fetal brains were isolated and analyzed for oxidative DNA damage, determined by 8-oxoG formation. In fetal brain, no significant differences were observed in DNA oxidation among saline-exposed wild type (+/+) *CSB*-normal, or +/- or homozygous *null* (-/-) *CSB*-deficient fetuses. However, with METH exposure, there was a 40% increase in 8-oxoG levels in -/- *CSB*-deficient fetuses compared to +/+ *CSB*-normal littermates ($p<0.05$). DNA oxidation also was elevated in fetal brain from METH-exposed +/- fetuses compared to saline-exposed controls of the same genotype ($p<0.05$), and in METH-exposed -/- fetal brains compared to saline-exposed -/- controls ($p=0.001$). This is the first evidence of increased DNA oxidation in drug-exposed fetuses lacking CSB, and suggests that in addition to Ogg1, CSB and the TCR pathway may contribute in protecting against METH-initiated developmental neurotoxicity. (Support: Canadian Institutes of Health Research; SOT Covance Fellowship [WJ])

1845 EFFECTS OF LOW DOSE PERINATAL VINCLOZOLIN EXPOSURE ON A BATTERY OF ANDROGEN-MEDIATED BEHAVIORS.

N. W. Colbert, J. B. Concannon and V. P. Markowski. *Environmental Toxicology Center, University of Southern Maine, Portland, ME.*

Vinclozolin (Vz) is a fungicide applied to industrial landscapes, golf turf, and foods intended for human consumption. Health concerns have arisen because metabolites of Vz bind to the androgen receptor, alter the expression of androgen-responsive genes and disrupt the development of male reproductive organs. Since androgens are also responsible for sex differentiation in the CNS, Vz could permanently affect sex-specific behaviors. Pregnant Long-Evans rats were administered 0, 1.5, 3, 6, or 12mg/kg Vz p.o. from gestation day 14 to postnatal day (PND) 3. Endpoints following parturition including pups per litter, sex ratio, mortality, male nipple retention, anogenital distance, and body weight throughout the preweaning period were not affected. Although we did not observe gross maternal or neonatal toxicity, Vz affected a number of behaviors in juvenile and adult male offspring. Two sex-differentiated functions were examined in immature males and females: social play behavior on PND22 and PND34 and the corticosterone stress response on PND34. In adult males, ex copula penile erections and copulatory behavior was also examined. During play behavior, males performed more of the target behaviors than females regardless of age. Although there were no exposure effects in PND22 offspring, the PND34 males showed evidence of dose-related demasculinization. In the stress procedure, female offspring responded to restraint with significantly higher plasma corticosterone compared to males. There was also a significant main effect of Vz exposure, although the relationship was complex and nonlinear. Adult males in each of the Vz dose groups produced significantly fewer erections than controls during ex copula tests. Low intensity erections, characterized by distension of the glans and tumescence of the base, were the most sensitive to exposure. As a consequence of Vz effects on social and erectile behaviors, exposed males showed numerous copulatory deficits during group mating and subsequently sired fewer offspring.

1846 AGE-DEPENDENT INCREASES IN MDMA-MEDIATED DOPAMINERGIC NEUROTOXICITY IN MICE.

M. E. Reveron[1], G. V. Erives[2], S. S. Lau[2] and T. J. Monks[2]. *[1]Division of Pharmacology and Toxicol., University of Texas at Austin, Austin, TX and [2]Department of Pharmacology and Toxicol., University of Arizona Health Sciences Center, Tucson, AZ.*

3, 4-Methylenedioxyamphetamine (MDA) and 3, 4-methylenedioxymethamphetamine (MDMA, ecstasy) are ring-substituted amphetamine derivatives with stimulant and hallucinogenic properties. The recreational use of these amphetamines, especially MDMA, is prevalent despite warnings of irreversible damage to the central nervous system. In rats and non-humans primates, MDMA elicits chronic damage to the serotonergic system, whereas in mice it causes long-term damage to the dopamine (DA) system. Although the extent of MDMA-mediated serotonergic neurotoxicity in rats is age-dependent, little is known of the factors regulating MDMA-mediated dopaminergic neurotoxicity in mice. We therefore examined the age-dependent MDMA-mediated dopaminergic toxicity in C57B1/6J mice (5 and 11 weeks old). Mice were treated with (+)-MDMA (20mg/kg, sc.) every two hours, for a total of four injections. MDMA elevates core body temperature, which appears to play an important role in MDMA-induced neurotoxicity, and increases in rectal temperature of 0.9°C and 2.1°C were observed in mice aged 5 and 11 weeks respectively. Significant reductions in striatal DA (87% decrease) concentrations, and its metabolites, homovanillic acid, and 3, 4-dihydroxyphenylacetic acid, were found in older mice, with reductions in DA of only 24% in the younger mice. Immunoblot analysis of the dopamine transporter, the vesicular monoamine transporter 2, and tyrosine hydroxylase revealed significant reductions of 70%, 19%, and 58% respectively in the older mice. Significant reductions in dopamine transporter expression (26%) also occurred in the younger mice. Interestingly, we documented a mild but significant (22%) decrease in striatal serotonin (5-HT) concentrations in the older mice. We conclude that age plays an important role in determining the magnitude of MDMA-mediated neurotoxicity in mice, and perhaps in determining the selectivity of the toxicity (DA vs 5-HT).

1847 THE EFFECT OF ACUTE ETHANOL EXPOSURE ON OXIDATIVE STRESS AND CASPASE-3 ACTIVE SUBUNIT EXPRESSION IN POSTNATAL DAY 4 RAT CEREBELLUM.

K. H. Horn[1], L. M. Kamendulis[2], C. R. Goodlett[3, 1] and J. E. Klaunig[2, 1]. *[1]Program in Medical Neurobiology, Indiana University School of Medicine, Indianapolis, IN, [2]Department of Pharmacology and Toxicology; Division of Toxicology, Indiana University School of Medicine, Indianapolis, IN and [3]Psychology, Indiana University Purdue Univeristy at Indianapolis, Indianapolis, IN.*

Microencephaly, a hallmark feature for the clinical diagnosis for Fetal Alcohol Spectrum Disorder results from the loss of cell populations within the CNS. Oxidative stress leading to apoptosis has been suggested as a potential mechanism underlying ethanol-induced cell death. Recent *in vitro* work demonstrated protection against ethanol-induced cerebellar granule cell loss following antioxidant supplementation. In addition, catalase activity was increased in the cerebellum 6- and 12-hours after ethanol exposure to postnatal day 4 (PD4) rat pups. In this study, we investigated ethanol's ability to decrease total antioxidant capability (TAC), increase reactive oxygen species (ROS), and increase capase-3 active subunit expression following ethanol exposure on PD4. Long Evans rat pups were matched for sex and rats within each pair received either milk intubations or 5.25g/kg ethanol (11.9% (v/v) solution in milk) in 2 intubations, 2 hours apart. Cerebellar tissue was collected and TAC, ROS, or caspase-3 active subunit expression was measured. Results indicated that a single ethanol exposure on PD4 had no effects on TAC. In contrast, ethanol treatment caused significant increases in cerebellar ROS levels [$F(1, 24)=4.939$, $p<0.05$] 12 hours after the initial intubation ($t(10)=2.564$, $p<0.05$). In addition, caspase-3 active subunit expression was significantly increased following ethanol exposure on PD4 [$F(1, 25)=19.817$, $p<0.01$], with post hoc analyses indicating significant increases at the 8- and 12-hour time points ($p<0.05$). These data, in conjunction with our previous findings that ethanol exposure increases catalase activity, suggest that oxidative stress may contribute to ethanol-induced cell loss within this model.

1848 POLYAROMATIC HYDROCARBON MIXTURE EFFECTS ON CENTRAL NERVOUS SYSTEM DEVELOPMENT, PLASTICITY, AND BEHAVIOR.

D. B. Hood, D. D. Wormley, T. Nayyar, J. Wu, T. Tu and S. Johnson. *pharmacology, meharry medical college, Nashville, TN.*

Environmental contaminants within the polyaromatic hydrocarbon class have been shown to cross the placenta, and by doing so, the fetus is exposed to the contaminant body burden of the mother. Consequently, a gestational exposure to environmental contaminant polyaromatic hydrocarbons may result in increased adverse

health outcomes, possibly affecting cognitive performance. Benzo(a)pyrene [B(a)P], and 2, 3, 7, 8, tetrachlorodibenzo-p-dioxin (TCDD) are two prototypical polyaromatic hydrocarbon environmental contaminants. A systematic review of the literature indicates that there is a relationship between health disparities and, normal and dysfunctional cognitive development and functioning. Gestational exposure studies were conducted where timed-pregnant rats were exposed to: 100µmg/m3 B(a)P (GD14-17; nose-only inhalation) and/or 700 ng/kg TCDD (GD 14; gavage). The results reveal polyaromatic hydrocarbon/mixture induced decrements in: 1) birth index, 2) NMDAR mRNA expression, 3) long-term potentiation (LTP), 4) fixed-ratio performance learning behavior, and 5) activity related cytoskeletal associated protein (Arc) mRNA and protein expression. These data provide a point of reference for neurotoxicological studies of polyaromatic hydrocarbon/ mixture effects on development in general, and on specific indices of learning and memory in particular. The data support associations between gestational exposure to environmental levels of these contaminants and neurodevelopmental delay that has been previously reported in historical cohort studies. The data suggest that the effect of gestational exposure to polyaromatic hydrocarbon/mixtures is to dysregulate hippocampal-based synaptic plasticity mechanisms.

1849 PRENATAL EXPOSURE OF TCDD DECREASED HIPPOCAMPAL ARC AND NMDAR1 EXPRESSION IN F1 GENERATION RATS.

J. Wu, T. Nayyar, T. Tu, S. Johnson and D. B. Hood. *pharmacology, meharry medical college, Nashville, TN.*

Activity-regulated cytoskeleton-associated protein (Arc) is an effector immediate-early gene that is proposed to involve synaptic plasticity. Sensory stimulation and placement in a novel environment induce expression of this gene, which is associated with learning new behaviors. Newly synthesized Arc mRNA migrates to dendrites and, following induction of long-term potentiation (LTP), localizes to activated post-synaptic dendritic domains in an N-methyl-D-aspartate receptor (NMDAR) dependent manner. TCDD (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin) is a prototypical polyaromatic hydrocarbon environmental contaminant that is widely distributed and persistent throughout the ecosystem. Our previous studies demonstrated that prenatal exposure to TCDD reduced LTP in the hippocampal dentate gyrus and caused the deficits in learning and memory behavior in F1 generation rats. To explore the molecular basis of these changes, timed-pregnant Long-Evans rats were exposed to TCDD (100 or 700ng/kg BW) by gavage on GD 14. The mRNA and protein expression of Arc and NMDAR1 were quantified by Real-time PCR and western blot analysis from the hippocampus of F1 generation rats that were specially trained for fix-ratio performance on PND70. The results showed that: (1) Arc mRNA and protein expression in trained rats were found to be significantly induced as compared to constitutive expression in untrained rats. (2) A dose-dependent decrease (20-30%) in Arc mRNA and protein expression was observed in TCDD exposed trained rats relative to unexposed trained rats. (3) The constitutive Arc mRNA and protein expression is also significantly decreased in TCDD exposed untrained rats relative to unexposed untrained rats. (4) A down-regulation (15-20%) in NMDAR1 mRNA and protein expression was found in TCDD exposed rats as compared to unexposed control rats. These data correlate well with our previous findings from LTP testing and the fixed-ratio performance learning behavior studies and suggest that prenatal TCDD exposure decreases the capacity for synaptic plasticity and learning ability in offspring.

1850 ASSESSING THE BIOLOGICAL AND ENVIRONMENTAL RISKS OF NANOPARTICULATES.

D. B. Warheit[1] and J. Everitt[1]. [1]*DuPont Haskell Lab., Newark, DE and* [2]*GlaxoSmithKline, Reseach Triangle Park, NC.*

This symposium is a basic primer on nanoparticles and health. After a brief introduction, Dr. Vicki Colvin, director of Rice University CBEN will discuss potential environmental risks related to nanomaterials. Few data exist on the environ. effects of engineered nanomaterials, yet some NGOs are calling for bans on these systems. Govt. policy issues will also be presented. Next, Dr. Sally Tinkle will discuss skin exposure to fine particulates: mechanism of entry and biological response. Previously, it was assumed that exposures to fine particles could not penetrate the stratum corneum of the skin; however, particle size may play a role in skin penetration potential. Dr. Gunter Oberdorster will discuss the pulmonary and extrapulmonary disposition of inhaled nanoparticles. Airborne particles <100 nm in diam. in urban air along with nanotechnol. particles (<10 nm) raise health concerns for humans. Toxicology. studies with different types of nano/ultrafine particles suggest a range of adverse effects. Translocation of 10 to 50 nm sized particles from the respiratory tract to other organs, including the CNS and heart, have also been demonstrated. Dr. Jeff Everitt will next discuss a recent interspecies study of lung responses to inhaled ultrafine TiO2 particles. Female rats, mice and hamsters were exposed to aerosols of ufTiO2 particles for 13 weeks and evaluated through 1 year. At higher doses the adverse lung responses in rats were significantly greater than the other 2 species. The final presentation will discuss lung bioassay studies of intratracheally instilled single wall carbon nanotubes (SWCNT). Individual SWCNT have dimensions of 1 nm (diameter) and lengths > 1 um, yet, SWCNT have a high electrostatic potential and thus agglomerate, forming bundles of 10 to 100 SWCNT, so-called nanoropes. There are very low respirable concentrations of SWCNT in the workplace (i.e., < 0.1 mg/m3). SWCNT instilled in the lungs of rats or mice have produced granulomas. Because of agglomeration, however, the relevance of these findings are questionable and should be confirmed *via* an inhalation study with SWCNT.

1851 ENVIRONMENTAL IMPACTS OF ENGINEERED NANOMATERIALS: RESEARCH FROM THE CENTER FOR BIOLOGICAL AND ENVIRONMENTAL NANOTECHNOLOGY.

V. Colvin. *Rice University, Houston, TX.* Sponsor: D. Warheit.

Traditionally, nanotechnology has been motivated by the growing importance of very small (diameter < 50 nm) computational and optical elements in diverse technologies. However, this length scale is also an important and powerful one for living systems. At Rice, we believe that the interface between the 'dry' side of inorganic nanostructures and the 'wet' side of biology offers enormous opportunities for medicine, environmental technologies, as well as entirely new types of nanomaterials. As part of our work on the potential biological applications, we also consider the unintended environmental implications of water soluble nanomaterials. Given the breadth of nanomaterial systems, we use a carefully selected group of model nanoparticles in our studies and focus on natural processes that occur in aqueous systems. We characterize the size and surface-dependent transport, fate and facilitated contaminant transport of these engineered nanomaterials. Models from larger colloidal particles can be extended into the nanometer size regime in some cases, while in others entirely new phenomena present themselves. We also consider biological interactions of nanoparticles and evaluate their biotransformation, bioremediation and accumulation under realistic environmental conditions. Finally, this exposure data is placed in the context of the known health effects of nanomaterials. In this regard, there is surprisingly little concrete data on the health or environmental effects of engineered nanomaterials, but that hasn't stopped a number of organizations from calling for bans on these systems. An overview of the latest policy and media coverage of this timely issue will be highlighted along with a summary of the current state of knowledge in the area.

1852 SKIN EXPOSURE TO PARTICLES: PENETRATION IS DEPENDENT ON PARTICLE SIZE.

S. S. Tinkle. *NIAID, NIH, Bethesda, MD.* Sponsor: D. Warheit.

Previously, skin exposure to fine or nanoparticles was not considered hazardous. The external layer of the skin, the stratum corneum (SC), is considered a mechanically strong and resilient structure that protects internal organs from environmental exposures. We tested the ability of particles of varying size to traverse intact SC and initiate an immunological response in the epidermis. Fluorospheres, 0.5 to 4 micron diameter, were applied to recovered human skin for 0, 15, 30 or 60 minutes, in the presence and absence of a 45 degree flexing motion, as occurs at the wrist. Location of these fluorescent particles in the skin was examined by laser scanning confocal microscopy. Fluorospheres, 1 micron or smaller, penetrated the SC and reached the epidermis in 41% and 54% of the skin samples flexed for 30 and 60 minutes, respectively. In addition, no fluorospheres were observed in the epidermis in the absence of flexing motion. We tested the ability of skin particulate exposure to induce murine cutaneous hypersensitivity using beryllium oxide. Beryllium (Be) stimulates an MHC Class II-restricted, CD4+ T cell-mediated immune response in approximately 3 to 5% of Be-exposed workers. Topical application of BeO to C3H/HeOuJ mice stimulated Be-specific lymphocyte proliferation and changes in the T lymphocyte cell activation markers CD44 and CD69L. To examine the effects of particulate exposure on gene expression, we compared 24 hour exposure of MH-S cells to both 1 micron chemically-inert polystyrene spheres and to size-selected beryllium oxide particles using the Affymetrix gene array. Polystyrene beads induced significant changes in structural proteins and cellular metabolism genes, whereas BeO particles induced changes in adhesion molecules and the MHC Class III region genes. These data support our hypothesis that skin exposure to smaller particles, in the size range of 0.5 - 1.0 um, can penetrate intact SC and induce a CMI response. Particles in the nanoscale size range (i.e., < 100 nm) have not yet been tested. Furthermore, inert particle exposure alters gene expression and cellular metabolism differently than exposure to a sensitizing particle.

1853 BIOLOGICAL EFFECTS AND FATE OF INHALED NANO/ULTRAFINE PARTICLES.

G. Oberdorster. *Environmental Medicine, University of Rochester, Rochester, NY.*

Airborne particulates <100 nm in diameter. occur at very high number concentrations in ambient air, specifically urban air, and at certain workplaces. More recently, nanotechnological developments have created new and even smaller sized spherical particles (<10 nm) concomitant with the development of nanotubes for applications ranging from electronics to biomedical fields. Their use in these areas raises concerns regarding potential exposures of humans and subsequent potential adverse health effects should they become airborne. Toxicology studies involving different types of nano- or ultrafine particles have revealed a wide range of adverse effects, ranging from mild to very potent acute pulmonary inflammatory responses, to effects on extrapulmonary organs such as the cardiovascular system. The large surface area per given mass of ultrafine particles is of toxicological importance because it correlates well with their potential to cause lung inflammation and oxidative stress. In addition, ultrafine particles at sizes below 50 nm have a high probability to be deposited throughout the respiratory tract upon inhalation. Following deposition, translocation of 10 to 50 nm sized particles from alveolar regions of the respiratory tract to interstitial sites as well as to other extrapulmonary organs, including the CNS, has also been demonstrated, yet many unanswered questions remain regarding their potency to cause health effects at these sites. Examples of anthropogenic ultrafine particles at the workplace and in the ambient air will illustrate human exposure scenarios. And results from toxicological investigations are presented to demonstrate their potential for causing adverse health effects.

1854 COMPARISON OF INTERSPECIES LUNG RESPONSES TO ULTRAFINE (NANO) TITANIUM DIOXIDE PARTICLES.

J. Everitt and E. Bermudez. *CIIT, Research Triangle Park, NC.*

The pulmonary responses of 3 rodent species to inhaled nanoTiO2 particles, i.e., ultrafine TiO2 (uf-TiO2) particles (15 to 40 nm particle size) were compared. Female rats, mice, and hamsters were exposed to aerosol concs. of 0.5, 2.0, or 10 mg/m3 uf-TiO2 particles 6 h per day, 5 days per week for 13 weeks. Following the exposure period, animals were held for recovery periods of 4, 13, 26 or 52 weeks (49 weeks for the uf-TiO2-exposed hamsters) and at each time point uf-TiO2 burdens in the lung and lymph nodes and selected lung responses were examined. Retained lung burdens were maximal at the end of exposure in all three species and increased in a dose-dependent manner. Lung burdens in all three species decreased with time after exposure and at the end of the recovery period the percentage of the lung particle burden remaining in the 10 mg/m3 group was 57, 45, and 3 % for rat, mouse, and hamster, respectively. The retardation of particle clearance from the lungs in mice and rats of the 10 mg/m3 group indicated that pulmonary particle overload had been achieved in these animals. Pulmonary inflammation was evident in rats and mice exposed to 10 mg/m3 as evidenced by increased numbers of macrophages and neutrophils and increased concentrations of soluble markers in bronchoalveolar lavage fluid (BALF). There were no significant changes in cellular response or of markers indicating toxicity in hamsters, reflecting the capacity of these animals to rapidly clear particles from the lung. Progressive epithelial and fibroproliferative changes were observed in rats of the 10 mg/m3 group and were limited to this species. Clearance of particles from the lung was markedly impaired in mice and rats exposed to 10 mg/m3 uf-TiO2, whereas clearance in hamsters did not appear to be affected at any of the administered doses. These data are consistent with the results of a companion study using inhaled pigmentary (fine mode) TiO2 (Bermudez et al., 2002) and demonstrate that the pulmonary responses of rats exposed to ultrafine particulate concentrations likely to induce pulmonary overload are different from similarly exposed mice and hamsters.

1855 PULMONARY BIOASSAY STUDIES WITH CARBON NANOTUBES IN RATS.

D. B. Warheit. *Pulmonary Toxicology, DuPont Haskell Lab., Newark, DE.*

The pulmonary toxicity of intratracheally instilled single wall carbon nanotubes (SWCNT) in rats was evaluated. The lungs of rats were instilled with either 1 or 5 mg/kg of 1) SWNT; 2) quartz particles, or 3) carbonyl iron particles (CI) Phosphate-buffered saline (PBS) + 1% Tween 80 instilled rats served as additional controls. For the lung tissue studies, graphite particles were added as an additional particle control. Following exposures, the lungs of PBS and particle-exposed rats were assessed both using bronchoalveolar lavage (BAL) fluid biomarkers, cell proliferation methods, and by histopathological evaluation of lung tissue at 24 hrs, 1 week, 1 month and 3 months post-instillation exposure. Exposures to high dose (5 mg/kg) SWCNT produced mortality in ~15% of the SWCNT-instilled rats within 24 hrs post-instillation due to mechanical blockage of the airways. The bronchoalveolar lavage and cell proliferation results demonstrated that lung exposures to quartz particles but not SWCNT or CI produced sustained increases vs. controls in pulmonary inflammation, cytotoxicity, and lung cell parenchymal cell proliferation indices. Histopathology indicated that pulmonary exposures to quartz particles (5 mg/kg) produced dose-dependent inflammatory responses, concomitant with foamy alveolar macrophage accumulation and lung tissue thickening at sites of normal particle deposition. Pulmonary exposures to SWCNT in rats produced a non dose-dependent series of multifocal granulomas in the airways, which was evidence of a foreign tissue body reaction. Due to the agglomerative nature of the SWCNT, the lack of lung toxicity by assessing lavage and cell proliferation parameters, the non-uniform distribution of lesions, and the low exposure potential measured in occupational exposure assessment studies, the physiological relevance of these results remains to be determined. Therefore, to reconcile the apparent results discrepancies in this pulmonary bioassay study, it is critical that the pulmonary effects of SWCNT soot in rats must by evaluated by conducting an inhalation toxicity study.

1856 MOLECULAR PROFILING AND COMPUTER MODELING IN EARLY DETECTION AND TREATMENT OF CANCER.

R. Thomas. *Canswers, McLean, VA.*

Cancer diagnosis is traditionally an ad hoc activity where the quality of diagnosis and treatment is often limited by experience and available tools. There exists an opportunity to provide clinicians with important new tools to increase the potential for patients to receive superior diagnosis and treatment. At the core of this opportunity are enhanced research and bioinformatics tools which allow toxicologists and other researchers to identify and exploit gene and molecular profiles associated with many stages of cancer development. For example, such profiles are currently being explored as methods for early detection of ovarian and breast cancer. In addition, gene and molecular profiles have shown promise in identifying aggressive tumor subtypes and in predicting patient outcomes. The purpose of this symposium will be to explore some of the most recent advances in gene profiling and bioinformatics tools now available to toxicologists, clinicians and other researchers.

1857 THE MOLECULAR STAGING OF COLORECTAL CANCER.

W. E. Grizzle[1], University. Manne[1], N. Jhala[1], C. Suarez-Cuervo[1], S. Meleth[2] and D. Alexander[3]. *[1]Pathology, University of Alabama at Birmingham, Birmingham, AL, [2]Medicine/Biostatistics Unit, University of Alabama at Birmingham, Birmingham, AL and [3]Epidemiology, University of Alabama at Birmingham, Birmingham, AL.* Sponsor: R. Thomas.

Outcomes of patients with adenocarcinomas of the colorectum (CRC) are best predicted by TNM staging and by race and age. The nodal component of stage (N) is the "gold standard" for predicting the clinical outcome of CRCs. We identified molecular features which are useful in predicting clinical outcome of patients with CRC. CRCs are separated into tumors of the proximal colon (cecum, ascending colon, hepatic flexture and first two thirds of transverse colon), distal colon (last one third of the transverse colon, splenic flexture, descending colon, and sigmoid colon), and rectum based on the embryologic derivation and different therapies for rectal cancers. We reported that the nuclear accumulation of p53 (p53-nac) in Caucasians with all stages of proximal colonic tumors and very low expression of Bcl-2 in all locations of Stage II CRC are correlated with a poor outcome. In Caucasians but not African-Americans, increased expression of MUC-1 but not MUC-2 in all locations of CRCs is correlated with a poor clinical outcome. Also, decreased expression of p27-kip-1 is correlated with a poor clinical outcome in Stage III CRC in both Caucasians and in African-Americans. Combinations of these molecular features are at least additive. Thus, for Stage II proximal adenocarcinomas of the colon in Caucasians, a poor clinical outcome is predicted by p53-nac, very low expression of Bcl-2 and high expression of MUC-1. For African-Americans, prognosis (hazard ratio) of CRCs is predicted primarily by nodal stage modified by low expression of p27-kip-1. We will demonstrate how hazard ratios for these molecular features of CRCs can be coupled with staging and demographic information for an overall hazard ratio for patients with CRCs.

1858 PREDICTING CLINICAL PROGNOSIS IN COLORECTAL CANCER PATIENTS USING MOLECULAR PROFILE DATA AND BIOINFORMATICS TECHNOLOGIES.

R. D. Thomas[1, 2], W. E. Grizzle[3] and University. Manne[3]. *[1]Canswers, Inc., McLean, VA, [2]INTERCET, LTD., McLean, VA and [3]Department of Pathology, University of Alabama at Birmingham, Birmingham, AL.*

Current cancer diagnostic and assessment benchmarks, such as tumor staging and grading are limited in their ability accurately assess the nature of disease and fail to provide oncologists with tools capable of predicting disease process and outcome.

Advances in molecular biology have provided insight into the mechanisms of cancer development and have led to the creation of gene and protein expression profile databases for various cancers. In addition, advances in bioinformatics have led to the creation of tools capable of identifying specific marker profiles capable of predicting such things as disease onset and clinical prognosis. The present study examines the use neural networks and data mining techniques to extract prognostic relationships within a molecular profile database containing immunohistochemical data on multiple cellular markers in 492 colorectal cancer patients. Multiple analysis approaches were used to identify which markers held the best prognostic potential and what cut-off values gave the most accurate prediction. Results of this study show that the certain markers, when analyzed in combination with particular diagnostic and demographic fields, are capable of predicting the likelihood of five year survival in a subset of the patient population. This presentation will focus on efforts to use bioinformatics tools to predict clinical prognosis using a combination of demographic, diagnostic and molecular profile data from historical cancer patients and will highlight the clinical potential of these technologies.

1859 THE EFFORTS TO UTILIZE SERUM PROTEIN PATTERNS AND ARTIFICIAL INTELLIGENCE BASED PATTERN RECOGNITION TOOLS FOR NCI-SPONSORED CLINICAL TRAILS OF OVARIAN AND PROSTATE CANCER DETECTION.

E. F. Petricoin. *National Cancer Institute, Bethesda , MD.* Sponsor: R. Thomas.

The FDA-NCI Clinical Proteomics Program has invented and is pioneering a new diagnostic paradigm: serum proteomic pattern diagnostics. The combined use of artificial intelligence-based pattern recognition tools and high throughput mass spec profiling of the low molecular weight (LMW) range of the blood proteome have allowed for the discovery of biomarkers that achieve much higher sensitivity and specificity for disease detection than any single biomarker alone. We have found that these LMW diagnostic fragments bind to specific carrier proteins that serve to enrich and amplify their signal. These discoveries are serving the basis for clinical trials which will begin at the end of 2003 for ovarian cancer detection. Ongoing work is also being extended to breast, prostate, colon, lung and pancreatic cancer early detection.

1860 THE INTEGRATION OF MOLECULAR PROFILING, TOXICOLOGY AND PATHOLOGY DATASETS FOR KNOWLEDGE DISCOVERY.

M. Waters[1], P. Bushel[1], G. Boorman[2], W. Eastin[2], S. Gustafson[4], P. Hurban[5], R. Irwin[2], A. Merrick[1], J. Nehls[3], K. Olden[3], R. Paules[1], J. Selkirk[1], S. Stasiewicz[1], N. Stegman[3], K. Tomer[3], B. Weis[3], J. Yost[4], S. Xirasagar[4] and R. Tennant[1]. *[1]National Center for Toxicogenomics (NCT), Research Triangle Park, NC, [2]National Toxicology Program (NTP), Research Triangle Park, NC, [3]NIEHS, NIH, DHHS, Research Triangle Park, NC, [4]Science Applications International Corporation, Germantown, MD and [5]Paradigm Genetics, Research Triangle Park, NC.*

The integration of multiple domain datasets for knowledge discovery is an important goal of the Chemical Effects in Biological Systems (CEBS) knowledge base (Waters, et al., 2003, *Environ Health Perspect* 111, 811-824). CEBS uses international database standards developed by the Microarray Gene Expression Data (MGED) Society, including those for data representation and exchange. By extending the microarray object model (MAGE-OM) to capture proteomics, metabonomics, toxicology and pathology data, CEBS SysTox-OM combines molecular expression and metabolite profiling with conventional toxicology and pathology to support hypothesis-driven and discovery research. To enable visualization of pharmacological and toxicological responses, molecular expression datasets (e.g., experimental outliers or determined proteins) are projected onto known biological pathways. Continuously updated gene and protein annotation is brought to CEBS datasets through the use of cancer bioinformatics infrastructure objects (caBIO) technology developed for the National Cancer Institute's Center for Bioinformatics. caBIO is a standards based set of genomic components including genes, chromosomes, sequences, libraries, clones, ontologies, etc. caBIO provides access to genomic data sources including GenBank, UniGene, LocusLink, HomoloGene, EnsEMBL, GoldenPath, BioCarta, dbSNP, among others. Improved gene annotation fidelity is achieved through maximal use of probe set sequence definition and alignment to respective genome scaffold sequences and vetted sets of synonym gene names for literature mining. CEBS is being designed to meet the information needs of systems toxicology.

1861 NOVEL APPROACHES TO ENGAGING TOXICOLOGISTS IN K-12 SCIENCE EDUCATION AND OUTREACH.

D. L. Eaton[1], C. Marcus[3], D. Dixon[2] and L. O'Fallon[2]. *[1]Occupational and Environmental Health Sciences, University of Washington, Seattle, WA, [2]NIEHS, Research Triangle Park, NC and [3]College of Pharmacy, University of New Mexico, Albuquerque, NM.*

Toxicologists are encouraged to become engaged in K-12 classroom activities with the goal of increasing student interest in and awareness of toxicology and environmental health. The SOT K-12 education subcommittee has been very supportive of providing toxicologists with tools and resources to help them prepare for K-12 classroom visits. This session will spotlight positive impacts of classroom visits by toxicologists, and will provide SOT members with novel approaches, tools and resources that can be used to facilitate toxicology education in the K-12 setting. During this workshop, there will be hands-on demonstrations of a variety of tools that toxicologists can use before and during classroom visits. Presenters will include toxicologists who have been actively engaged in K-12 outreach as well as K-12 teachers who have utilized toxicology in the classroom. The presenters will highlight successful models for taking students into the field to learn more about local issues that affect their life and health. The purpose of the session is to show how toxicologists are making a positive impact; provide them with, and direct them to, locations where they can obtain tools; and show them how specific tools can be used effectively in the classroom. In addition, SOT members will learn how to take the classroom learning experience into the field to study local environmental health issues.

1862 POSITIVE IMPACTS OF TOXICOLOGIST VISITS TO THE CLASSROOM: A TEACHER'S PERSPECTIVE.

D. Becker[1], A. Renkwitz[1], F. Ross[2] and B. Tharp[3]. *[1]Cambridge-South Dorchester High School, Baltimore, MD, [2]Robert Poole Middle School #56, Baltimore, MD and [3]Baylor College of Medicine, Houston, TX.* Sponsor: D. Eaton.

Panelists will present a summary of environmental health sciences (EHS)/toxicology projects with their students to demonstrate how toxicologists can become positively engaged in learning activities/projects to increase student awareness of and interest in EHS/toxicology and addressing: * The interdisciplinary nature of EHS topics * How students receive and engage in EHS/toxicology information and how they perceive it as an extension of their science, social studies, mathematics, and/or language arts. * State Standards and assessments, with suggestions on how scientists can make impact/inroads on their local state curricula agenda. * Positive impacts (for teachers and students) of classroom visits by/interactions with scientists/toxicologists, featuring examples of student work. * The importance of "Community Connections" to local EHS issues: -taking students into the field to learn about/study -students sharing with others in schools, homes, and communities

1863 TOXICOLOGISTS IN THE CLASSROOM: SUCCESSFUL MODELS FOR K-12 OUTREACH.

D. L. Eaton[1], N. I. Kerkvliet[2], C. Marcus[3], S. H. Safe[4] and M. A. Trush[5]. *[1]Environment Occup. Health Sciences., University Washington, Seattle, WA, [2]Environment Molec.Toxicology., Oregon St. University, Corvallis, OR, [3]College of Pharmacy, University New Mexico, Albuquerque, NM, [4]Vet. Physiol. & Pharmacology, Texas A & M University, College Station, TX and [5]Environment Health Sciences., Johns Hopkins University, Baltimore, MD.*

A panel of toxicologists with extensive K-12 classroom experience will share insights and provide examples of successful approaches and potential problems which can be employed by toxicologists for effectively conveying toxicology information to K-12 students and their teachers. Successful communication of toxicology to K-12 students is dependent on the specific grade, age and location of the students, cooperation of teachers and principals, and integration of toxicology with other disciplines in the curriculum. The KIS approach (keep it simple!) has been found to be important when communicating basic toxicology to K-12 audiences. Examples of simple experiments using dye indicators in weak buffer solutions can be used to engage students, while illustrating such concepts as dose-response, thresholds, cumulative toxicity, and individual susceptibility, in an engaging hands-on way. Students from urban and rural areas have a different appreciation of real-life toxicological issues such as air pollution and pesticide spraying. The Texas A&M PEER program has developed an extensive rural education program that includes a series of hands-on learning modules that illustrate relevant toxicology problems to student (grades 6 - 8) in rural schools. "Intuitive risk assessment" is used by middle and high school students as they form opinions about risk and make personal health-related decisions. However, most teachers lack sufficient knowledge of toxicology to be comfortable interjecting principles of toxicology, risk assessment and risk perception

into their everyday teaching in the classroom. Examples of "teacher-friendly" toxicology and risk assessment presentations to teachers will be presented as other types of successful outreach activities for professional toxicologists.

1864 HANDS-ON FUN: TOOLS FOR TOXICOLOGISTS ENTERING THE K-12 CLASSROOM.

L. O'Fallon. *NIEHS, Research Triangle Park, NC.* Sponsor: D. Eaton.

During this interactive session, toxicologists will be introduced to simple, inexpensive activities and tools that can be used when visiting classrooms. The 6 tables of hands-on activities will be organized by grade-level appropriateness. Bio Buildup and Separating Solutions, K-3 Bio Buildup is a math investigation that toxicologists can use with elementary students to simulate bioaccumulation in a simple aquatic ecosystem using stick-on dots. Separating Solutions is an engaging activity where elementary students use coffee filter "paper chromotography" to investigate a mysterious liquid. Toxicologists can use this activity to show students that many different substances can be in solution simultaneously. Peak Flow Lab., High School This activity provides toxicologists a creative way of using a peak flow meter to discuss the effects of toxic respiratory exposures (e.g., tobacco smoke and occupational exposures) on peak flow and lung function within and across individuals. Bioassay Basics, Middle & High School Bioassay activities using lettuce seeds and brine shrimp are demonstrated. These simple and inexpensive investigations can be used by toxicologists with middle and high school students. Enviromysteries, Middle School Toxicologists can use videos and video clips from 'Breaking the Mold' at the Enviromysteries website with grade 5-9 students when addressing sources of air pollution or health outcomes, e.g., asthma and water-born illnesses. Toxicology-in-a-box and EH Fact Files, Middle School Toxicology-in-a-Box is a literal and virtual suitcase of materials that can be used by toxicologists for many presentations to a wide range of students. The kit includes activities, slides, demonstrations, and a script of explicit instructions and tips for the presenter. EH Fact Files is a resource that can be used by toxicologists to prepare for a classroom visit. This tool helps toxicologists distill complex scientific and historical information about specific health topics into age-appropriate language. Respiratory System, Middle School Toxicologists will learn about an engaging activity used in rural Texas middle schools.

1865 CLASSROOM TO FIELD: PUTTING TOXICOLOGY IN A LOCAL CONTEXT.

J. Lewis[1] and B. Sattler[2]. *[1]University of New Mexico Health Sciences Center, Albuquerque, NM and [2]University of Maryland School of Nursing, Baltimore, MD.*

Teaching students about toxicology and environmental health can be very challenging. Sometimes methods used are fairly dry and the topics may appear remote to the students, especially when the focus is on the processes of toxicology and environmental contamination, rather than on public health impacts. Therefore, finding novel ways of highlighting, and/or involving students directly in, real issues in their community can serve to engage the students and thereby more effectively teach them about the science of toxicology and environmental health. In addition, it can also give students the added benefit of being seen as resources in their community and serve as a means of raising the awareness of parents, friends, and family members. The presenters in this session will highlight their efforts in taking students into the field to teach them about toxicology through local environmental health issues in New Mexico and Masachusetts. In New Mexico, environmental health concerns include extensive uranium and other mine-related pollutants, as well as air and water pollution in this primarily rural and poverty-stricken state. University of New Mexico has built lasting relationships with the diverse population to address the health disparity in chronic diseases such as asthma, diabetes and cancers of unknown etiology that provide an excellent opportunity to make toxicology relevent to k-12 sutdents and teachers. Urban environmental health issues such as asthma and children's health have been a primary focus at the University of Maryland. Programs that engage nursing students in the environmental health sciences have been particularly successful in building community and addressing local environmental health issues. The presenters will discuss 'nuts and bolts' of this approach and the benefits to students, teachers, toxicologists, and communities.

1866 THE NATIONAL CHILDREN'S STUDY: PROGRESS DEVELOPING METHODS APPROPRIATE FOR ASSESSING CHILDREN'S EXPOSURE, BIOMARKERS AND GENETIC SUSCEPTIBILITY.

C. A. Kimmel[2] and B. D. Abbott[1]. *[1]RTD, NHEERL, ORD, USEPA, Durham, NC and [2]NCEA, ORD, USEPA, Silver Spring, MD.*

The National Children's Study is a long term prospective study of the effects of environmental influences on the development and health of children across the United States. This study is a collaborative effort authorized by the Children's Health Act of 2000. The National Institute of Child Health and Human Development (NICHD), the National Institute of Environmental Health Sciences (NIEHS), the Center for Disease Control and Prevention (CDC), and the USEPA (EPA) are involved in planning and conduct of the study. This study will include approximately 100, 000 children, following them from before birth to adulthood. Social, behavioral, cultural, chemical, physical, and genetic factors need to be considered to assess the broad and complex influences of the environment on child health and development. In this symposium, individuals involved in various aspects of study planning and/or advisory groups will present recent progress in developing improved methods for identifying biomarkers, evaluating genetic susceptibility, and modeling children's exposure. A final presentation will discuss the issues and concerns related to childhood asthma, one of the major themes of the study, and progress in that research area. The NCS and the ongoing pilot studies to develop the final form of that study, represent a rich resource for the toxicological and epidemiological community. This symposium provides an introduction to this vast resource, an update on research in this arena and an indication of future research directions in studies of children's environmental health.

1867 AN OVERVIEW OF THE NATIONAL CHILDREN'S STUDY.

P. Mendola. *Human Studies Division, USEPA, Research Triangle Park, NC.* Sponsor: B. Abbott.

There is substantial public health concern that aspects of the environment adversely impact child health and development. Children may be more vulnerable to environmental threats but little comprehensive research exists to identify which factors are harmful, harmless or helpful. Research with a life-course approach is needed to explore the links between multiple exposures and multiple health outcomes over time. The National Children's Study will "investigate basic mechanisms of developmental disorders and environmental factors, both risk and protective, that influence health and developmental processes" (Children's Health Act, 2000). A large sample size of approximately 100, 000 is planned to allow the evaluation of links between low level environmental exposures, social and behavioral factors with less common outcomes, as well as the interaction between genetics and the environment. The study will focus on children's environmental health themes of major public health concern that are best suited to this type of longitudinal study, such as pregnancy outcomes, neurobehavioral development, asthma, obesity and altered physical development, and injury. Pregnant women from across the United States will be enrolled as early as possible in pregnancy (or before pregnancy) and their children will be evaluated during prenatal development, through birth, childhood, and into adulthood. Strong partnerships between federal and non-federal scientists and community, parent, advocacy, and industry groups are being emphasized throughout the planning process. Planning and organization of the study are underway with a chartered federal advisory committee and working groups considering issues such as hypotheses and study design, ethics, development and behavior, chemical and physical exposures, injuries, emerging technologies to measure exposures and outcomes, and community outreach/participation. This study, similar in importance to the Framingham study and the Women's Helath Initiative, will provide a legacy for future generations on the role of environmental factors on children's health and development, and well-being.

1868 VALIDATION OF NON-INVASIVE BIOLOGICAL SAMPLES: PILOT PROJECTS RELEVANT TO THE NATIONAL CHILDREN STUDY.

J. E. Gallagher[1], T. Lehman[2], R. Modali[2], S. Rhoney[1], J. Rockett[1], M. Clas[1], J. Inmon[1], D. Dix[1], C. Mamay[1], S. Fenton[1], S. McMaster[1], S. Barone[1] and R. Sams[1]. *[1]NHEERL, USEPA, Research Triangle Park, NC and [2]Bioserve Biotechnologies LTD, Laurel, MD.*

Advances in genetic technology and the work of the human genome project to map and sequence the human genome are expediting our understanding of genetic determinants of common diseases. Determination of most genetic polymorphisms associated with disease susceptibility has been accomplished utilizing DNA from tissue such as whole blood or buffy coats. MALDI-TOF (matrix assisted laser desorption/ionization time of flight mass spectrometry)combines enzymatic amplification reactions, bioinformatics and a miniaturized chip based format which together provide high throughput genetic analyses. To date, we have successfully applied MALDI -TOF for genotyping DNA isolated from fingernails, buccal swabs, saliva, sputum, mouthwash lavages and urine. We have extended our studies to evaluate the degree to which specific metabolism, detoxification and repair polymorphisms account for variation in genetic expression and sensitivity/responsiveness. To accomplish our goals, we obtained whole blood samples from children, adult and elderly through ongoing EPA clinical and field studies. Ex-vivo treatment of cells was then conducted with environmental toxicants. Recognizing that cumu-

lative risk to an individual is the combined risk from aggregate (multi-pathway, multi-source and multi-route) exposures to multiple agents, we focused on measuring gene expression and immune and oxidative/antioxidant measurements since they likely reflect the effects of cumulative exposures/stressors. They may also be early exposure/effects biomarkers for chronic diseases such as cancer, cardiovascular disease, diabetes and inflammatory disease. Understanding the extent to which genetic differences account for individual variations in response, will assist in better understanding gene-environment interactions, a major focus of the National Children's Study. This abstract does not necessarily represent EPA policy.

1869 DEVELOPMENT AND USE OF BIOMARKERS OF EXPOSURE FOR CDC'S NATIONAL EXPOSURE REPORT.

L. L. Needham. *Organic Analytical Toxicology, Centers for Disease Control and Prevention, Atlanta, GA.* Sponsor: B. Abbot.

In environmental public health involving potential exposure to chemicals, it is essential to assess exposure and any effects in an accurate manner. Our laboratory has developed analytical methods for measuring chemicals or their metabolites in biological specimens. We have used these to support epidemiological investigations, emergency response investigations, and for exposure assessment studies. In each of these types of studies, it is very helpful to have a reference value for these chemicals in the general population. We recently reported reference range concentrations of several chemicals in segments of the US population. The National Health and Nutrition Examination Survey was used to collect these urine and blood samples. The results were examined based on age, sex, and race/ethnicity. These results, applications of these results, and future plans for collecting such data will be discussed.

1870 STUDY DESIGN CONSIDERATIONS FOR THE EXPOSURE COMPONENT OF THE NATIONAL CHILDREN'S STUDY.

H. Ozkaynak[1], L. Needham[2], R. Whyatt[3] and J. Quackenboss[4]. *[1]NERL, USEPA, Washington, DC, [2]CDC, Atlanta, GA, [3]Columbia University, New York, NY and [4]NERL, USEPA, Las Vegas, NV.* Sponsor: H. Ozkaynak.

An ideal strategy for the exposure monitoring component of the planned National Children's Study (NCS) is to measure indoor and outdoor concentrations and personal exposures of children to a variety of pollutants, including ambient particulate and gaseous pollutants, biologicals, endocrine disrupting chemicals, metals, pesticides, among others. However, due to the large sample size of the study (about 100, 000 children), it is not feasible to measure every possible exposure of every child in the study. Thus, an important technical and logistical challenge is to develop an appropriate study design with adequate statistical power that will permit detection of exposure-related health effects, based on exposure and biomonitoring measurements on a smaller subset of the target population. Since the study will follow a longitudinal design, a related challenge will be deciding: a) which exposure-related measurements need to be assessed repeatedly over time; b) the timing of such repeated exposure assessments, and c) the appropriate type of direct and/or indirect (e.g., survey based) monitoring methodology to be employed, given various resource and participant burden considerations. The Chemical Exposure Work Group of the NCS project is currently undertaking an evaluation of these issues by utilizing the expertise represented within the group, as well as, those of outside contractors, in evaluating the available information on exposure monitoring in the context of an epidemiological study design. This presentation will summarize the recent findings from this NCS sponsored work group activity regarding potential alternatives for exposure and biological sampling, and inference of subject-specific exposures in the context of an epidemiologic study design. Disclaimer: This work has been partially funded by the United States Environmental Protection. It has been subjected to Agency review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

1871 ENVIRONMENTAL EXPOSURE AND ASTHMA: HYPOTHESES FOR THE NATIONAL CHILDREN'S STUDY.

S. J. London. *Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC.* Sponsor: B. Abbott.

Asthma hypotheses for the National Children's Study were developed by the Interagency Coordinating committee with input from the Asthma Working Group which developed hypotheses and coordinated the work of many other working groups that also submitted asthma hypotheses. The goal was to identify the most important hypotheses for asthma regarding the environment, both physical and social. We also focused on hypotheses which must be addressed in the context of a longitudinal study beginning as early in life as possible. Out of the two year process came the following asthma hypotheses: 1. Exposure to outdoor and indoor air pollution and bioaerosols (allergens, fungi) is associated with increased risk of developing asthma. 2. Respiratory viral infection in early life is associated with increased risk of developing asthma. 3. Maternal stress during pregnancy and in the early life of the child is associated with increased risk of developing asthma. 4. Dietary factors influence the risk of asthma. In particular, dietary antioxidants and other micronutrients decrease the risk of asthma, while obesity increases the risk of asthma. 5. Early exposure to certain bacteria and their products (ex: endotoxin) decreases the risk of asthma. This encompasses the "Hygiene Hypothesis" of asthma. 6. Access to health care and management of asthma are strongly related to asthma hospitalization and morbidity. The asthma working group has been organizing workshops aimed at developing methodologies needed to address these hypotheses in a cost-efficient manner in the National Children's Study.

1872 DEVELOPING THE USE OF THRESHOLD CONCEPT FOR PROTEIN ALLERGENS.

J. Vodela[1] and T. D. Landry[2]. *[1]Residue Branch, USDA/FSIS, Washington DC, DC and [2]Environment, Health and Safety, Dow Chemical Company, Midland, MI.*

Public exposure guidelines are meant to protect all segments of the population, including the very young and the very old, pregnant women, and hypersensitive individuals. Each year the Food & Drug Administration receives reports of consumers who experienced adverse reactions following exposure to an allergenic substance in foods. Food allergies are abnormal responses of the immune system, especially involving the production of allergen specific IgE antibodies, to naturally occurring proteins in certain foods that most individuals can eat safely. Although the number of food proteins with allergenic potential is not clearly established, there are a limited number of proteins involved in commonly observed food allergy. When the immune system recognizes a food protein as foreign and harmful, cellular and biochemical cascades are initiated that may lead to sensitization and ultimately allergic reactions. There is a significant incidence of food allergy to naturally occurring allergens in children (5% to 6 % in US) and adults (2% US). Although protection may require exposure avoidance (e.g. to peanuts in peanut sensitive persons), it would be preferable to be able to apply the "dose makes the poison" concept to induction and / or elicitation. This round table will provide recent scientific information on the health effects of protein allergens and will consider how the "threshold" concept for sensitization to allergens may be applied to establishing non-zero exposure guidelines.

1873 FOOD ALLERGENS-THE FDA PERSPECTIVE.

K. Falci. *US Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD.* Sponsor: J. Vodela.

Food allergies affect an estimated 6% of young children and 2% of adults in the United States. A food allergy is an IgE-mediated immunologic reaction to an ingested food and may result in a variety of symptoms including the skin, gastrointestinal tract, and respiratory tract. Eight major foods account for 90% of all food allergies: milk, egg, peanut, tree nuts, fish, crustacea, soy, and wheat. As the Agency responsible for the safety of the nation s food supply, the United States Food and Drug Administration (FDA) is actively involved in efforts focused on food allergy for the eight most common allergenic foods. Currently regulations require the declaration of all ingredients on the food label, yet undeclared allergens remain an important issue. Failure to declare ingredients, collective naming of flavors, colors, and spices, advisory labeling statements, recalls, and test kits for allergen protein detection are just some of the issues of concern to both the FDA and allergic consumers. Through allergen inspections, outreach to industry and consumers, and enforcement of regulations, USFDA continues efforts to improve food allergen activities

1874 FACTORS AFFECTING THE DETERMINATION OF THRESHOLD DOSES FOR ALLERGENIC FOODS: TOXICOLOGICAL ASPECTS.

S. Hefle. *Food Allergy Research and Resource Program, University of Nebraska, Lincoln, NE.* Sponsor: T. Landry.

While there are numerous anecdotal reports of activities such as opening packages of the offending food or kissing the lips of someone who has eaten an allergenic food being capable of eliciting allergic responses in food-allergic individuals, the

majority of experiences indicate that non-zero thresholds exist and that severe allergic reactions have usually been associated with high levels of contamination. There are several factors that affect how studies for minimum eliciting doses of allergenic foods are designed and the resulting data interpreted. A description of the challenges that a group that sought to determine whether existing data on threshold doses for commonly allergenic foods were sufficient to allow consensus to be reached on the establishment of threshold doses for specific foods (Taylor et al. (2002); J. Allergy Clin. Immunol.) found and the development of a globally recognized consensus protocol for doing threshold studies will be covered. Problems of interpretation of results and assigning safety factors will be discussed, such as comparing subjective reactions to objective reactions, study design issues, the uncertainty in using LOAEL to establish threshold doses for allergenic foods, and the large variability of individual threshold doses. One mathematical model for thresholds that has been proposed (Bindslev-Jensen et al. (2002) Allergy) will be discussed. Additional issues will be covered such as the decision to use adults vs. children (and the challenges associated with outgrowth of childhood food allergies), issues with the nature of the challenge material or allergen content of the challenge materials and the fact there are no validated animal models for allergenic food thresholds to date. There is no widespread agreement on whether the current statistics used in threshold studies will be acceptable to regulatory agencies; evolving ideas on what the safety factors should be will also be presented.

1875 FACTORS AFFECTING THE DETERMINATION OF THRESHOLD DOSES FOR ALLERGENIC FOODS-CLINICAL ASPECTS.

J. Hourihane. *Infection Inflammation and Repair, University of Southampton, Southampton, United Kingdom.* Sponsor: J. Hourihane.

There are many factors that contribute to a variation of eliciting doses in an individual exposed to a food allergy by accident in the community or deliberately during a formal food challenge. Some of these factors are intrinsic and unavoidable: age and gender, for example, or asthma. Other factors may be predictable but not easily controllable, such as the degree of asthma control (which appears critical), exposure to allergens during the pollen season (reactions may be more likely if the food allergen is associated with pollens) and predicting situations that may be associated with higher risk (eating the allergen after exercise or while consuming alcohol). Other factors may be out of the control of the individual. The most important one of these is the adequate training and awareness of manufacturers and caterers who aim to provide safe and nutritious meals to their allergic and non-allergic customers alike. Subjects can react in different ways to the same dose, if the allergen is presented in different food combinations or after different cooking processes: egg becomes less allergenic with cooking, peanut becomes more allergenic. Exploration of eliciting doses during food challenges is now a widely accepted clinical practice. People can volunteer for research motivated food challnges or may have clinically-motivated (physician-directed) challenges modified to embrace exploration of lowest eliciting doses. Safety considerations mean that challenge subjects must be in optimal physical condition with stable asthma and atopic dermatitis. They are also self-selected to a degree in that subjects who consider themselves high risk do not volunteer for studies. This means they may not be representative of the group of patients at highest risk in the community. Threshold data generated in formal challenges represent the current state of the art but several confounding variables affect these data and a broader view needs to be taken before legislating about threshold amounts of allergens in foods .

1876 COMMUNICATING RISKS ASSOCIATED WITH FOOD ALLERGENS.

D. W. Achesson. *Center for Food Safety and Applied Nutrition, Food and Drug Administraion, College Park, MD.* Sponsor: J. Vodela.

Allergy to foods is a significant cause of morbidity and mortality in the United States. Current estimates of the burden on the health care system are difficult to determine due to an apparent underreporting of serious adverse events and death associated with exposure to food allergens. Part of a successful strategy to combat allergy from foods is to ensure both consumers and health care professionals are well educated regarding the risks and preventative strategies that could be adopted. Education campaigns are frequently more successful if focused on targeted populations. To that end gaining a better understanding of the at-risk populations as well as the true disease burden would facilitate a more directed consumer message. FDA continues to explore ways to improve communication to stakeholders regarding allergens as well as methods to improve understanding of the overall morbidity and mortality associated with food allergy

1877 EVIDENCE FOR THRESHOLDS FOR TYPE 1 ALLERGY TO ENZYMES USED IN THE DETERGENT INDUSTRY.

K. Sarlo. *Central Product Safety, Procter & Gamble Company, Cincinnati, OH.*

Occupational allergy to enzymes used in the detergent industry is well recognized. The industry has been able to control the development of IgE antibody to enzymes and the onset of disease *via* strict adherence to exposure guidelines. These guidelines help to minimize exposure *via* management of product formula, equipment, ventilation, clean-up procedures, air and medical monitoring and employee training and education. Decades of prospective monitoring of the workforce has shown that individuals with enzyme-specific IgE antibody can continue to work in the industry with a greatly reduced risk for developing symptoms of allergy caused by enzymes. During the late 1960s, approximately 50-60% of the workforce had IgE antibody and 15% of these individuals developed asthma within 1 year of use of enzyme in the detergent industry. Currently, only 10-20% of the workforce has IgE antibody to enzyme and there have been only 2-3 cases of enzyme-induced asthma over the past 12 years. These observations show that the intensity of exposure needed to induce the generation of IgE is less than the intensity needed to elicit symptoms. Studies in animal models of enzyme allergy support the observations made in man: low exposure to enzyme will induce antibody but is not sufficient to elicit respiratory reactions. Prospective and retrospective clinical evaluations of consumer populations exposed to different enzyme-containing products also show that thresholds exist for induction of IgE antibody. Exposure to enzyme *via* use of detergents for hand laundry or machine laundry does not lead to induction of IgE antibody yet exposure to enzyme *via* personal care cosmetic products can. The exposure from detergent use is very different from the exposure from personal care use, supporting the existence of thresholds for induction of IgE. The experience of managing type 1 allergy to enzymes tell us that there are different thresholds of exposure for inducing IgE and for eliciting allergy symptoms and that the development of IgE antibody does not equate to disease.

1878 AN *IN VITRO* SURROGATE FOR DRUG-INDUCED PHOSPHOLIPIDOSIS.

R. E. Morgan, A. Kriauciunas, B. Berridge, J. Sullivan and D. K. Monteith. *Lead Optimization Toxicology, Eli Lilly Research Labs, Greenfield, IN.*

Drug-induced Phospholipidosis (PL) is the lysosomal accumulation of phospholipids likely due to interference in phospholipase activity, though other mechanisms have been described. The disorder has not been causally linked to aberrant cell function, however its occurrence is of concern in drug development. This condition is characterized by the presence of intralysosomal lamellar inclusions, appearing as electron-dense whorls in electron micrographs, and can occur in concert with organ toxicity. Drug molecules that possess a polar motif comprised of a lipophilic region and a charged region are aptly named cationic amphiphilic drugs (CAD), and have been shown to induce PL in a variety of tissues. Current *in vitro* screening strategies for PL are qualitative and afford limited throughput. Therefore, a 96-well method of screening for PL was developed and validated utilizing primary rat hepatocytes, a phospholipid-conjugated dye (NBD-PE), a laser-scanning fluorescence detection system, and several CAD. Concentration-response curves were established for chloroquine, amiodarone, propranolol, imipramine, maprotiline, two proprietary molecules, and two negative controls (amikacin & isoniazid) to validate the assay. EM confirmed the ability of the *in vitro* hepatocyte assay to detect PL. This assay was then employed to screen candidate drugs from various platforms for PL; the results correlated well with *in vivo* findings. In screening one drug platform, several molecules induced the formation of vacuoles that were absent of NBD-PE fluorescence and appeared as intracellular dark-holes under fluorescence microscopy. EM of hepatocytes exposed to these drug candidates revealed the presence of clear cytoplasmic vacuoles, not consistent with PL. Subsequent *in vivo* studies confirmed the presence of clear cytoplasmic vacuoles in several tissues in the absence of typical PL. In summary, this assay quantified PL *in vitro* with accuracy, reproducibility and moderate throughput, and was shown to correlate well with *in vivo* histologic changes.

1879 MODELLING THE ESTROUS CYCLE *IN VITRO* USING 3-D RAT VAGINAL EPITHELIUM CELL CULTURES.

R. Caldelari[1], B. D. Car[2], L. D. Lehman-McKeeman[2], E. J. Muller[1] and M. M. Suter[1]. *[1]CELLnTEC advanced cell systems, Bern, Switzerland and [2]Bristol Myers Squibb, Princeton, NJ.*

The vaginal epithelium undergoes significant, hormone induced morphological changes during the estrus cycle. To date, assessment of estrous cycle disruption has relied on animal-intensive procedures, including cytology of cells from vaginal lavage, direct histology of the vaginal epithelium, or the uterotrophic assay measuring uterine weight change. Here we describe a dynamic 3-D *in vitro* model that accurately mimics the cyclical structural changes exhibited by the multilayered strati-

fied squamous vaginal epithelium during the estrous cycle *in vivo*. This model was established from long-term (40 passage) cultures of rat vaginal keratinocytes using CELLnTEC's serum-free medium CnT-03. Following cyclical alteration of the progesterone and estradiol concentrations in the culture medium over a 4-day period, histological investigation revealed characteristic morphological alterations including transient production of mucous cells, followed by cornification. The cyclical morphology observed closely matched the stages of diestrous 1, diestrous 2, proestrous, and estrous as seen *in vivo*. Addition of progesterone alone was not sufficient to induce these cyclical changes. Overall, based on histological examination this model appears to accurately mimic the structural changes seen during the estrous cycle *in vivo*, and therefore represents the first *in vitro* alternative to the use of live animals for assessment of estrous cycle disruption.

1880 PREDICTION OF OVARIAN FUNCTION DEFECTS BY MOUSE FOLLICLE CULTURE.

J. E. SMITZ[1], I. Hellinckx[2] and R. Cortvrindt[1, 2]. [1]*Reprod Med., AZ-VUB, BRUSSELS, Belgium and* [2]*EggCentris NV, Zellik, Belgium.* Sponsor: P. McAnulty.

Regulatory agencies demand for the development of *in vitro* systems to replace current *in vivo* testing protocols. Today most often *in vitro* assays use isolated cells / cell lines of the target organ.The ovary is composed of follicles, functional units to grow and mature the oocyte and produce steroid hormones. Folliculogenesis is a lengthy process consisting of oocyte growth and maturation along with theca cell recruitment, granulosa cell proliferation and differentiation. Follicle development is characterised by an intense paracrine signalling between the different cell types. Disruption of the unit results in dedifferentiation of the somatic cells and block growth and maturation of the oocyte. The only relevant assay to correctly assess ovarian function *in vitro* is follicle culture. Preantral mouse follicles were cultured singly for 12 days in αMEM with 5 % FCS, ITS and gonadotrophins. The preantral follicles attach by outgrowh of the theca cells and undergo a remodelling process. By day 8 granulosa cells differentiate into mural and cumulus cells separated by antral-like cavities. By day 12, follicles reach the preovulatory stage and respond to an hCG/LH stimulus. Denuded oocytes were analysed for nuclear maturation. Results were expressed in % of follicles that reached the criterion within the set of follicles (10/15). 330 sets were collected over time and analysed for reproducibility. Defined end-parameters (Mean +/-SD): day 12:Follicle Survival (97 +/- 6%), Antrum formation (80 +/- 17%); day 13: Mucification (97 +/- 5%); PB oocytes (85 +/- 11%). Steroidogenesis: estrogen on day 4(41 ng/l) (preantral stage), day 8 (3969 ng/L), day 12 (17256 ng/L). Progesterone levels on day 8 and 12 (0.37 and 3, 97 μg/L) and increased steeply 16 h after the hCG stimulus to 247.2 μg/L. Conclusion: Cultured preantral mouse follicles progress *in vitro* as *in vivo* within a strictly defined time frame. Under defined culture conditions the outcome is reproducible and can be used to study ovarian function *in vitro*. This multi-parametric assay is suitable for female fertility risk assessment.

1881 AN *IN VITRO* SURROGATE ASSAY FOR MITOCHONDRIAL TOXICITY.

B. Li, D. Watson, R. Morgan and D. Monteith. *Lilly Research Laboratories, Greenfield, IN.*

Nucleoside analogs are prominent agents in antiviral and anticancer therapies; however, these compounds have been implicated in a variety of organ toxicities in patients. These dose-limiting toxicities occur after prolonged treatment and have been linked to effects on the mitochondria (Mt). Therefore, it is considered important to evaluate nucleoside analogs for their effect on Mt in human cell systems. A mechanism for the Mt effects involves the inhibition of DNA polymerase gamma. In the present research, several endpoints were evaluated to determine cytotoxicity in proliferating and static cultures of human hepatocytes. In addition, specific Mt effects were assessed by determination of MtDNA content using a novel TaqMan® analysis of MtDNA and nuclear DNA. Confluent and low-density cultures of HepG2 cells were treated for 4 to 8 days with a range of nucleoside analog concentrations. AZT, dideoxycytidine (ddC), and ribavirin (Rbv) were selected as compounds with different mechanisms for inhibiting DNA synthesis. The test compounds did not induce cytotoxicity in static cell cultures at concentrations up to 300 microM by the assessment of cellular ATP or neutral red uptake. All three compounds induced effects on ATP and neutral red uptake in proliferating cells. Effects were concentration-dependent, evident at 4 days and progressed with increasing duration of treatment. Rank order potency of the compound-mediated cellular effects was Rbv>AZT>ddC. At 8 Days, cytotoxicity in the proliferating cultures was observed at 30 to 100 microM. Interestingly, cytotoxicity or potential growth inhibition was not associated with effects on MtDNA content. AZT or Rbv did not affect MtDNA content. In contrast, ddC did decrease MtDNA content in a concentration-dependent manner in both proliferating and static cultures and this effect was not associated with cytotoxicity. In summary, the *in vitro* assay quantified MtDNA content associated with cellular effects with accuracy, reproducibility and moderate throughput.

1882 INVESTIGATING APOPTOTIC MECHANISMS OF CELL DEATH USING POSITIONAL BIOSENSORS.

J. R. Haskins, A. M. Peters, M. Weiss, J. Strauss, R. DeBiasio and Y. Chen. *Assay Development, Cellomics, Inc., Pittsburgh, PA.* Sponsor: E. McGuire.

Apoptosis is integral to many normal developmental processes, but can lead to premature cell death when triggered by xenobiotics. There are several key criteria for determining whether a cell is undergoing apoptosis, including distinct changes in cellular morphology and alterations in specific biochemical and molecular markers. The general patterns of apoptotic signals are comparable, but mechanistic drivers can vary significantly depending on cell type and causative agent. Among key proteases that can trigger apoptosis are the caspases. We have developed two positional biosensors for *in vitro* monitoring intracellular caspase 3 and 8 activity in living or fixed cells. Both biosensor designs are based on measuring the intracellular redistribution of fluorescent reporters from an initial subcellular compartment to a targeted compartment upon activation. These biosensors are composed of recombinant polypeptides containing several key components, including reporting, sensing and targeting elements. Following proteolytic cleavage of the sensing element by the activated caspase, the targeting and reporting elements are driven by a nuclear localization signal to the nucleus where they are easily detected using quantitative image analysis. To demonstrate applicability of this approach, a 240 compound library of known toxins was screened against HeLa cells stably expressing either the caspase 3 or 8 biosensor at concentrations of 1, 10 or 50 μM for up to 4 hours. By adding additional fluorescent probes for detecting changes in nuclear morphology, mitochondrial transmembrane potential and membrane permeability, further details were elucidated that allowed differentiation of apoptotic mechanisms. Individual cellular responses were quantitated using automated fluorescence microcopy. Results indicated the value of positional biosensors as a new generation of fluorescent protein reagents that function as temporal and spatial reporters of cellular processes *in vitro*.

1883 C. ELEGANS RESPONSE TO MAMALIAN ANTIOXIDANT RESPONSE ELEMENT INDUCER TBHQ.

M. Calkins[1, 3] and J. A. Johnson[1, 2, 3]. [1]*Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI,* [2]*Waisman Center, University of Wisconsin, Madison, WI and* [3]*School of Pharmacy, University of Wisconsin, Madison, WI.*

Caenorhabditis elegans has proved to be a useful model for many human dieases and conditions. The response of c. elegans to pharmacological agents is often very similar to the human response. As such c. elegans can provide a simple, easily manipulated whole organism model for pharmacological processes that are relevant to humans. In this regard we have begun to evaluate the response of c. elegans to known antioxidant response element (ARE) inducers, especially tert butyl hydroquinone (tBHQ). The ARE is a cis-acting enhancer sequence that mediates expression of phase II detoxification enzymes in response to oxidative stress. This response is centered on the cap n collar transcription factor Nrf2. tBHQ has been shown to induce Nrf2 activity and activate the ARE in mammalian systems by a mechanism that is independent of oxidative stress. Early microarry data has revealed a set of genes in c. elegans that are inducible by tBHQ treatment in mixed population cultures. Many of the identified genes are involved in antioxidant protection and phase II conjugation. Additionally, we have shown that tBHQ treatment extends lifespan and that pretreatment can protect against oxidative insults. These results suggest that there is a homologous ARE type response in c. elegans. Furthermore, it is possible that the mechanism by which tBHQ activates ARE gene expression in mammals is conserved as well. Since this mechanism is unknown in mammalian systems, c. elegans may prove to be a useful model for elucidating this response. In order to further characterize the response to tBHQ we conducted RT-PCR analysis of staged cultures at different time points, and found that tBHQ dependant induction occurs in larval and adult worms. In dauer worms however, we find a much different response. Specifically there is a downregulation of the transcription factor Skn-1, which is known to be homologous to Nrf2 in structure and function. We are currently investigating the mechanism by which tBHQ activates gene expression in the nematode c. elegans.

1884 WOUND HEALING RESPONSE OF THE EPIDERM FULL THICKNESS (EPIDERM-FT) *IN VITRO* HUMAN SKIN EQUIVALENT AFTER SOLAR UV IRRADIATION: COMPARISON TO EXCISED HUMAN SKIN.

P. J. Hayden, B. Burnham, M. Klausner, J. Kubilus and J. E. Sheasgreen. *MatTek Corp., Ashland, MA.*

Normal human epidermal keratinocytes (KC) and dermal fibroblasts (FB) were cultured to produce full-thickness skin equivalents consisting of FB-containing dermal and stratified epidermal components with a fully developed basement membrane at

the dermal/epidermal junction (EpiDerm-FT 200, EFT). The wound healing response of EFT after solar UV-irradiation was compared to excised human skin. H&E stained paraffin sections of EFT displayed a dose-dependent increase in apoptotic sunburn cells at 24 h post-irradiation. After 48 h, high dose (61 J/cm2, metal halide lamp) samples showed extensive epidermal damage and some dermal damage. After 72 h, sunburn cells still persisted in mid dose samples (40 J/cm2), which were thinner than controls (indicating a major decrease in KC proliferation) but still without major epidermal damage. Epidermal destruction of high dose samples was nearly complete at 72 h with significant loss of dermal matrix also evident. However *via*ble basal cells remained in some areas, with signs of proliferation and epidermal regeneration. MMP-1 activity in the culture media was also evaluated by ELISA. A 50% increase in activity was observed in irradiated samples at 24 h. By 48 h, mid dose samples showed a 100% increase in activity, while high dose samples showed a 150% increase. At 72 h, mid dose sample MMP-1 activity was comparable to controls, while high dose sample activity remained elevated by 125%. Similar experiments were conducted with excised human skin. The response was similar to EFT. The earliest response was increased sunburn cell formation, with tissue thinning at 40 J/cm2 and extensive damage at 61 J/cm2. Basal cell proliferation and epidermal regeneration was also observed in the excised human skin by 72 h in high dose samples. These results show that the EFT human skin equivalent behaves similarly to excised human skin in terms of solar UV induced damage and wound healing. The model may thus prove useful for additional applications in dermal/epidermal wound healing phenomena.

1885 MURINE EMBRYRONIC STEM CELLS AS A MODEL TO IDENTIFY BIOMARKER PROFILES OF PPAR AGONISTS.

M. Pang[1], T. Downey[2], S. Kim[3], K. L. Rose[1], R. Snodgrass[3] and A. E. Vickers[1]. *[1]BioMarker Development, Novartis Pharmaceuticals, East Hanover, NJ, [2]Partek, Inc., St. Charles, MO and [3]VistaGen Therapeutics, Inc., Burlingame, CA.*

Murine embryonic stem (ES) cell differentiation represents a dynamic biological system for investigating compound induced effects on a wide range of cell types and tissue-tissue interactions *in vitro*. Our goal is to identify biomarker profiles that are characteristic of PPAR activators. The PPAR activators included in this study are PPARγ-agonists (troglitazone, pioglitazone and rosiglitazone), PPARα-agonists (WY14643, fenofibrate, bezafibrate), and the mixed PPARα-γ agonists (KPR297 and AZ242). Previously, concentration profiles eliciting a 20-50% decrease in cell number at the end of a 6-day differentiation embryoid body (EB) assay using the murine embryonic stem cell line CCE-Lena (derived from 129Sv mouse strain) were determined. Here, we investigate the effect of each compound on ES cell differentiation and development by comparative analysis of the gene expression profile of cultures exposed for either 6-days, or for the last 3-days following an initial 3-day EB development period. Gene expression profiles were established using the Affymetrix murine genome array (U74Av2) followed by statistical analysis of the data *via* hierarchical clustering, principal components analysis, and 3-way analysis of variance based on exposure time, type of PPAR agonist (α-agonists, γ- agonists, or mixed), and individual compound. Preliminary findings indicate that it is possible to separate the PPAR-agonists from each other according to the type of PPAR activator and length of compound exposure. Global gene expression and quantitative RT-PCR analysis of marker genes suggest a PPAR activator related effect on murine stem cells, increasing immature mesoderm differentiation and suppression of liver and blood-related differentiation.

1886 EMBRYONIC STEM CELL-DERIVED HEPATOCYTE CULTURES AS A MODEL TO IDENTIFY BIOMARKER PROFILES OF PPAR AGONISTS.

H. R. Snodgrass[1], Y. S. Kim[1], O. A. Callan[1], G. Keller[2], A. Kubo[3] and A. Vickers[4]. *[1]VistaGen Therapeutics, Inc., Burlingame, CA, [2]Mount Sinai School of Medicine, New Youk, NY, [3]Nara Medical University, Nara, Japan and [4]Novartis Pharmaceuticals, East Hanover, NJ.*

The goals of these studies are to identify biomarker profiles that are characteristic of PPAR activators, and to evaluate the utility of *in vitro* cultures of hepatocytes differentiated from murine embryonic stem (ES) cells as a system for early toxicity assessment. ES cell differentiation systems represent a dynamic *in vitro* biological system for investigating compound induced effects on a wide range of cell types and tissue-tissue interactions. The PPAR activators included in this study are PPARγ-agonists (troglitazone, pioglitazone, rosiglitazone and KRP297), PPARα-agonists (WY-14643 and fenofibrate), and the mixed PPAR agonists (bezafibrate and AZ242). Here, we report on the effect of each compound on an ES cell culture system, designed to optimize hepatocyte differentiation, by adding the compound during the last 2 days of a 14 day culture. The effects of the compounds were evaluated by monitoring gene expression using microarrays. The data were analyzed by a variety of statistical tools including analysis of variance, hierarchical clustering, principal components analysis, and class prediction algorithms. Preliminary analyses indicate that, contrary to the results with the 6-day EB assay (presented in another abstract), troglitazone had a more pronounced effect, than pioglitazone and rosiglitazone, on the differentiation of these cultures. The expression of a significant number of the genes relating to liver development was modified by these compounds. Predictive gene biomarkers were identified which had an 89% success rate of distinguishing troglitazone from pioglitazone and rosiglitazone effects, and a 85% success rate of distinguishing PPARα from PPARγ agonists. These data suggest that, in combination with the 6-day EB system, the ES differentiation system may be a very useful early screen for compound toxicity and pathway effects.

1887 CHEMICALLY INDUCED CELL DEATH IN HUMAN KERATINOCYTES.

O. E. Clark, E. W. Nealley, K. L. Finke, R. E. Roberts and W. J. Smith. *Biochem Pharmacology Br, US Army Med. Rsch Inst of Chem Defense, Aberdeen Proving Ground, MD.* Sponsor: A. Brimfield.

Our laboratory studies the mechanism of toxicity of the chemical blistering agent sulfur mustard (SM) for the purpose of developing medical countermeasures to this chemical threat agent. SM is a cytotoxic alkylating agent with mutagenic and vesicating properties. The exact mechanism of SM-induced skin vesication is not completely understood. The human epidermal keratinocyte (HEK) in culture is the *in vitro* equivalent of the basal epidermal cell of human skin, a principal target for the toxicity of SM in tissue. In this report, our laboratory evaluated several markers of cytotoxicity in HEK exposed to SM and a variety of chemical toxicants. The markers studied were protein content, cellular morphology, membrane integrity, annexin V labeling and DNA fragmentation. The chemical toxicants were calcimycin (A23187), staurosporine (STR), cycloheximide (CHX), actinomycin D (ActD) and camptothecin (CPT). All six compounds lowered cellular protein levels at 24 hours post-exposure, with A23187 and STR having significant effects as early as 4 hours after exposure. A23178 caused an early (1 hr) annexin V response, but these cells rapidly die as determined by an uptake of propidium iodide (PI). By 4 hours, only STR shows an increase of annexin positive cells. SM failed to generate annexin staining at either 4 or 24 hours post-exposure. As for morphological changes, only STR and A23187 led to changes at 4 hours. STR resulted in stellate-shaped cells, whereas A23187 yielded cells that rounded up and had contracted cytoplasm. At 24 hours, A23187 caused DNA-containing cytoplasmic blebs, whereas SM-exposed cells had blebbing without DNA content. Comet analysis of DNA fragmentation did not discriminate different types of death processes by the different toxicants. These results help elucidate the contributions of various death processes following toxicant exposure and hopefully will contribute to a better understanding of critical events in SM pathology.

1888 SULFUR MUSTARD ALTERS LAMININ 5 AND GELATINASE MNRA LEVELS AND INCREASES GELATINASE ACTIVITY IN A MOUSE EAR VESICANT MODEL.

D. R. Gerecke[1], P. Bhatt[1], C. L. Sabourin[2], T. L. Rudge[2], R. P. Casillas[2], R. C. Kiser[2], S. L. Casbohm[2], M. K. Gordon[1], D. J. Riley[3] and M. P. Shakarjian[3]. *[1]Pharmacology & Toxicology, Rutgers University, Piscataway, NJ, [2]Medical Research and Evaluation Facility, Battelle Memorial Institute, Columbus, OH and [3]Medicine, UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ.*

Bis(2-chloroethyl)sulfide (sulfur mustard, HD), is a chemical warfare agent that penetrates the skin rapidly, and, after several hours, causes extensive blistering. Epidermolysis bullosa (EB) is a genetic disease with a similar skin phenotype as that of HD blistered-skin. Some forms of EB are caused by mutations in the genes for any of the three polypeptides (laminin-α3, laminin-β3 and laminin-γ2) that form the basement membrane protein, laminin 5. Matrix metalloproteinases (MMPs) may play a role in the chronic structural damage incurred in EB. We treated mouse ears with 80μg of liquid HD and examined whether the expression of the laminin 5 chains or MMPs are altered after HD exposure. Ear specimens were taken at 6, 12, 24, and 72 hours after mustard exposure for histology, real-time PCR and gelatinase activity assays. HD caused a time-dependent increase in skin weight and damage relative to vehicle controls. HD exposure increased mRNA levels for MMP-9 (gelatinase B), laminin-α3, laminin-β3 and laminin-γ2, whereas MMP-2 (gelatinase A) mRNA levels decreased. There was a time-related increase in overall gelatinase activity observable 6 hours after HD exposure. The enzyme activity peaked at 24 hours, and persisted throughout the study. This increased MMP activity may contribute to the blister damage. An understanding of these *in vivo* MMP patterns following HD skin exposure may lead to defining the pathogenic mechanisms of HD injury and the development of pharmacological countermeasures. *This work is supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-02-C-0091

1889 GENOMIC ANALYSIS OF SULFUR MUSTARD-INDUCED LUNG INJURY.

C. S. Phillips[1], J. F. Dillman[1], L. M. Dorsch[1], M. D. Croxton[1], Z. Hess[2], T. S. Moran[2] and A. M. Sciuto[2]. *[1]Applied Pharmacology, USAMRICD, Aberdeen Proving Ground, MD and [2]Neurotoxicology, USAMRICD, Aberdeen Proving Ground, MD.*

Sulfur mustard (SM) is an alkylating agent that has been used as a chemical warfare agent in numerous conflicts around the world. The lung is a primary target of SM exposure. To understand the mechanism of SM-induced lung injury we analyzed global changes in gene expression in a rat lung SM exposure model. Male Sprague-Dawley rats were injected in the femoral vein with liquid SM, which circulates directly to the pulmonary vein and then to the lung. This results in a deep lung SM exposure. Rats were exposed to 1 mg, 3 mg or 6 mg of SM, and lung tissue was harvested at 0.5, 1, 3, 6 and 24 hours post-injection. RNA was extracted from the lung and used as starting material for the probing of oligonucleotide microarrays. The gene expression data were analyzed using principal component analysis and two-way analysis of variance to identify the genes most significantly changed as a result of variation over time and dose. These genes were rank ordered by p-value and categorized based on molecular function and biological process. Previously, inflammation has been shown to play a role in SM injury, and our data corroborate these findings. However, the most significant changes in gene expression reflect alterations in the cell cycle and vesicular trafficking. In particular, the changes in cell cycle reflect a potential cell cycle block at the G1/S transition due to activation of the p53 pathway. Although our data do not determine directly the activation of p53, many of the genes that are significantly upregulated in a dose dependent fashion have been shown by other investigators to be p53 responsive genes. Thus, SM exposure appears to induce a robust p53 response in the lung.

1890 GENOMIC ANALYSIS OF THE MECHANISM OF ACTION OF POTENTIAL VESICANT COUNTERMEASURES.

J. F. Dillman[1], L. M. Dorsch[1], A. I. Hege[1], C. S. Phillips[1], Y. W. Choi[2], R. C. Kiser[2] and C. L. Sabourin[2]. *[1]Applied Pharmacology, USAMRICD, Aberdeen Proving Ground, MD and [2]Medical Research and Evaluation Facility, Battelle Memorial Institute, Columbus, OH.*

Sulfur mustard (SM) is a potent alkylating agent that induces cutaneous vesication. We are developing medical countermeasures to reduce the injury caused by SM exposure. Screening in the mouse ear vesicant model has identified three lead compounds: British anti-lewisite (BAL), indomethacin, and octyl homovanillamide. To understand the molecular mechanism of action of these compounds we used oligonucleotide microarrays to compare gene expression profiles in unexposed skin (vehicle control), SM-exposed skin, and skin pretreated with each compound before SM exposure. Male CD-1 mice were topically exposed on the inner surface of the right ear to SM alone or pretreated for 15 minutes with one of the lead compounds and then exposed to SM. Left ears were vehicle-exposed. Skin tissues were harvested 24 hours later for ear weight determination (an endpoint indicating compound efficacy). RNA was extracted from the tissues and used as starting material to probe oligonucleotide microarrays. The gene expression data were analyzed using principal component analysis (PCA) to identify sources of data variability. Our PCA results reveal partitioning of the samples based on drug treatment and SM exposure. Vehicle-exposed mouse ears cluster together away from the other treatment groups. Mouse ears pretreated with BAL or octyl homovanillamide cluster more closely with vehicle-exposed ears, while indomethacin-treated mouse ears cluster more closely with SM-exposed ears. This clustering of the samples is supported by the relative ear weights, in which the indomethacin group has ear weights closer to the SM-exposed group, whereas the BAL and octyl homovanillamide groups have ear weights closer to the vehicle-exposed group. These data provide the basis for understanding what gene expression changes are important in the development of efficacious SM exposure treatments.

1891 TIME- AND DOSE-DEPENDENT ANALYSIS OF GENE EXPRESSION IN SULFUR MUSTARD-EXPOSED MICE.

C. L. Sabourin[1], J. V. Rogers[1], Y. W. Choi[1], R. C. Kiser[1], R. P. Casillas[1], M. C. Babin[1] and J. J. Schlager[2]. *[1]Medical Research & Evaluation Facility, Battelle Memorial Institute, Columbus, OH and [2]Pharmacology Division, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*

The chemical warfare agent sulfur mustard (SM) produces blister formation with a severe inflammatory reaction in skin of exposed individuals. The development of efficacious countermeasures against SM vesication requires an understanding of the cellular and molecular mechanism of SM-induced tissue injury. This study examined SM-induced alterations in gene expression using microarrays (5002 genes) to identify transcriptional events associated with SM skin injury. Mice (n=3) were exposed topically to SM (0.04, 0.08, and 0.16 mg) on the inner surface of the right ear and skin tissues were harvested at 1.5, 3, 6, and 12 h. Genes were selected based on the three mice in the same dose group demonstrating a ≥2 fold increase or decrease in gene expression for the SM-exposed tissue when compared to the methylene chloride vehicle control ear at all 3 doses and 4 time points. At the 0.04 mg SM dose, the genes observed were primarily involved in inflammation, apoptosis, and cell cycle regulation. Exposure to 0.08 mg SM increased the expression of genes related to inflammation and cell cycle regulation. Exposure to 0.16 mg SM led to a total of six genes that were changed at all observed time periods; however these genes do not appear to be directly influential in biological mechanisms such as inflammation, apoptosis, and cell cycle regulation as was observed at the lower SM doses of 0.04 and 0.08 mg. These functional categories have been observed in previous studies utilizing both *in vivo* and *in vitro* model systems of SM-induced dermal injury, suggesting that molecular mechanisms associated with inflammation, apoptosis, and cell cycle regulation may be appropriate targets for developing prophylactic/therapeutic treatments for SM skin injury. Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-99-D-0010, Task Order 0007.

1892 LOW-LEVEL INHALATION EXPOSURE TO SARIN AND CYCLOSARIN LEADS TO ENHANCED EXPRESSION OF NEURONAL CELL DEATH AND REGENERATION RELATED GENES.

J. W. Sekowski[1], J. Bucher[1], M. Orehek[1], M. Horsmon[1], D. Menking[1], C. E. Whalley[2], B. Benton[2], M. Vahey[3], M. Nau[3], D. Burnett[2], J. Jarvis[2], B. Gaviola[2], R. Mioduszewski[2], S. Thomson[2] and J. J. Valdes[1]. *[1]Molecular Engineering Team, US Army RD&E Command, APG-EA, MD, [2]Toxicology Team, US Army RD&E Command, APG-EA, MD and [3]WRAIR, US Army MRMC, Rockville, MD.*

The acute effects of chemical weapons have been widely studied since World War I and are well understood. Since the description of Gulf War and Balkans Syndromes, however, there has been increased concern for exposure of personnel to sub-acute and low-level doses of chemical warfare (CW) agents that may occur as a result of decontamination procedures or due to use of such agents by an adversary. There is a considerable body of literature suggesting that exposures to organophosphorus (OP) compounds used as pesticides result in significant cognitive and neurological decrements. However, there is a paucity of literature regarding the molecular effects of low-level exposures to OP nerve agents. In this study, male and female Sprague-Dawley rats were exposed to aerosolized sarin (GB) and cyclosarin (GF) *via* whole body inhalation for 240 minutes at several concentrations near the ECT50 for miosis. Controls were exposed to air under the same conditions for the same time period. Alteration of gene expression levels in the brain of the exposed animals was assessed using DNA microarray analysis, RT-PCR, and Western blot. To date, our results indicate that low-level inhalation exposure to GB and GF results in the persistent differential expression of a number of important genes at one week post-exposure. Many of the altered genes participate in cellular processes critical to neuronal injury, death, and regeneration. Furthermore, our analyses reveal several differently altered genes between the female and male animals. Additional studies with nerve agents such as soman and VX will reveal whether these differences translate into true sex-linked susceptibility to increased OP toxicity, and whether a genetic fingerprint can be defined for a class or a particular OP agent.

1893 TOXIC EFFECTS OF A WHOLE-BODY INHALATION SARIN (GB) VAPOR EXPOSURE IN THE GOTTINGEN MINIPIG.

S. W. Hulet[1], E. M. Jakubowski[1], J. S. Forster[1], M. B. Dennis[2], B. J. Benton[1], W. T. Muse[1], P. A. Dabisch[2], R. A. Way[1], J. L. Edwards[2], J. M. McGuire[2], J. A. Scotto[1], D. C. Burnett[1], B. I. Gaviola[1], J. R. Jarvis[2], R. A. Evans[2], K. L. Matson[2], C. L. Crouse[2], J. H. Manthei[1], R. J. Mioduszewski[1] and S. A. Thomson[1]. *[1]Toxicology, US Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD and [2]Geo-Centers, Inc., Aberdeen Proving Ground, MD.*

Due to a steep dose-response slope, there is a fine line between nerve agent doses that cause minimal signs of exposure and those that are potentially lethal. In order to assess chemical defense materiel requirements, it is essential to fill gaps in toxicological databases that define the physiological progression; from the first noticeable effect (miosis) to potentially fatal effects of inhalation exposure. In this study a sexually mature male Gottingen minipig was implanted with a jugular cannula. The pig was placed in a dynamic, whole-body inhalation chamber and exposed to a fixed, toxic dosage of sarin (GB) vapor. Pupil constriction was assessed using infrared pupillometry. Internal dose of GB (regenerated GB) was estimated in serial blood samples drawn through a jugular catheter. Changes in RBC cholinesterase activity were quantified along with other physiological parameters (glucose, Na, K, lactate, pH, etc.). The pig was also monitored for real-time changes in EEG spectra, respiratory rate and cardiac function. The first noticeable sign of GB exposure was

pupil constriction followed thereafter by muscle tremors and labored respiration. Following the exposure, a necropsy was performed and tissue analysis for regenerated GB (R-GB) yielded the highest levels in the lungs and RBCs, with other organs such as the liver containing lesser amounts. Sections of the brain with high-levels of cholinergic activity exhibited measurable levels of R-GB (caudate >frontal cortex>temporal cortex).

1894 DEVELOPMENT OF A MICROFLUIDIC MICROARRAY FOR THE RAPID DETECTION OF TOXICOGENOMIC SIGNATURES.

J. West[1], R. M. DeVay[1] and S. Micheal[2]. *[1]Microfluidics Research Group, Sandia National Laboratories, Livermore, CA and [2]VetMed: Molecular Biosciences, University of California, Davis, CA.*

The development of toxicogenomic microarrays capable of detecting alterations in expression of thousands of genes resulting from a toxic chemical/drug exposure in single experiment has rapidly advanced and become a widespread application technology. Two significant drawbacks to this technology in its current format are the long and tedious processing time for RNA/DNA sample preparation, taking up to 2 days, and exquisite sensitivity to of the aforementioned samples to degradation from ambient DNA and RNA nucleases. In order to tackle these inherent weaknesses in microarray analysis we are developing a microfluidic microarray for detecting toxic chemical or biological exposures. This single Chip will concentrate, fluorescently label and hyribridize target genes on a single device. The potential advantages of such a device are the dramatically reduced sample throughput time, decrease sample degradation, and small size, which is amenable to portable devices. This device will combine the use of microfluidics and microarrays to facilitate the detection of unknown target genes. Here we present an optimized strategy for DNA microarray probe synthesis requires less than thirty minutes in bench top synthesis. We demonstrate comparable hybridization efficiencies with much reduced time of analysis. Moreover, the current method will be more amenable to integration with the fabricated microfluidic chip as the labeling reaction is enzyme free. We further report the fabrication of the first integrated microfluidic chip with both sample-processing channels for monolith polymerization and the fabrication of a high-density microarray on the same chip.

1895 LACK OF DNA SINGLE STRAND BREAKS IN A LUNG EPITHELIAL CELL LINE AFTER EXPOSURE TO ARSENIC.

A. R. Molinelli[1], J. Nakamura[2], J. A. Swenberg[1, 2] and M. C. Madden[3]. *[1]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC, [2]Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC and [3]Human Studies Division, USEPA, NHEERL, Research Triangle Park, NC.*

Arsenic (As) is a carcinogen whose most important target organs include the skin and lungs. Exposure can occur *via* water ingestion, or inhalation, as As is a by-product of fossil fuel combustion and other industrial activities. The carcinogenic mechanism of action for As remains unclear. One hypothesis proposes As induces cancer by creating oxidative stress. In previous studies we found modest evidence of increased peroxidation after exposure of cells to inorganic As as arsenite (iAs). To study the possible mechanistic link between As exposure and lung carcinogenesis, we examined iAs induction of DNA single strand breaks (SSBs) using a human bronchial epithelial cell line (BEAS-2B). SSBs were assessed *via* the comet assay, and a novel colorimetric assay that indirectly measures SSBs repair. This assay is based on the premise that DNA SSBs induce the activation of the repair enzyme poly(ADP-ribose)polymerase, which in turn depletes intracellular NAD+/NAD(P)H (Nucleic Acids Res. 31(17):e104, 2003). We did not find any statistically significant differences in DNA SSBs between the control and iAs (1nM-10μM) treated cells with the comet and NAD(P)H assays with up to 4h exposures at 37°C. In addition, no increase in SSBs was observed when the cells were exposed at 4°C, which inhibits DNA repair. In these studies, H_2O_2 and methyl methanesulfonate induced increased SSBs. These data suggest that iAs does not directly induce an increase in SSBs in this cell line, possibly because BEAS-2B cells may be more resistant to damage than other extrapulmonary cell types shown to have increased SSBs upon iAs exposure. Furthermore, iAs can be converted *in vivo* to methylated organic species that may be more potent inducers of oxidative stress and SSBs. Further analyses into the possible contributions of the aforementioned factors is underway. [This abstract may not necessarily reflect official EPA policy. Supported by EPA/UNC CT827206].

1896 CYTOGENETIC EVALUATION OF ARSENIC TRIOXIDE TOXICITY IN SPRAGUE-DAWLEY RATS.

A. K. Patlolla and P. B. Tchounwou. *Center for Environmental Health, Jackson State University, Jackson, MS.*

Arsenic is metalloid that exhibits a high degree of toxicity to human. It has been shown to induce cardiovascular diseases, developmental abnormalities, neurobehavioral disorders, diabetes, hearing loss, hematologic disorders, gastrointestinal disorders, and various types of cancer including skin, liver, lung, bladder, and kidney neoplasms. However, studies of its carcinogenic potential in animals have not been conclusive. In this study, we investigated the genotoxic potential of arsenic trioxide in bone marrow cells obtained from Sprague-Dawley rats; using structural chromosomal aberrations and mitotic index as toxicological end-points. Four groups of six male rats each, weighing approximately 50 g per rat, were injected intraperitoneally, once a day for five days with doses of 5, 10, 15, 20 mg/kg body weight (b.wt) of arsenic trioxide dissolved in distilled water. A control group was also made of 6 animals injected with distilled water without chemical. All the animals were sacrificed on day 6, and chromosome preparation was obtained from bone marrow cells following standard protocols. Chromatid and chromosome aberrations were investigated in 300 metaphase cells per animal. Arsenic trioxide exposure resulted in a significant increase ($p<0.05$) of the number of structural chromosomal aberrations that included ring chromosomes, chromosome gaps, chromosome breaks, multilocus deletions and chromosome fragments. Mitotic indices of 11.76+/-0.88%, 6.93 +/- 0.66%, 6.86 +/- 0.57%, 5.59 +/- 0.156%, and 4.01 +/- 0.24% were recorded for the control group, 5, 10, 15, and 20 mg/kg b.wt., respectively; indicating a gradual decrease in mitotic index, with increasing dose of arsenic trioxide. These findings demonstrate that arsenic trioxide has a strong clastogenic/genotoxic potential as evidenced by the induction of structural chromosomal aberrations, and reduction of mitotic index in the bone marrow cells of Sprague-Dawley rats

1897 ARSENITE DEPRESSES POLY(ADP-RIBOSYL)ATION IN HUMAN SKIN KERATINOCYTES AND IN MOUSE SKIN.

T. G. Rossman, E. V. Komissarova, A. N. Uddin and P. Li. *Environmental Medicine, New York University School of Medicine, Tuxedo, NY.*

Drinking arsenic-contaminated water is associated with neoplasias of the skin, lung, bladder, and possibly other sites. Previously, we demonstrated that arsenite in drinking water enhances solar ultraviolet irradiation-induced skin carcinoma in the mouse. We suggest that alterations in DNA repair and genomic stability play a role in arsenite carcinogenesis. Although DNA repair enzymes are not inhibited by arsenite, evidence suggests that poly(ADP-ribose) (PAR) synthesis is inhibited in arsenite treated cells. Poly(ADP-ribosyl)ation of proteins contributes to DNA repair and maintenance of genomic stability. Human skin keratinocytes (HaCaT) were exposed to 0.1μM sodium arsenite for different times, lysed, and levels of PAR and poly(ADP-ribose)polymerase (PARP-1) were analyzed by Western blotting. PARP-1 protein levels increased up to 2.5 fold after 4 days exposure and remained high for at least 14 days. During this time, growth in arsenite caused decreases in total protein poly(ADP-ribosyl)ation, suggesting that arsenite inhibits PARP-1 enzyme activity. Because PARP-1 regulates its own transcription *via* PARP-1 auto-poly(ADP-ribosyl)ation, we suggest that the inhibition of PARP-1 activity by arsenite resulted in enhanced PARP-1 transcription. Arsenite's effects on protein poly(ADP-ribosyl)ation were also demonstrated in mice. Skh1 hairless mice were given 10 mg/l (non-toxic concentration) of sodium arsenite in drinking water for 29 weeks. Immunohistochemistry of normal skin obtained at the end of the experiment showed an increased epidermal thickness and decreased level of nuclear protein poly(ADP-ribosyl)ation, compared with control mice. Our results suggest that inhibition of poly(ADP-ribosyl)ation by arsenite may contribute to arsenite-associated carcinogenesis by its effects on DNA repair, DNA-damage-signaling, and transcription. We have constructed a vector for PARP-1 RNA interference to test the role of PARP-1 in arsenite-induced cell transformation.

1898 P-GLYCOPROTEIN EXPRESSION LEVEL HAS A SIGNIFICANT IMPACT ON CLASTOGENICITY *IN VITRO.*

A. M. Peters, J. Strauss, M. Weiss and J. Haskins. *Assay Development, Cellomics, Inc., Pittsburgh, PA.* Sponsor: E. McGuire.

The micronucleus assay is among a new set of genetic toxicology assays wherein cultured cells are treated with compounds and scored for evidence of clastogenicity through monitoring micronucleus formation. This *in vitro* clastogenicity assay helps enable evaluation of compounds earlier in the drug discovery process. Automated measurement of genotoxicity in intact cells represents a faster, more functional, and accurate assay as compared to the time-consuming, manual process of scoring slides. By using the cytokinesis-block method, a quantitative software module, and automated fluorescence imaging microscopy, we were able to implement an optimized assay to quickly evaluate genotoxicity in multiple cell lines. Through these investigations, it was discovered that the clastogenic activity of certain positive control compounds was cell-type dependent. For example, cells exposed to equimolar concentrations of vinblastine sulfate (13 nM) had micronucleus frequencies of 6%, 12% and 39% for CHO-K1, V79-4, and Balb/3T3 cells, respectively. Since vinblastine is a P-glycoprotein (P-gp) substrate, it was theorized that differences in P-gp expression between the various cell lines might be responsi-

ble for the differential induction of clastogenicity. To investigate this phenomenon, each cell line was evaluated for P-gp expression level through functional and biochemical methods. Using a fluorescent substrate for P-gp, a kinetic live-cell assay employing an automated imaging system was designed to assess functional P-gp expression. Each cell line was evaluated in the presence or absence of specific inhibitors of P-gp, including cyclosporin A and verapamil. Quantitative analysis of kinetic response profiles revealed that a strong relationship existed between P-gp expression level and micronucleus induction. These results eloquently demonstrate the potential need to evaluate compounds for clastogenicity in multiple cell types. Evaluations of this magnitude are only mad possible through utilization of automated imaging systems.

1899 *N*-ETHYL-*N*-NITROSOUREA (ENU) INCREASED BRAIN MUTATIONS IN PRENATAL AND INFANT MICE BUT NOT IN THE ADULTS.

W. Slikker III[1], N. Mei[2] and T. Chen[2]. *[1]College of Letters and Science, University of California, Los Angeles, CA and [2]Division of Genetic and Reproductive Toxicology, NCTR/FDA, Jefferson, AR.*

There are rising trends in the incidence of childhood cancer. The reported incidence of gliomas, one of the most common cancers for children, in the under-15 age group rose by about 40% (Ries et al., 1997). The rising trend of childhood cancer in brain may be associated with environmental exposure to genotoxins and susceptibility to mutation induction in early development. To investigate age-dependent mutagenic sensitivity of brain to genotoxins, the Big Blue mouse model was utilized in this study. Groups of 5 Big Blue transgenic mice were treated with 120 mg/kg ENU, a known potent mutagen and carcinogen, at three days before birth (prenatal), and eight days (infant) and eighteen weeks (adult) after birth. The animals were sacrificed 6 weeks after the treatment. The mutant frequencies (MFs) and types of mutations in the brain *cII* gene from ENU-treated and concurrent control mice were determined. A significant increase in MF over control was found in the prenatal and infant groups (235×10^{-6} in prenatal and 117×10^{-6} in infant vs. 44×10^{-6} in control, $p < 0.001$ respectively), whereas there was no significant difference between the adult group and its control (62×10^{-6} in treatment vs. 35×10^{-6} in control). Molecular analysis of the mutants also indicated that the mutational spectra from the ENU-treated mice were age-dependent. A:T → T:A transversion, the signature mutation induced by ENU, was the major type of mutation in mice from prenatal and infant ENU-treated groups, while G:C → A: T transition, a typical mutation type for spontaneous mutation, was the main type of mutation in the adult and control groups. These results demonstrate a differential mutagenic effect of ENU on mouse brain depending on the age and suggest a potential brain cancer hazard for perinatal exposure to genotoxins. It also indicates that future mutagenesis assays should take into account the age-related differential mutagenicity response of age to a test agent, especially in early development.

1900 A MODEL OF SENSITIVITY: 1, 3-BUTADIENE INDUCES HPRT MUTANTS IN MICE LACKING MICROSOMAL EPOXIDE HYDROLASE ACTIVITY.

J. Wickliffe, M. M. Ammenheuser, L. Galbert, J. Salazar and J. Ward. *University of Texas Medical Branch, Galveston, TX.*

Increased sensitivity to the genotoxic effects of 1, 3-butadiene (BD) is associated with a human polymorphism in the xenobiotic metabolizing enzyme, microsomal epoxide hydrolase (mEH). There exists a lack of mechanistic research that sufficiently explains the variation in sensitivity attributable to this polymorphism. A targeted knockout mouse was developed in which the function of mEH has been abolished. To evaluate this model as a surrogate for increased human sensitivity to BD, we examined *Hprt* mutant frequencies (MFs) following exposure of wild-type and knockout mice to BD (inhalation) and to the principal mutagenic metabolite of BD, butadiene diepoxide (BDO_2, IP). In both experiments, unexposed wild-type and knockout mice had MFs that were not significantly different from one another. Knockout mice (n = 6) exposed to 20 ppm BD (6 hours/day, 5 days/week for 4 weeks) exhibited a significant ($p < 0.05$) 12.4-fold increase in MF over unexposed controls (n = 10) and a significant 5.4-fold increase in MF over exposed wild-type mice (n = 6). Knockout mice (n = 3) exposed to 30 mg/kg BDO_2 (2 x 15 mg/kg IP) exhibited a significant 5.2-fold increase in MF over unexposed controls (n = 5) and a significant 1.7-fold increase in MF over exposed wild-type mice (n = 3). In the latter experiment, however, there was some evidence of acute toxicity, which may have led to a depressed response. Additional research examining the mutagenic effects of the initial reactive epoxide, butadiene monoepoxide (BDO), in this knockout mouse is currently underway. Our initial experiments demonstrate that mice lacking mEH function are substantially more sensitive to BD and BDO_2 than their wild-type counterparts. This model should serve as an appropriate, initial surrogate for studies assessing variation in human susceptibility to BD.

1901 FORMATION OF DNA ADDUCTS IN F344 RAT NASAL TISSUE BY 2, 6-DIMETHYLANILINE AND 2, 6-DIETHYLANILINE, BUT NOT ALACHLOR.

J. D. Duan[1], M. Genter[2], A. M. Jeffrey[1] and G. M. Williams[1]. *[1]Department of Pathology, New York Medical College, Valhalla, NY and [2]Department of Environmental Health, University of Cincinnati, Cincinnati, OH.*

Alachlor (AC), a chloracetanilide herbicide, is an N substituted 2, 6-diethylaniline (DEA) which has produced increases in tumors of the nasal mucosa (NM) in rats. Whole body autoradiograms of the distribution in rats with oral administration showed that radiolabeled AC was specifically associated with the NM. The oncogenicity of chloroacetanilides has been linked to the formation of 2, 6-dialkylbenzoquinoneimines. These metabolites bind extensively to glutathione and thiols in proteins, resulting in sustained proliferation of the tissue, which is postulated to be the basis of carcinogenicity, although weak genotoxicity of AC has been reported. Moreover AC is biotransformed to DEA which is closely structurally related to 2, 6-dimethylaniline (DMA), which is also a rodent nasal carcinogen, and yields well-characterized DNA adducts in the NM (Drug Chem Toxicol, 25: 93, 2002; Chem Res. Toxicol, 14: 165, 2001). In the present experiments rats were administered DMA or DEA by gavage, and AC in the diet (310, 382, and 126 mg/kg/day for 7, 7 and 90 days, respectively) and the nasal epithelium DNA was extracted using Qiagen columns. For adduct analysis, the DNA was digested, adducts enriched using OASIS HLB columns (Waters Corp) and postlabeled. DNA isolated from both DMA- and DEA-treated rats gave clear spots by postlabeling while DNA from AC-treated rats did not. In these studies the amount of DEA administered was greater per day than AC, although the cumulative exposure to AC was greater. Additional studies are underway to measure the dose response for DNA adduct formation to determine if this was the cause for the apparent lack of DNA binding of AC. The finding of DEA adducts in the nasal mucosa suggests that it, like DMA, could be a nasal carcinogen. Supported in part by NIH grants ES-08799.

1902 THE ROLE OF O6 METHYLGUANINE DNA REPAIR METHYLTRANSFERASE AND MOUSE 3-METHYLADENINE DNA GLYCOSYLASE IN REPAIRING ANTHRACYCLINE-INDUCED DNA ADDUCTS IN SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI.

W. J. Mackay, J. Armagost, R. Robinson and E. Scully. *Biology & Health Services, Edinboro University of PA, Edinboro, PA.*

For more than thirty years, the natural anthracycline antibiotics represent one of the most commonly used classes of anticancer drugs. However, the clinical usefulness of these drugs is limited due to acute cardiotoxic effects and a dose-related cardiomyopathy. It is known that anthracyclines interact with DNA in a very complex manner. Studies in our lab have shown that anthracyclines can induce mutations in Salmonella typhimurium. In future attempts to improve the clinical efficacies and reduce the pre-carcinogenic effects of these compounds, it will be important to better understand the role of DNA repair pathways, which are involved in correcting anthracycline-induced DNA damage. Current research involves the analysis of the protective functional role of O6methylguanine DNA repair methyltransferase (O6 MTase) and 3-methyladenine DNA glycosylase (Aag) in recognizing and repairing anthracycline-induced DNA adducts. E. coli strains (MB1900), which lack Aag (alkA tag), are extremely sensitive (107-fold) to adriamycin-induced cell killing relative to wild type (AB1157) strains. In addition, mutant Salmonella strains (YG7108), which lack MTase (ada ogt), exhibit an increase of daunomycin-induce mutations (8.2-fold) relative to parental strains (TA1535) that display normal levels of MTase activity.

1903 INVESTIGATION OF THE MUTAGENIC POTENTIAL OF EMISSIONS FROM ASPHALT FORMULATIONS WITH AND WITHOUT CRUMB-RUBBER MODIFICATION.

L. D. Olsen[1], V. S. Houk[2], S. H. Warren[2], L. D. Claxton[2], K. W. Hanley[1], A. K. Miller[3], G. A. Burr[1], D. Almaguer[1] and G. M. Kinnes[1]. *[1]NIOSH, Cincinnati, OH, [2]USEPA, Research Triangle Park, NC and [3]USEPA, Denver, CO.*

In 1991, Congress enacted legislation that required each state to use a minimum amount of crumb-rubber modified (CRM) hot-mix asphalt in road paving operations. However, because of concerns over the lack of information on the environmental and human health effects caused by CRM asphalts, Congress passed a temporary moratorium and directed that these effects be evaluated. In 1995, Congress eliminated the requirement to use CRM asphalts but continued the requirement to conduct research. Because asphalt emissions may contain mutagenic compounds (e.g., polycyclic aromatic hydrocarbons), their mutagenic potential was investigated using a spiral *Salmonella* mutagenicity assay. Asphalt emissions were collected above

open ports of heated asphalt storage tanks, at seven hot-mix plants in six states. At each site, asphalt emissions from the same asphalt formulation, with or without crumb-rubber, were collected on 37-mm polytetrafluoroethylene filters at 28.3 L/min over approximately 8 to 10 hrs. Asphalt samples were extracted with methylene chloride, evaporated to dryness for total mass determination, and dosing solutions were prepared in dimethyl sulfoxide. The spiral *Salmonella* mutagenicity assay was conducted using tester strains TA98 and TA100, with and without 10% S9 metabolic activation. Because of dispersion problems (sample formed beads on the plate) with samples from the first three sites, the remaining samples were extracted using hexane and half of the extract was fractionated into aliphatic compounds, polycyclic aromatic compounds, and polar compounds using NIOSH Method 5800. These fractions and the remaining extract were dried for total mass determination. As before, dosing solutions were prepared, and the spiral *Salmonella* assay was conducted. Test doses ranged from 13 to 900 µ/mL equivalents per plate. None of the asphalt emissions or fractions were found to be mutagenic at the doses tested. (This is an abstract and does not necessarily reflect EPA policy.)

1904 PBDES IN US NURSING MOTHERS MILK, FOOD, AND ELECTRONIC APPLIANCES: LEVELS AND ESTIMATED INTAKE BY VARIOUS ROUTES.

A. J. Schecter[1], O. Paepke[2], J. J. Ryan[3], J. Olson[4], R. Malisch[5], L. Birnbaum[6] and M. Pavuk[1]. *[1]Environmental Sciences, University of Texas School of Public Health, Dallas, TX, [2]ERGO Research Laboratory, Hamburg, Germany, [3]Health Canada, Ottawa, ON, Canada, [4]Pharmacology & Toxicology, University at Buffalo, Buffalo, NY, [5]State Laboratory for Chemical and Veterinary Analysis, Freiburg, Germany and [6]Experimental Toxicology Division, USEPA, Research Triangle Park, NC.*

Polybrominated diphenylether (PBDE) flame retardants are currently used in the USA to a larger extent than in Europe. They have recently been measured in human tissues, fish, dust and other environmental samples. Although animal studies find toxicity including carcinogenicity, endocrine disruption, reproductive/developmental alterations, nervous system alterations, and immune system perturbation, no human health studies have yet been published. To determine dose and routes of intake in humans from the USA, milk from nursing Texas mothers, food from Texas supermarkets and wipe samples from Texas electrical products including computers and computer monitor cases, were analyzed for up to 13 polybrominated diphenyl ethers(17, 28, 47, 66, 77, 85, 99, 100, 138, 153, 154, 183, and 209). Milk was obtained from a Dallas clinic and an Austin, Texas, milk bank. All nursing mothers milk was contaminated with PBDEs. Combined congener levels for the first 47 samples varied from 6-419 ppb lipid. The mean was 74 and the median was 34 ppb. These human milk levels are higher than in other countries, up to 10-100 times those in Europe. No correlations with the mothers age, ethnicity, or length of time nursing were found. Findings were in the range previously reported in human tissue from elsewhere in the USA. Our working hypothesis based on dioxin, dibenzofuran and PCB data and the limited PBDE data, is that most intake into humans in the US is through a food route, particularly food containing animal fat. Preliminary analysis supports this conclusion. (This abstract does not reflect the policy of the USEPA.)

1905 DEVELOPMENTAL NEUROTOXICITY OF POLYBROMINATED DIPHENYLETHERS (PBDE): STEROID-DEPENDENT BEHAVIOR, SEXUAL DEVELOPMENT AND CIRCULATING STEROIDS.

H. Lilienthal, A. Hack, A. Roth-Haerer, L. Altmann, G. Winneke and H. Wiegand. *Neurobehavioral Toxicology, Med. Institute of Environmental Hygiene, Duesseldorf, Germany.*

Recent studies detected increasing levels of brominated flame retardants, including PBDEs in human breast milk. The purpose of the present investigation was to test if maternal PBDE exposure affects steroid-dependent behavior, markers of sexual development, and serum concentrations of steroids. Pregnant rats were exposed by daily SC injections with 0, 1, or 10 mg PBDE 99/kg body wt. from gestational day 10-18. An additional group received the technical PCB mixture Aroclor 1254 (30 mg/kg body wt.) for comparison. Exposure to PBDE 99 and PCBs caused reductions in circulating concentrations of estradiol and testosterone in male offspring at weaning which became even more pronounced in adult males. The higher dose of PBDE 99 decreased anogenital distance only in male rats. The higher dose also delayed puberty onset in females, while the lower dose caused an acceleration in males. Dose-dependent elevations in sweet preference were detected in PBDE-exposed adult male offspring, indicating a feminization of this sexually dimorphic behavior. Since several interactions between the nervous system and the active form of vitamin D3 (1, 25-dihydroxyvitamin D3 - 1, 25-D) have been recently described and since reductions in circulating concentrations of this steroid were found after exposure to a reconstituted PCB mixture in a previous study of our lab, we wanted to test if PBDE exposure alters 1, 25-D dependent behavior. First, it was shown that 1, 25-D caused dose-related increases in conditioned taste aversion (CTA) in non-exposed rats, demonstrating affective properties of this steroid. Then, an intermediate dose of 1, 25-D was selected for induction of CTA in exposed rats. PBDE exposure resulted in dose-related increases in 1, 25-D induced CTA. Taken together, these results indicate endocrine-modulating effects of PBDE 99 which influence the development of the nervous system. (supported by EU project QLK4-CT-1999-01562 and Federal Environmental Agency, Germany, project F+E 29965221/03).

1906 DEVELOPMENTAL NEUROTOXICITY OF PBDES:IMPAIRMENT OF SYNAPTIC PLASTICITY IN RAT CORTEX AND HIPPOCAMPUS. WIEGAND, H., ALTMANN, L., AND LILIENTHAL, H. MED. INST. ENVIRONM. HYG. AT THE HEINRICH-HEINE-UNIVERSITY, DUESSELDORF, GERMANY.

H. Wiegand[1], L. Altmann[2] and H. Lilienthal[3]. *[1]Med.Inst.Environm.Hyg., Heinrich-Heine-University, Duesseldorf, Germany, [2]Med.Inst.Environm.Hyg., Heinrich-Heine-University, Duesseldorf, Germany and [3]Med.Inst.Environm.Hyg., Heinrich-Heine-University, Duesseldorf, Germany.*

Epidemiological studies indicate a marked increase of PDBE levels in breast milk during the last decade in european and north-american populations. However, mechanisms of actions of PBDEs in the developing CNS are largely unknown. Therefore, we assessed synaptic plasticity in different rat brain regions of immature and aged offspring after prenatal PBDE99 treatment (1 or 10 mg/kg during gestational day 10-18). Recordings from brain slices were performed *ex vivo*. Samples of brain and fat tissues were analyzed for PBDE99 content. Results in immature rats:No effects were found for body weight (- gain) in dams or offspring as well as in the excitability of neurons in both brain regions tested (10mg/kg and control group, respectively). In cortical slices long-term potentiation (LTP) and paired-pulse facilitation (PPF) differed from controls. While LTP was reduced mainly in the deeper cortex layers III to IV, PPF was only affected in layers II to III In the hippocampal CA1 region only LTP of the EPSP slopes was significantly reduced in the 10 mg/kg animals while LTP of the somatic population spikes as well as the EPSP amplitudes were not affected by exposure. No effects of exposure were found on the amount of hippocampal PPF. Results in aged rats: The amount of LTP measured in cortical slices was reduced in the 10 mg/kg group in comparison to the control or 1 mg/kg group. PPF was reduced. In the hippocampal CA1 region the amount of LTP was found to be statistically significantly reduced in the 10 mg/kg group as compared to the controls. PPF was unaffected. Conclusion: There is clear cut evidence for developmental neurotoxicity of prenatal PBDE99 treatment producing fat tissue levels in an order of magnitude not far from PBDE levels of north-american population mother milk samples. (supported by EU grant # QLK4-CT-1999-01562)

1907 COMPARATIVE DEVELOPMENTAL NEUROTOXICITY OF PBDE 99 IN TWO DIFFERENT MOUSE STRAINS AND RAT.

H. Viberg, A. Fredriksson and P. Eriksson. *Environmental Toxicology, Uppsala University, Uppsala, Sweden.*

Flame-retardants are used to suppress or inhibit combustion processes in order to reduce the risk of fire. One class of flame-retardants, polybrominated diphenyl ethers (PBDEs), have been found to increase in the environment and in human mothers milk. In recent studies we have seen that neonatal exposure to some brominated flame-retardants, such as PBDE 47, PBDE 99, PBDE 153 and PBDE 209, can cause permanent aberrations in spontaneous behaviour in NMRI mice, an effect that worsen with age, and affect the cholinergic system. The present study compares the neurotoxic effect between different speices, mouse strains and genders. Male NMRI mice, male and female C57 Bl. mice and male Sprague-Dawley rats were exposed to PBDE 99 in the dose range 0.8 to 16.0 mg/kg b.wt., as one single oral dose, on postnatal day 10. Spontaneous behaviour was observed in adult NMRI and C57 Bl. mice and adult SD rats. Male NMRI mice exposed to 0.8 or 12.0 mg PBDE 99/kg b.wt. on postnatal day 10 showed significantly impaired spontaneous motor behaviour as adults. This effect was dose-response related. Male and female C57 Bl. mice exposed to 0.8 8.0 or 16.0 mg PBDE 99/kg b.wt. on postnatal day 10 showed significantly impaired spontaneous motor behaviour as adults. This effect was dose-response related. Male Sprague-Dawley rats, exposed to 0.8, 8.0 or 16.0 mg PBDE 99/kg b.wt. on postnatal day 10 showed significantly impaired spontaneous motor behaviour as adults. This effect was dose-response related. In conclusion, neonatal exposure to PBDE 99 induces behavioural derangements in adult mice and rats, an effect that is dose-response dependent. PBDE 99 causes similar developmental neurotoxic effects in rats, compared to male and female mice of different strains.

1908 EXPOSURE TO AN ENVIRONMENTALLY RELEVANT DOSE OF PBDE 99 DISRUPTS THYROID HORMONE HOMEOSTASIS AND CAUSES NEUROBEHAVIOR DISTURBANCES IN RAT OFFSPRING.

S. N. Kuriyama, C. Talsness, W. Wittfoht and I. Chahoud. *Department of Toxicology, Institute of Clinical Pharmacology and Toxicology, Charite University Medical School Berlin, Campus Benjamin Franklin, Berlin, Germany.* Sponsor: E. Silbergeld.

Thyroid hormone levels and locomotor activity were evaluated in offspring rats exposed *in utero* to a low dose of PBDE 99. The two doses were selected to be 100- and 500-fold higher than the average found in human adipose tissue in Europe. Wistar dams (n=10) were treated by gavage on gestation day 6 with a single dose of 60 or 300 μg PBDE 99/kg body weight or peanut oil (control). A reference control group composed of dams treated with 0.5‰ of PTU in drinking water from GD 7 to 21 was included. Serum samples obtained from dams (PND1 and PND 22) and offspring (PNDs 1, 14 and 22) were analyzed for circulating total and free T4, T3 and TSH. On PNDs 36 and 71, the locomotor activity of two males and two females per litter, were monitored during a 24 h period using the Mobiltron apparatus. A statistically significant reduction in T4 levels was observed in PBDE treated offspring at the end of lactation (PND 22). PTU-exposed offspring showed a decrease in T4 and TSH levels at the beginning of lactation (PND 1), which has restored to normal levels at weaning. The analyses of the locomotor activity show a statistically significant hyperactivity in PBDE-exposed offspring after weaning (median LBC / day: control - 434; PBDE300 - 570) and at puberty (median LBC / day: control - 903; PBDE60 - 1014; PBDE300 - 1005). Transient hyperactivity was noted in PTU-treated animals with normal activity being restored at puberty. Neonatal PTU-induced hypothyroidism has been shown to be associated with hippocampal dysfunction in hyperactive rats. Since we have observed decreases in thyroxin level during lactation in both PBDE and PTU groups, our data suggest that transient neonatal hypothyroidism in PBDE-treated rats leads to a persistent hyperactive state. *In utero* exposure to an environmentally relevant dose of PBDE 99 interferes with thyroid hormone homeostasis and neurobehavior development in rat offspring.

1909 2, 2', 4, 4'-TETRABROMODIPHENYL ETHER (PBDE-47) ALTERS THYROID FUNCTION IN RATS.

J. M. Hedge[1], K. M. Crofton[1], S. C. Laws[1], M. J. DeVito[1], D. G. Ross[1] and P. C. Das[2]. *[1]NHEERL, ORD, USEPA, Research Triangle Park, NC and [2]Curriculum in Toxicology, UNC, Chapel Hill, NC.*

Two commercial PBDE mixtures, DE-71 and DE-79, cause dose-dependent depletion of serum T4 *via* induction of UGTs and increased CYP1A1 activity. This work characterized the effect of a major congener, PBDE-47, in DE-71 for effects on hepatic enzymes and thyroid hormones. Female 27 day-old LE rats (n=8-14/group) were gavaged with 0, 0.3, 1, 3, 10, 30, 100 mg/kg bw PBDE-47 for 4 days. An additional group was treated with 30 mg/kg/day DE-71 (n=12) as a comparative control. Serum and liver tissues were collected on the 5th day. Serum total T4, T3, and TSH were assayed by RIA, and hepatic microsomal EROD, PROD, and UDPGT enzyme activities were determined. PBDE-47 dose-dependently decreased serum total T3 and T4 (~25%, and ~75% at the highest dose), and failed to alter TSH concentration. DE-71 was slightly more potent (10-15%) compared to the same dose of PBDE-47. These data clearly demonstrate the PBDE-47 is a thyroid hormone disrupter. *This is an abstract of a proposed presentation and does not necessarily reflect University.S.EPA policy.*

1910 DISPOSITION OF 2, 2′, 4, 4′-TETRABROMODIPHENYL EITHER (BDE 47) IN FEMALE MICE.

D. Staskal[1], J. Diliberto[2], M. DeVito[2] and L. Birnbaum[2]. *[1]Curriculum in Toxicology, UNC, Chapel Hill, NC and [2]ETD, NHEERL, ORD, USEPA, Research Triangle Park, NC.*

2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE 47) is present in commercial mixtures of polybrominated diphenyl ethers (PBDEs), which are used as a flame retardant in a wide variety of consumer products. Levels of BDE 47, the major PBDE congener in wildlife and human tissues, are steadily increasing in North America. The continued production and use of PBDEs suggests that peak body burdens have not yet been reached and concern has risen because the effects and fate of these chemicals are unknown. Information on the toxicokinetics of BDE 47 is limited. In this study, the disposition of a single oral dose of [14C]BDE 47 (0.0, 0.1, 1.0, 10, or 100 mg/kg) was assessed in female C57BL/6N mice five days following exposure. Residual radioactivity was measured in excreta, liver, lung, kidney, adipose, blood, muscle, skin, and brain. Cumulative excretion in the urine after five days was 41, 40, 32, and 14% of the dose (0.1, 1.0, 10, or 100 mg/kg, respectively). In feces, 46% was excreted in the highest dose group (100 mg/kg), and approximately 35% in all others. This data demonstrates a rapid elimination of BDE 47 in mice; at the low dose (0.1mg/kg), over half of the administered dose was eliminated in two days. Preliminary chromatographic data suggests that most of the [14C]BDE 47 in excreta is parent compound, which raises several mechanistic questions concerning its disposition. We estimate that at least 80% of the dose was absorbed at all dose levels; however, the overall elimination of BDE 47 is dose dependent. This toxicokinetic behavior has important implications for extrapolation of toxicological studies to the assessment of health risk in humans. (This abstract does not reflect EPA policy. This work was partially funded by EPA NHEERL-DESE CT826513).

1911 EFFECTS OF POLYBROMINATED DIPHENYL ETHERS (PBDES) ON BASAL AND TCDD-INDUCED CYTOCHROME P450 1A1 ACTIVITY IN MCF7, HEPG2 AND H4IIE CELLS.

L. Peters[1], M. van den Berg[1], A. Bergman[2] and T. Sanderson[1]. *[1]Institute for Risk Assessment Sciences, University of Utrecht, Utrecht, Netherlands and [2]Department of Environmental Chemistry, University of Sweden, Stockholm, Sweden.*

Polybrominated diphenylethers (PBDEs) are used as additive flame-retardants in consumer products to reduce the chances of ignition and burning. Levels of some PBDE congeners have been increasing in fish, wildlife, and human tissues during the last decades. Some are highly lipophilic and persistent, resulting in bioaccumulation in the environment. The structural similarity of PBDEs to other polyhalogenated aromatic hydrocarbons such as certain PCBs, has raised concerns that PBDEs might act as agonists for the aryl hydrocarbon receptor (AhR). To study the possible dioxin-like effects of the environmentally relevant PBDEs (BDE47, 77, 99, 100, 153, 154, 183, 209), the Ah receptor-mediated induction of the cytochrome P450 enzyme CYP1A1 was studied in human breast carcinoma cells (MCF7), human hepatocellular carcinoma cells (HepG2), and rat hepatoma cells (H4IIE). 7-Ethoxyresorufin-O-deethylase (EROD) was used as a marker for CYP1A1 activity. The cells were exposed to several concentrations (0.01-10 μM) of these PBDEs. Positive controls were 2, 3, 7, 8-TCDD (0.001-2.5 nM) and PCB126 (1-10 μM). EROD activity was measured after 72 hours. None of these PBDEs was capable of inducing EROD activity; this was confirmed by real time RT-PCR for CYP1A1 mRNA. However, when the cells were exposed to PBDEs in combination with TCDD, there was a concentration dependent inhibition of TCDD-induced EROD activity. At maximum TCDD-induced CYP1A1 (EROD) activity, BDE153 reduced this AhR-mediated activity by as much as 40% at a concentration of 10 μM. All PBDEs tested showed the same effect in all three cell lines, though quantitative differences were observed. Catalytic inhibition and cytotoxicity by MTT assay was not observed and did not appear to explain the observed decrease in CYP1A1 activity. Whether this antagonism is due to direct interaction with the Ah receptor or some other unknown mechanism of downregulation will be further assessed.

1912 EFFECTS OF BROMINATED FLAME RETARDANTS ON THE ACTIVITY OF THE STEROIDOGENIC ENZYME AROMATASE (CYP19) IN H295R HUMAN ADRENOCORTICAL CARCINOMA CELLS IN CULTURE.

R. Fernandez Canton[1], T. Sanderson[1], R. Letcher[3], A. Bergman[2] and M. Berg[1]. *[1]Institute for Risk Assessment Sciences, Utrecht, Netherlands, [2]Department of Environmental Chemistry and Analytical Chemistry, Stockholm University, Stockholm, Sweden and [3]Great Lakes Institute for environmental Research, Windsor, ON, Canada.*

Flame retardants are substances used in plastics, textiles, electronic circuitry and other materials to prevent fire ignition processes. Brominated flame retardants (BFRS) are ubiquitous chemicals with large and global industrial use. Due to this fact, several BFRs are found in quantifiable levels in wildlife and humans. Several *in vitro* studies have shown that these chemicals have endocrinological effects. Our study focuses on the potential interactions of a wide range of these BFRs with aromatase (CYP19). We used the human H295R adrenocortical carcinoma cell line to assess the effects on aromatase activity after a 24h exposure to twenty different BFRs. These included polybrominated diphenyl ethers (PBDEs) and several of their hydroxylated metabolites (OH-BDEs, MeO-BDEs), tetrabromobisphenol-A (TBBPA), hexabromocyclodocedane (HBCD) and brominated phenols (BP). Effects were studied in the concentration range from 0.5 to 7.5 μM. Aromatase activity was measured using the tritiated water-release assay. MTT and LDH assays were used to measure cytotoxicity of these BFRs. Exposure of H295R cells to 6OH-BDE47 and 6OH-BDE99 showed an inhibitory effect on aromatase activity at concentrations > 2.5 μM and > 5 μM, respectively, although 6OH-BDE47 caused a decrease in cell *via*bility at concentrations > 2.5 μM possibly explaining the aromatase inhibition. Replacement of the 6OH group by a methoxy group eliminated cytotoxicity, while significant aromatase inhibition remained. A concentration-dependent induction of aromatase activity was caused by 2, 4, 6-tribromophenol(TBP) between 0.5 and 7.5 μM. These inductive properties were lost

when the OH-group was replaced by a MeOH-group or adjacent bromines were removed. These results give a first indication of possible structure activity relationships for interaction of BFRs with aromatase.

1913 POLYBROMINATED DIPHENYLETHERS INHIBIT TCDD-INDUCED EROD-ACTIVITY IN CARP HEPATOCYTES.

R. V. Kuiper[1, 2, 3], J. G. Vos[3, 2], A. Bergman[4] and M. van den Berg[1]. *[1]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands, [2]Pathobiology, Utrecht University, Utrecht, Netherlands, [3]National Institute for Public Health and the Environment, Bilthoven, Netherlands and [4]Environmental Chemistry, Stockholm University, Stockholm, Sweden.*

Ethoxyresorufin-O-deethylase (EROD) activity is a popular biomarker for exposure to AhR agonists. In environmental studies, complex mixtures of xenobiotics are subject to investigation where a number of pollutants inhibiting EROD activity may hamper adequate exposure assessment. This study investigates the effect of the increasingly environmentally detected polybrominated diphenylethers (PBDEs) on EROD activity in carp hepatocytes. BDEs-47, -99, -100 and -153 were selected based on their environmental abundance. Commercial penta-BDE (DE-71, Great Lakes Chemical Corp.) untreated and after clean up to remove planar impurities, a BDE-47 metabolite, 6OH-BDE-47, and PCB-153, a known inhibitor of EROD activity, were included. Freshly isolated carp hepatocytes were co-exposed for 5 days to 2, 3, 7, 8-TCDD (0, 1, 3, 10, 30 and 100 pM), and one of the pure PBDE/PCB congeners (0, 0.25 and 2.5μM) or either DE-71 fraction (0, 0.1 and 10 μM), and EROD activity was determined. Exposure to TCDD alone resulted in dose-dependent increase of EROD activity. This induction was significantly reduced in the presence of BDEs-47, -99, -153, and both DE-71 fractions, dependent on PBDE dose, and generally not paralleled by reduced catalytic conversion of MTT as *via*bility parameter. Particularly strong inhibition was observed for the environmentally most abundant congener BDE-47 (down to 6% of the corresponding control at 2.5μM BDE), and cleaned-up DE-71 (to 4% of the control at 1.0 μM DE-71). BDEs-47 and -153 added shortly prior to measurement did not reduce EROD activity, indicating that inhibition is not catalytic. PCB-153 did not affect EROD activity in this study. Thus, environmentally relevant PBDEs may interfere with EROD measurement *in vitro*, resulting in underestimation of toxic hazard in environmental samples. Supported by FIRE, EU contract number QLK4-CT-2002-00896.

1914 CHILDREN'S HEALTH RISK ASSESSMENT OF THE COMMERCIAL PENTABROMODIPHENYL ETHER PRODUCT.

T. L. Serex[1], R. J. Wenning[2], J. A. Biesemeier[1], A. Von Burg[2], S. Braithwaite[2], A. M. Shipp[3] and G. Lawrence[3]. *[1]Regulatory Affairs, Great Lakes Chemical Corp., West Lafayette, IN, [2]ENVIRON International Corp., Emeryville, CA and [3]ENVIRON International Corp., Ruston, LA.*

A health risk assessment of the commercial pentabromodiphenyl ether (pentaBDE) product was conducted for the United States Environmental Protection Agency (USEPA) Voluntary Children's Chemical Evaluation Program Pilot (VCCEPP). The commercial pentaBDE product is made in the United States and used almost exclusively as a flame retardant in flexible polyurethane foam (FPUF) in bed mattresses and cushioning in upholstered products. Hazard information was compared with screening-level exposure models to evaluate all plausible pathways by which children and prospective parents might be exposed in the workplace, home, school, office, and ambient environments. Three non-cancer health effect benchmark values were used for comparison to theoretical daily intakes. Changes observed in thyroid T4 levels and the incidence of thyroid hyperplasia in rats were used to develop screening toxicity values. The BMDL10 for thyroid hyperplasia ranged from 4 mg/kg/day in male rats to 9.6 mg/k/day in female rats. Applying a 100-fold uncertainty factor to the lowest BMDL10 (4 mg/kg/day for thyroid hyperplasia), resulted in a screening toxicity value of 0.04 mg/kg/day. The BMDL10 for changes in T4 levels was 4.5 mg/kg/day for data from PND 4 and 5.9 mg/kg/day for PND 14 in weanling rats. By applying a 30-fold uncertainty factor, the resulting toxicity value calculated for changes in T4 homeostasis was 0.2 mg/kg/day. Liver enzyme induction was identified as the basis for USEPA's oral reference dose (RfD; 0.002 mg/kg/day), this endpoint was used in the assessment to provide an upper-bound estimate of the potential health hazards. The results of the screening-level exposure assessment indicated that the highest hypothetical exposures occurred in children <1 year, 1-2 years and 3-5 years old. Total daily intakes ranged from 0.00004 to 0.0009 mg/kg/day. Calculated daily intakes for children and adults were well below the three screening toxicity benchmarks.

1915 DEVELOPMENTAL EXPOSURE TO POLYBROMINATED DIPHENYL ETHERS IMPAIRS SYNAPTIC TRANSMISSION AND LTP IN HIPPOCAMPUS.

M. E. Gilbert[1], L. Sui[2, 1] and K. M. Crofton[1]. *[1]Neurotoxicology, USEPA, Research Triangle Pk, NC and [2]National Research Council, Washington, DC.*

Polybrominated diphenyl ether (PBDE) flame retardants bioaccumulate in wildlife and in humans and reduce circulating levels of thyroxine (T4). The present work examined hippocampal function in adult offspring of LE rats treated daily by oral gavage with 0, 30 or 100 mg/kg of a commercial PBDE mixture, DE71 from gestational day 6 to weaning on postnatal day (PND) 21. This regimen produced moderate decreases in T4 (40-70%) during the early postnatal period with recovery to control levels by PND36. Spatial learning and hippocampal synaptic transmission were evaluated in adult male offspring. Learning was assessed in a Morris water maze (2 trials/day for 15 days). Several weeks later, electrodes were implanted in the perforant path and dentate gyrus under urethane anesthesia, to examine synaptic function. Input/output (I/O) and paired-pulse functions were collected to assess baseline synaptic transmission and short-term facilitation. Long-term potentiation (LTP) was induced by delivering theta burst stimulation at a modest (300μA)and a high (1500μA) stimulus intensity. No effect of DE71 was seen on spatial learning. In baseline I/O recordings, population spike amplitude was increased in the 100 mg/kg group relative to controls, with no effect on EPSP slope amplitude. Despite an increase in baseline synaptic transmission, LTP of the population spike was impaired in response to both stimulus strengths in the 100 mg/kg dose group. The data suggest that the reductions in T4 induced by a 100 mg/kg dose of DE71 were sufficient to alter synaptic transmission in the hippocampus. Effects of DE71 on hippocampal synaptic function are similar to those produced by developmental exposure to the polychlorinated biphenyl mixture, A1254, which also failed to affect learning in the Morris water maze. Collectively, these results suggest that identifying behavioral correlates of subtle changes in hippocampal synaptic function may require more sophisticated tests of learning and memory. *(This abstract does not reflect USEPA policy).*

1916 PERFLUOROOCTANE SULFONATE (PFOS) ALTERS LUNG DEVELOPMENT IN THE NEONATAL RAT.

R. C. Grasty[1, 2], N. Roberts[1], B. E. Grey[1], C. Lau[1] and J. M. Rogers[1, 2]. *[1]Reproductive Toxicology Division, NHEERL, ORD, USEPA, Research Triangle Park, NC and [2]Curriculum in Toxicology, UNC Chapel Hill, Chapel Hill, NC.*

Perfluorooctane sulfonate is an environmentally stable compound used widely in industrial and household products; its presence has been detected in both humans and wildlife. Chronic prenatal exposure to PFOS in rodents leads to mortality in newborns within hours to days after birth. We have previously demonstrated that treatment on gestation days (GD) 19-20 is sufficient to induce neonatal death in the rat; while the underlying pathophysiological mechanism remains to be determined. Here, timed-pregnant Sprague-Dawley rats were treated by oral gavage with 0, 25, or 50 mg/kg/d PFOS/K+ on GD 19-20; and lungs from fetuses and newborns (within 1 h after birth) were harvested for evaluation. Histological examination of the fetal lungs taken on GD 19-21 did not reveal any difference between control and treated animals. Lungs from newborns indicated morphological abnormalities in some of the treated animals (A) in both dose groups, whereas no abnormalities were apparent in others (B), as compared to controls (C). Lungs from control newborns and 50 mg/kg group were then compared using morphometric analysis. In all measurements taken, no differences were found between samples from groups B and C. In contrast, the proportion of cross-sectional lung area occupied by solid tissue was 0.43 in group A samples and 0.26 in group C ($p<0.05$). The proportion of airway space was significantly decreased in group A as was the ratio of airway:tissue area (2.41 in A vs. 2.94 in C). Ultrastructural evaluation of the alveolar epithelial cells with electron microscopy confirmed the histological findings, and indicated subcellular disorganization in the newborn lungs exposed to PFOS. These results thus demonstrate that exposure to PFOS on GD 19-20 critically impacts normal lung development in the rat, potentially leading to pulmonary dysfunction in the newborns. This abstract does not reflect EPA policy.

1917 EFFECTS OF PERFLUOROOCTANE SULFONATE (PFOS) ON THYROID HORMONE STATUS IN ADULT AND NEONATAL RATS.

M. N. Logan[1], J. R. Thibodeaux[2], R. G. Hanson[2] and C. Lau[2]. *[1]Biology, North Carolina Central University, Durham, NC and [2]USEPA NHEERL, Research Triangle Park, NC.* Sponsor: J. Rogers.

Perfluorooctane sulfonate is an environmentally persistent compound ubiquitously found in humans and wildlife. Previous studies have suggested that PFOS alters thyroid hormone status in laboratory animals. The current study examines whether hormone metabolism is involved in PFOS-mediated thyroid deficiency. Adult

Sprague-Dawley rats were given (oral gavage) 3 or 5 mg/kg PFOS/K+ for 21 days, while controls received 0.5% Tween-20 vehicle. Serum thyroxine (T4) and triiodothyronine (T3), and liver uridine diphosphate-glucuronosyl transferase (UDP-GT), a microsomal enzyme that metabolizes (T4), were determined at days 3, 7, 14 and 21 after initiation of treatment. Significant reductions in serum total T4 (58%), free T4 (70%), and total T3 (23%) were noted in the PFOS-treated rats in as little as three days in both dose groups. Hormonal deficits were sustained throughout the study. However, no significant alterations in T4-UDP-GT activities were detected; at day 21, mean T4-UDP-GT activities were 0.614, 0.571, and 0.654 pmol/mg protein/min for control, 3, and 5 mg/kg PFOS groups, respectively. Previously, we have shown that prenatal exposure to PFOS led to hypothyroxinemia in rat pups during postnatal development, while serum T3 levels were not affected. The current study extended the chemical exposure to the postnatal period. PFOS (10 mg/kg, p.o.) was given to rat pups daily from PD1 to PD48. All pups survived the chemical treatment, but serum tT4, fT4 and tT3 were significantly reduced by 64%, 68% and 29% of controls, respectively. The hormonal deficits persisted into young adulthood. These results suggest that PFOS reduces circulating thyroid hormones effectively in both mature and developing rats, but enhancement of hepatic metabolism of the hormones is not likely involved in altering the hormone economy. (Funded by EPA/NCCU Toxicology research program, training agreement CT 829460 with the Department of Biology, NCCU. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1918 PERFLUOROOCTANOIC ACID: RELATIONSHIP BETWEEN REPEATED INHALATION EXPOSURES AND PLASMA PFOA CONCENTRATION IN THE RAT.

P. M. Hinderliter, M. P. DeLorme and G. W. Jepson. *Haskell Laboratory for Health and Environmental Sciences, Newark, DE.*

A large pharmacokinetic database exists describing the behavior of perfluorooctanoic acid (PFOA) following oral exposure. The objective of this study was to quantify plasma PFOA concentrations in male and female rats, following single and repeated inhalation exposures, for the purpose of bridging the oral and inhalation exposure data sets. The study was comprised of two separate experiments: single (6 hour) and repeated nose only exposures (6 hours per day, 5 days per week for three weeks) to an aqueous aerosol of 0, 1, 10, or 25 mg/m^3 PFOA. Levels were selected to produce plasma concentrations similar to those observed in previous oral gavage studies. For the single exposure, blood was drawn pre-exposure, during exposure and post-exposure. For the repeated exposure, blood was collected immediately before and after the daily inhalation exposure period three days per week. Plasma derived from the whole blood samples was analyzed by liquid chromatography-mass spectrometry (LC-MS). PFOA appeared rapidly in the blood of both male and female rats exposed *via* the inhalation route. PFOA elimination was sex-dependent, with female rats eliminating PFOA from the plasma much more efficiently than male rats. PFOA plasma concentrations were proportional to atmospheric exposure concentrations from 1 to 25 mg/m^3. Repeated daily inhalation exposures produced little plasma carryover in female rats, but significant carryover in male rats. Male rats reached a steady state plasma concentration by three weeks with plasma concentrations of 8, 21, and 36 µg/mL respectively when exposed to 1, 10, and 25 mg/m^3 PFOA. Female rats reached post exposure plasma concentrations of 1, 2, and 4 µg/mL respectively but returned to baseline levels for the pre-exposure time points. This study provides the data necessary to relate external atmospheric concentrations of PFOA to PFOA levels in male and female rat plasma. This research was sponsored by the Association of Plastics Manufacturers in Europe (APME) Fluoropolymers Committee.

1919 CONSIDERATIONS RELEVANT TO CONSTRUCTING A HUMAN PBPK MODEL FOR PERFLUOROOCTANOIC ACID (PFOA).

D. J. Paustenbach[1] and G. W. Jepson[2]. *[1]ChemRisk, San Francisco, CA and [2]Biochemical and Molecular Toxicology, Haskell Laboratory for Health and Environmental Sciences, Newark, DE.*

Perfluorooctanoic acid (PFOA) is a surfactant, which has received significant interest in recent years due to its reported prevalence in blood samples from many US citizens. Consequently, there has been some activity associated with constructing a human PBPK model to relate inhalation and oral exposures to PFOA levels in plasma. While there have been many kinetic studies conducted on rodents, the rodent kinetic data have not been useful in extrapolation to PFOA kinetics in humans. There are several considerations relevant to the construction of a human PBPK model that will determine how, or even if, such a model is appropriate for PFOA. First, PFOA is a surface-active chemical, which will affect its behavior in biological systems and has thus far complicated any efforts to determine tissue to blood partition coefficients. Second, PFOA is reported to be unmetabolized in mammalian systems, thereby eliminating the need for explicit description of metabolizing organs or tissues. Third, the elimination rate is dramatically different between humans and rodents. The reported half-life in humans is approximately 4 years as compared to 2 hours in the female rat. Further, rats have sex dependent elimination rates, which do not appear to be relevant to humans. Fourth, controlled human exposures to PFOA are lacking; therefore, any attempts to validate a PBPK model would be severely limited. This is further complicated by the fact that elimination mechanisms have not been completely elucidated in rats and humans. These considerations and gaps in the understanding of species-specific elimination of PFOA make it impractical to construct and apply a human PBPK or generic kinetic model to predict PFOA concentrations in plasma following ingestion or inhalation of PFOA. The basis for constructing and validating a human PBPK model for PFOA was described and evaluated.

1920 A LONG-TERM TREND OF SERUM LEVELS OF PERFLUOROOCTANE SULFONATE (PFOS) AND PERFLUOROOCTANOATE (PFOA) IN JAPANESE.

K. Harada, A. Koizumi, T. Yoshinaga, K. Inoue and N. Saito. *Health Environmental Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan.* Sponsor: R. Reitz.

PFOS and PFOA are chemicals of a new class of Pops. They have been used from 1950s as various purposes. The aim of this study is to assess a long-term trend of PFOS and PFOA exposures using serum samples collected from late 1970s to 2003 in Japan. Methods.Human serum. We used samples in the sample bank. We collected serum samples in Miyagi (Miyagi samples), Akita (Akita samples) and Kyoto (Kyoto samples). In Miyagi, Akita, Kyoto, we collected samples in 1970s and 2003, in the early 1990s and 2003, in 2002 and 2003 respectively. Analysis: the 0.5 ml of serum were used for determination of PFOA and PFOS by LC/MS (Saito et al., 2003). Results. The PFOS and PFOA levels in serums [GM(GSD)](PFOS and PFOA in order :microgram/L) were; in Miyagi samples in 1970s 1.1(1.8) and 0.2(2.0) for females (n=40); in 2003 5.7(1.8) and 3.3(2.0) for males (n=32) and 3.5(2.9) and 2.8(1.5) for females (n=23). In Akita samples they were; in 1991, 10.2(1.5) and 2.2(1.4) for males (n=16) and 8.0(1.4) and 1.7(1.5) for females (n=40) and in 2003 12.9(1.5) and 3.4(1.5) for males (n=66) and 5.0(3.2) and 2.4(1.7) for females (n=54). In Kyoto, they were in 2003; 21.8(1.7) and 9.4(1.5) for males (n=28) and 12.7(1.5) and 6.1(1.5) for females (n=26) . In Miyagi PFOS and PFOA concentrations have increased 3 times and 14 times, respectively. In Akita, there are no increase between 1990s and 2003 for PFOS but the level of PFOA was increasing. There are large gender differences in the concentrations of PFOS and PFOA at three locations. Furthermore, there are predominant regional differences for both PFOS and PFOA concentrations. Systematic collection of samples at different time periods, genders, and locality enabled us to analyze the exposure trends.Reference.Saito et al.; (2003) Arch. Environment Contam. Toxicol. 45: 149-58

1921 PERFLUOROOCTANOATE AND PERFLUOROOCTANE SULFONATE CONCENTRATIONS IN SURFACE WATERS IN JAPAN.

N. Saito[2], A. Koizumi[1], T. Yoshinaga[1], K. Harada[1] and K. Inoue[1]. *[1]Health Environmental Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan and [2]Health and Environmental Sciences, Iwate Environmental Institute, Morioka, Japan.* Sponsor: R. Reitz.

Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are Pops widely used in Japan.We analyzed their concentrations in surface water samples collected from all over Japan. Methods: Water samples were collected from rivers, coastal sea waters and tap waters. For all sampling, a two-L sample was collected. Samples were passed through the Presep-C Agri column at a flow rate of 10 mL /min using a Waters Concentrator System (Concentrator Plus, Waters, Tokyo, Japan). Presep-C cartridges were then eluted with 1.5 mL of methanol and concentrated at room temperature. The methanol extracts were chromatographed using HPLC and Mass spectra were taken on an LC/MS.The fragment ions for PFOA m/z 413 (C7F15CO2-) and for PFOS m/z 499 (C8F17SO3-) were monitored for quantification. Results and discussion: The lowest limits of detection (LOD) (ng/L) were 0.06 for PFOA and 0.04 for PFOS. The lowest limits of quantification (LOQ) (ng/L) were 0.1 for both analytes. The levels [geometric mean (GM); geometric standard de*via*tion (GS)] (ng/L) of PFOA and PFOS in the surface waters were GM (GS): 0.97 (3.06) and 1.19 (2.44) for Hokkaido-Tohoku (n=16); 2.84(3.56) and 3.69 (3.93) for Kanto (n=14); 2.50 (2.23) and 1.07 (2.36) for Chubu (n=17); 21.5 (2.28) and 5.73 (3.61) for Kinki (n=8); 1.51 (2.28) and 1.00 (3.42) for Chugoku (n=9); 1.93 (2.40) and 0.89 (3.09) for Kyushu-Shikoku (n=15). The GM of PFOA in Kinki was significantly higher than in other areas (ANOVA $p<0.01$). Systematic searches of Yodo and Kanzaki Rivers revealed two potential sources, a public-water-disposal site for PFOA and an airport for PFOS. The former was estimated to release 18 kg of PFOA/day. PFOA in drinking water in Osaka city [40

(1.07) ng/L] was significantly higher than in other areas. The present study confirms that a large amount of PFOA is produced and released in Kink, and causes drinking water contamination.

1922 PAHS EXPOSURE AND BIOMARKERS: SIMULTANEOUS ANALYSIS OF 1-HYDROXYPYRENE AND ITS CONJUGATES IN URINE.

Y. Hu[1], X. Xue[1], Z. Zhou[2], J. Fu[2], B. S. Cohen[1], A. A. Melikian[3], M. Desai[1], X. Li[1], E. Tang[1], X. Huang[1], N. K. Roy[1] and Q. Qu[1]. *[1]Institute of Environmental medicine, New York University School of Medicine, Tuxedo, NY, [2]Department of Toxicology, School of Public Health, Peking University, Beijing, China and [3]American Health Foundation, Valhalla, NY.* Sponsor: L. Chen.

The present study is part of the biomarker validation project conducted in a Chinese occupational population. 57 subjects, including 28 exposed workers and 29 controls, were recruited and monitored for their personal exposures to PAHs and excretions of 1-hydroxypyrene (1-OHP) in urine. The average personal exposure levels of pyrene, benzo(a)anthracene (BaA) and BaP in exposed workers were 15.2 ± 9.1 ug/m3, 4.4 ± 1.6 ug/m3 and 4.3 ±1.6 ug/m3, respectively. The corresponding levels in control subjects were 0.016 ± 0.017 ug/m3, 0.022 ± 0.011 ug/m3, and 0.011 ± 0.011 ug/m3. A variety forms of 1-OHP, including free 1-OHP and its conjugates of glucocorunide (1-OHP-G) and sulphate (1-OHP-S), were analyzed simultaneously in urine samples collected from participating subjects after work. The average levels of total urinary 1-OHP were 7.15 ± 5.35 umol/mole creatinine in exposed workers and 0.71 ± 0.43 umol/mole creatinine in the controls. Among the three forms, 1-OHP-G was found to be predominant, which represents 93.7% and 74.2% of total 1-OHP excretions in exposed and control subjects, respectively. A strong correlation was observed between urinary concentrations of 1-OHP-G and personal exposure levels of pyrene, BaA and BaP (r=0.896, 0.904 and 0.907 respectively). Multiple linear regression analyses indicated that after adjustment for potential confounding by the amount of smoking, there was still a strong association between PAHs exposure and levels of 1-OHP in urine ($p<0.01$). The estimated half-life of 1-OHP in urine is about 15.2 hours after cessation of exposure. Further validation of 1-OHP and other biomarkers for low level exposures is being investigated in humans exposed to PAHs at or above ambient levels.

1923 THE CARCINOGEN 7, 8-DIHYDRO-9, 10-EPOXY-7, 8, 9, 10-TETRAHDROBENZO[A]PYRENE AND BENZO(A)PYRENE REDUCED ANDROGEN RECEPTOR EXPRESSION IN HUMAN LUNG CELLS.

P. Lin and J. Ko. *Toxicology, Chung Shan Medical University, Taichung, Taiwan.*

5-Alpha dihydrotestosterone significantly increased cell growth of lung adenocarcinoma cell lines H1355. Benzo[a]pyrene (BaP) is a pulmonary carcinogen found in cigarette smoke. Treatment with 1 mM BaP tremendously reduced androgen receptor (AR) expression in H1355 cells, as determined with Western immunoblot and the real-time RT-PCR assay. Similarly, 1 mM BaP significantly reduced AR mRNA levels in human bronchial epithelial cells BEAS-2B. Although BaP, 2, 3, 7, 8-tetrachlorodibenzo-p-dixin (TCDD) and polychlorinated biphenyl 126 (PCB126) activated aryl hydrocarbon receptor (AhR) which subsequently induced cytochrome P4501A1 (CYP1A1) and 1B1 (CYP1B1) expression in H1355 cells, neither TCDD nor PCB126 reduced AR expression. Antagonizing AhR activation with alpha-naphthoflavone, or inhibiting CYP1B1 activity with 2, 4, 3, 5-tetramethoxystilbene, however, prevented BaP-induced AR reduction. We also found that 7, 8-dihydro-9, 10-epoxy-7, 8, 9, 10-tetrahydrobenzo[a]pyrene (BPDE), a BaP carcinogenic metabolite catalyzed by CYP1A1 and CYP1B1, significantly reduced AR expression in H1355 cells and human lung fibroblasts WI-38. Furthermore, inhibiting tyrosine phosphorylation with genestein tremendously prevented BaP-induced AR reduction in H1355 cells. This is the first study reported that BaP and BPDE reduced endogenous AR expression. These data suggest that metabolically activated BaP may disrupt androgen function by reducing AR levels in androgen-responsive organs.

1924 DEVELOPMENT OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF PYRENE-1, 6- AND -1, 8-DIONE IN ANIMAL AND HUMAN URINE.

A. Ruzgyte, M. Bouchard and C. Viau. *Environmental & Occupational Health, University of Montreal, Montreal, QC, Canada.*

Pyrene is a major and ubiquitous representative of most mixtures of polycyclic aromatic hydrocarbons (PAHs). A number of field studies indicated that 1-hydroxypyrene (1-OHP) in urine is a good biomarker of occupational exposure to pyrene, while it lacks sensitivity as an indicator of low-dose environmental exposure. Recent mass-balance and kinetics studies in rats exposed to ^{14}C-labelled pyrene suggest that 1-OHP is not the major excreted metabolite of pyrene. The purpose of this study was thus to identify and quantify metabolites of pyrene other than 1-OHP, in animal and human urine. Using a liquid chromatography-electrospray-mass spectrometry method, it was first observed that dioxygenated pyrene metabolites (m/z = 233) were present in significant amounts in urine samples of rats treated with pyrene. A high-performance liquid chromatography method with fluorescence detection techniques was then developed to simultaneously determine pyrene-1, 6-dione (P16D) and pyrene-1, 8-dione (P18D) metabolites. Interestingly, results using both rat and human samples showed that P16D molar concentrations exceed that of 1-OHP by 30 to 90 times and P18D concentrations exceed 1-OHP by 4 to 9 times. These urinary metabolites thus appear as potentially useful biomarker of low-dose environmental exposure to pyrene.

1925 EFFECTS OF POLYAROMATIC HYDROCARBON CONTENT IN VEHICLE GASOLINE EMISSION EXHAUST ON GSH/GSSG RATIO *IN VITRO*.

R. P. Balan[1], J. L. Garcia-Tavera[1], A. Zambrano-Garcia[2], J. L. Arriaga[2], University. Gonzalez-Macias[2], I. Zapata-Penazco[2], M. E. Cebrian[1], E. S. Calderon-Aranda[1] and A. De Vizcaya-Ruiz[1]. *[1]Toxicology, CINVESTAV-IPN, Mexico D.F., Mexico and [2]Ecotoxicology, IMP, Mexico D.F., Mexico.*

Mobile sources contribute with an 84% of total emissions to the air in Mexico City, among their components are polyaromatic hydrocarbons (PAHs), which have been described as the responsible components of most of the toxic effects from diesel emissions. Limited information is available on the effects from PAHs mixtures from vehicle gasoline emissions exhaust on mammalian cells. The objective of this study is to provide information on the toxic effects on J774A.1 macrophages exposed to PAHs organic extracts (PAH-VEE) from gasoline emissions using oxidative stress as a potential biomarker of toxicity. Gaseous and particulated emissions were obtained from a 1.7 L gasoline engine vehicle operated under the FTP-75 method using two Mexican commercial gasolines and analyzed under US-EPA TO13. Average PAHs content in the organic extracts of the vehicle emissions were 42.43ng/μL in the gaseous and 1.97ng/μL in the particulated phase, two ring compounds being the most abundant. J774A.1 cells were exposed to the PAH-VEE from the gaseous and particulated phases at a dose equivalent to a 160m of urban operation conditions. No cytotoxicity assayed by MTT was observed. The GSH/GSSG ratio was determined using the recycling method with 2-VP as a scavenger in J774A.1 cells exposed to the PAH-VEE at 06, 12 and 24h. No evident alteration on GSH/GSSG ratio, in cells exposed to both phases of gasoline emissions was observed under these conditions. The lack of effect could be attributed to a low concentration of hydrophilic compounds, or to the triggering of other antioxidant elements involved in the antioxidant response in macrophages, such as γ-GCS, GSH synthetase, GST, SOD and catalase. Further research using higher concentrations of PAHs in the organic extracts and the use of GSH synthesis inhibitors could be performed to rule out a possible antioxidant compensation response. (Financed by FIES-IMP 98-119-VI).

1926 *IN VIVO* AND *IN VITRO* IMMUNOSUPPRESSIVE EFFECTS OF BENZO[K]FLUORANTHENE IN FEMALE BALB/C MICE.

T. Jeon, C. Jin, S. Lee, D. Lee, N. Kim, S. Hyun and T. Jeong. *Pharmacy, Yeungnam University, Kyungsan, Kyungbuk, South Korea.*

Although polycyclic aromatic hydrocarbons (PAHs) have been known to suppress immune responses, few studies have addressed the immunotoxicity of benzo[k]fluoranthene (B[k]F). In this study, we investigated the immunosuppression of B[k]F, both *in vivo* and *in vitro*, in female BALB/c mice. To assess the effects of B[k]F on humoral immunity as splenic antibody response to sheep red blood cells (SRBCs), B[k]F was given a single dose or once daily for 7 consecutive days po with 30, 60 and 120 μmoles/kg. Acute oral administration of B[k]F induced a dose-related increase in liver weight. B[k]F also induced activities of hepatic ethoxyresorufin O-deethylase and pentoxyresorufin O-depentylase. Oral B[k]F reduced the number of antibody forming cells (AFCs) in a dose-dependent manner. Subacute treatment with B[k]F caused weight increases in liver and decreases in spleen and thymus. Interestingly, subacute B[k]F inhibited the hepatic activity of methoxyresorufin O-demethylase. The number of AFCs was dramatically decreased by B[k]F in a dose-dependent manner. In a subsequent study, mice were subacutely exposed to same doses of B[k]F without an immunization with SRBCs, followed by splenic and thymic lymphocyte phenotypings using a flow cytometry and *ex vivo* mitogen-stimulated proliferation. B[k]F exposed mice exhibited reduced splenic and thymic cellularity, decreased numbers of total T cells, $CD4^+$ cells and $CD8^+$ cells in spleen and immature $CD4^+CD8^+$ cells, $CD4^+CD8^-$ cells and $CD8^+CD4^-$ cells in thymus. In *ex vivo* lymphocyte proliferation assay, B[k]F inhibited splenocyte proliferation by

LPS and Con A. In *in vitro* mitogen-stimulated proliferation by untreated splenic suspensions, B[k]F only suppressed splenocyte proliferation to LPS. These results suggest that B[k]F might have immunotoxic potential by mechanisms associated with metabolic processes. (Supported by a grant from Korea Food and Drug Administration).

1927 DNA ADDUCT AS BIOLOGICAL EFFECT MARKER IN A CHINESE POPULATION WITH ENVIRONMENTAL EXPOSURES TO PAHS.

Q. Qu[1], Y. Hu[1], X. Xue[1], Z. Zhou[2], J. Fu[2], B. Cohen[1], D. Li[3], X. Li[1], E. Tang[1] and N. Roy[1]. *[1]Environmental Medicine, NYU School of Medicine, Tuxedo, NY, [2]Toxicology, Peking University School of Public Health, Beijing, Beijing, China and [3]Gastrointestinal Medical Oncolog, University of Texas, M.D Anderson Cancer Center, Houston, TX.* Sponsor: L. Chen.

A biomarker validation study has been being conducted in a Chinese population with occupational or environmental exposures to PAHs. So far, 142 subjects (99 male and 43 female) have been recruited from coke oven workers, residents in a metropolitan area and surrounding suburb. All participating subjects were monitored for their personal exposures to PAHs and DNA adduct levels in lymphocytes. The average personal exposure levels of pyrene, benzo(a)anthracene (BaA) and BaP were 3.28, 1.044, and 0.957 ug/m3 with corresponding median levels of 0.155, 0.137, and 0.072 ug/m3, respectively. The bulky PAH-DNA adducts were determined by 32p-postlabeling analysis (P1 enrichment procedure). A moderate significant correlations were observed between DNA adduct levels and personal exposure levels of BaP ($r=0.355$, $P<0.0001$) and total PAHs ($r=0.335$, $P<0.0001$). The multiple regression analyses of DNA adducts on PAHs exposure indicated that smoking status and cotinine levels had no significant effect on DNA adducts. Genotype assays are now being conducted to evaluate whether or not the polymorphisms of genes, including CYP1A1, mEH, GSTT1, GSTM1, etc., play roles in determining individual variations in susceptibility to PAHs-induced DNA adducts formation.

1928 BENZO(A)PYRENE INDUCES ATM-DEPENDENT P53 PHOSPHORYLATION AND CELL CYCLE ARREST IN HUMAN PLACENTAL AND ENDOMETRIAL CELL LINES.

J. M. Kitzman, T. A. Medrano and K. T. Shiverick. *Pharmacology & Therapeutics, University of Florida, Gainesville, FL.*

Maternal cigarette smoking disrupts placental growth and function, and is linked to uterine disease and infertility. Benzo(a)pyrene (BaP), a major toxicant in cigarette smoke, has been previously shown to inhibit proliferation of human placental JEG-3 and uterine endometrial RL95-2 cells. ATM, a serine/threonine kinase, is known to function as a primary sensor of DNA damage that activates p53 through phosphorylation on serine-15 (p53 ser-15phos). This study investigated whether BaP inhibition of cell cycle progression involves ATM-dependent p53 serine-15 phososphorylation in JEG-3 and RL95-2 cells. In trophoblastic JEG-3 cells, flow cytometry analysis shows that 10 uM BaP produced G2/M phase accumulation by 24 hr that continued through 96 hr. Western immunoblot analysis showed that BaP significantly increased p53 ser-15phos at both 24 and 48 hr, followed by increased expression of p21Cip1 (p21), a key cyclin dependent kinase inhibitor, at 48 hr. The presence of 20 uM wortmannin, a specific inhibitor of ATM kinase, markedly inhibited BaP-induced p53 ser-15phos at 24 hr, as well as p21 expression at 48 hr. In endometrial RL95-2 cells, BaP (10 uM) treatment produced G2/M cell cycle accumulation at 48 and 72 hrs accompanied with a 600% increase in p53 ser-15phos at 48 hr. Again, the presence of 20 uM wortmannin markedly inhibited the BaP-mediated effect on p53 phosphorylation. In summary, data indicate that the anti-proliferative effects of BaP in JEG-3 and RL95-2 cells involve G2/M cell cycle arrest in association with ATM-dependent p53 serine-15 phosphorylation. (Supported by NIH ES07375)

1929 *IN VIVO* AND *IN VITRO* COMPARISONS OF POLYCYCLIC AROMATIC HYDROCARBONS-INDUCED IMMUNOTOXICITY IN MICE.

D. Lee, T. Jeon, C. Jin, S. Lee, N. Kim, S. Hyun and T. Jeong. *Pharmacy, Yeungnam University, Kyungsan, Kyungbuk, South Korea.*

Effects of five PAHs, such as benz[e]acephenanthrylene (B[e]A), 1, 2-benzanthracene (1, 2-BA), pyrene, chrysene and benzo[a]pyrene (B[a]P), on immune functions were compared in female BALB/c mice. PAHs were given a single dose or once daily for 7 consecutive days po at either 30, 60 or 120 μmoles/kg. Activities of hepatic CYP-dependent enzymes were also determined following the administration of PAHs. Additionally, subacute effects of PAHs on splenic and thymic lymphocyte phenotypes and *ex vivo* mitogen-stimulated proliferation were determined. Finally, *in vitro* splenocyte proliferation, following a direct addition of PAHs, was measured. Acute oral administration of pyrene and B[e]A induced a dose-related decrease and increase in thymus and liver weights, respectively. Oral B[a]P and B[e]A suppressed the antibody response most potently. Subacute treatment with B[a]P, B[e]A and 1, 2-BA induced weight decreases in spleen. All PAHs significantly induced hepatic EROD activities. B[e]A, pyrene and chrysene induced PROD activities. Subacute oral treatment with most PAHs suppressed the antibody response dose-dependently. The subacute exposure to B[a]P, B[e]A and 1, 2-BA without an immunization showed decreases in thymus weights. In addition, B[a]P, B[e]A and 1, 2-BA exposed mice exhibited characteristic suppression profile of splenic and thymic cellularity, numbers of total T, T-subset and B cells in spleen and $CD4^+CD8^+$, $CD4^+CD8^-$ and $CD4^-CD8^+$ cells in thymus. In *ex vivo* lymphocyte proliferation assays, B[a]P and 1, 2-BA suppressed splenocyte proliferation by LPS and Con A. No PAHs had effects on *in vitro* mitogen-stimulated proliferation directly. These findings suggest that the immunotoxic potential of PAHs studied would be ranked as B[e]A, B[a]P > 1, 2-BA > chrysene, pyrene, and that B[a]P and 1, 2-BA might require metabolic activation for their immunotoxicity. (Supported by a grant from Korea Food and Drug Administration).

1930 DIETARY FAT MODULATED METABOLISM OF FLUORANTHENE (FLA) IN F-344 RATS.

S. A. WALKER and A. RAMESH. *PHARMACOLOGY, MEHARRY MEDICAL COLLEGE, NASHVILLE, TN.*

Chemically contaminated diets rich in fat leads to a greater intake and retaining of toxic chemicals in the body with an increased risk to human health. This study was conducted to measure the metabolism of fluoranthene (FLA), a polycyclic aromatic hydrocarbon compound, subsequent to acute oral exposure under different dietary regimens. F-344 male rats were given 50 μg/kg FLA *via* oral gavage using test meals comprising peanut, corn, and coconut oils as representatives of monounsaturated, polyunsaturated and saturated dietary fats respectively. Control animals were either exposed to vehicle only or unexposed. After exposure, rats were sacrificed at different time points; trunk blood, urine and feces were collected. Stomach, intestine, liver, lung, testis and brain tissues were removed. Samples were subjected to liquid-liquid extraction using water, methanol and chloroform. The extracts were analyzed for FLA and metabolites by reverse-phase HPLC with fluorescence detection. The FLA metabolite concentrations varied with the type of dietary fat intake. For e.g. FLA dosed in test meal containing saturated fat produced the greatest quantity of metabolites followed by test meals containing polyunsaturated and monounsaturated fat. The FLA 2, 3-diol is the predominant metabolite followed by i) trans-2, 3-dihydroxy-1, 10b-epoxy-1, 2, 3, 10b tetrahydro FLA (2, 3 D FLA), ii) FLA-2, 3-dione, iii) 3-hydroxy FLA and iv) 8-hydroxy FLA. The concentrations of FLA 2, 3-diol and 2, 3 D FLA were high in plasma of rats that were administered saturated fat, followed by polyunsaturated and monounsaturated fat. On the other hand, the concentrations of 3 (OH) FLA (detoxification product of FLA) were low in plasma of rats that ingested saturated fat compared to those that ingested poly, and monounsaturated fat. Our findings indicate a) dietary fat type influences the overall concentration of FLA metabolites b) the qualitative distribution of FLA metabolites in plasma and target tissues also depend on lipid type (Supported by NIEHS grant #1R15ES12168-01, NIGMS-SCORE grant #2S06GM08037-32, RISE grant #2R25GM59994 and NCRR-RCMI grant #G12RR03032).

1931 DMBA BONE MARROW TOXICITY SELECTIVELY AFFECTS THE GRANULOCYTE POPULATION, MONOCYTE VS. NEUTROPHIL.

N. Galvan[1, 2], C. J. Czuprynski[1, 3] and C. R. Jefcoate[1, 2]. *[1]Molecular and Environmental Toxicology, UW-Madison, Madison, WI, [2]Pharmacology, UW-Madison, Madison, WI and [3]Pathological Sciences, UW-Madison, Madison, WI.*

Treatment of mice with the prototypical polycyclic aromatic hydrocarbons (PAHs), 7, 12-dimethylbenz(a)anthrecene (DMBA) and benzo(a)pyrene (BP), leads to bone marrow toxicity, lymphoid tumors and immunosuppression. Resistance to the DMBA toxicity with the CYP1B1-/- and TNFR-/- mice has provided compelling evidence that implicate CYP1B1 and TNFR (and by implication TNFα) in this response. We have used in situ hybridization and RT-PCR to show the presence of CYP1B1 and induction by PAHs in the bone marrow. In addition, we demonstrated that CYP1A1 is not present in bone marrow, and that low levels of CYP1B1 are sufficient for DMBA bone marrow toxicity. Our previous *in vitro* studies show that bone marrow stromal fibroblasts activate PAHs *via* CYP1B1. Others have shown that leukocytes can bioactive PAHs *via* CYP1B1 metabolism, while mononuclear phagocytes secrete TNFα following PAH treatment. We have demonstrated that 48 hours after DMBA treatment of C57Bl/6 mice is then followed by a decrease in the granulocyte (Gr-1+) population. In this study, we aimed to identify the specific type of hematopoietic cells that express CYP1B1 within the

bone marrow and populations that are susceptible to DMBA toxicity. The use of 7/4 and Gr-1 monoclonal antibodies has allowed us to identify and separate by FACS the myeloid population into monocytes (Gr-1lo/7/4hi) and neutrophils (Gr-1hi/7/4hi). These data will allow us to determine the susceptibility of these bone marrow populations to DMBA. In addition, RT-PCR analysis will determine their relative CYP1B1 expression, and of inflammatory cytokines that further modulate DMBA bone marrow toxicity.

1932 SPECIES-SPECIFIC INTESTINAL MICROSOMAL METABOLISM OF FLUORANTHENE.

A. RAMESH, S. A. WALKER, L. WHITTEN and G. SEALS.
PHARMACOLOGY, MEHARRY MEDICAL COLLEGE, NASHVILLE, TN.

Several species respond differently to toxic chemical exposure. The magnitude of susceptibility to toxicant exposure may depend on the ability of animals to metabolize the chemicals. The present study has been undertaken to see whether any differences exist among mammals in the metabolism of fluoranthene (FLA), a polycyclic aromatic hydrocarbon (PAH) compound. Since intestine is the functional interface between orally ingested FLA and the body, microsomes were isolated from the small intestine of rat, mouse, hamster, guinea pig, goat, sheep, pig, dog, and humans (commercially procured) and incubated for 15 min with 125 µl of FLA solution (0.807 mg/ml). The reaction was stopped by the addition of 8 ml ethyl acetate containing butylated hydroxytoluene. The post-incubation samples were extracted twice with ethyl acetate, concentrated, and analyzed for FLA/metabolites by reverse-phase HPLC with fluorescence detection. The concentrations of FLA metabolites produced by microsomes varied among the species. The invitro metabolism of FLA was in the order- human > dog > pig > goat > sheep > guinea pig > hamster > rat > mouse. The FLA metabolites identified were FLA 2, 3-diol, trans-2, 3-dihydroxy-1, 10b-epoxy-1, 2, 3, 10b tetrahydro FLA (2, 3 D FLA), FLA-2, 3-dione, 3-hydroxy FLA, and 8-hydroxy FLA. The rodent microsomes produced considerably higher proportion of FLA 2, 3-diol, and 2, 3 D FLA than did pig, dog, and humans. On the other hand, the pig, dog, and human microsomes converted a greater proportion of FLA to 3-hydroxy FLA, the detoxification product of FLA. Our results revealed that the metabolism of widely distributed PAHs such as FLA is species-specific. Overall, our findings suggest that dog, pig, and humans are the species best equipped to process FLA. These findings will be of help in extrapolation of metabolic data from one species to another and identify the susceptible species from a risk assessment perspective (Supported by NIEHS grant #1R15ES12168-01, NIGMS-SCORE grant #2S06GM08037-32, RISE grant #2R25GM59994 and NCRR-RCMI grant # G12RR03032).

1933 *IN VITRO* AND *IN VIVO* GENOTOXICITY OF ISOLATED FRACTIONS FROM PAH AND CHLOROPHENOL MIXTURES.

T. D. Phillips, A. M. Gillespie, L. Cizmas, L. He, G. Zhou, T. J. McDonald, Y. Qian and K. C. Donnelly. *Texas A&M University, College Station, TX.*

Creosote and pentachlorophenol are chemicals found in wood preserving waste (WPW). Improper disposal of WPW has lead to contamination of soil and groundwater in many locations. This study compares the *in vitro* and *in vivo* genotoxicity of isolated fractions of WPW with chemical composition. The WPW was fractionated using liquid-liquid extraction to obtain acid, base, and neutral fractions following the EPA 3650B Method. The neutral fraction was then enriched for chlorinated dioxins (PCDDs) using a mixed bed silica column followed by a carbon column; and enriched for polycyclic aromatic hydrocarbons (PAHs) using sequentially an alumina-silica column and a carbon column. The fractions were then analyzed for their chemical content using GC/MS, and tested in the Salmonella/microsome assay, E.coli prophage induction assay, and ^{32}P postlabeling assay to characterize genotoxicity. The chemical analyses indicate that the chlorophenols were concentrated in the acid fraction, the base fraction contained PAHs and phenolic compounds; and the neutral fraction contained PAHs and PCDDs. The PCDD fraction contained primarily hepta and octa chlorinated PCDDs along with the low molecular weight PAHs. The PAH fraction contained a large amount of the higher molecular weight and carcinogenic PAHs. In the Salmonella/microsome assay, the base fraction induced 119 net revertants at a dose of 0.1 mg/plate, while crude extract induced 19 net revertants at the same dose. In the prophage induction assay, both the acid and base fractions induced positive genotoxic responses. In the ^{32}P postlabeling assay, the PAH fraction produced approximately 3 times the DNA adduct frequency observed in the other fractions. Although the base fraction contained the lowest amount of benzo(a)pyrene, it induced the maximum genotoxic response *in vitro*. The data indicate that isolation of the most genotoxic compounds from a complex mixture may be necessary to obtain a more accurate measurement of potential carcinogenicity.

1934 PAH *O*-QUINONES PRODUCE SIGNIFICANT AMOUNTS OF 8-OXO-7, 8-DIHYDRO-2′-DEOXYGUANOSINE (8-OXO-DG) IN SALMON TESTIS DNA.

J. Park, L. M. Szewczuk and T. M. Penning. *pharmacology, school of medicine, university of pennsylvania, philadelphia, PA.* Sponsor: S. burchiel.

Polycyclic aromatic hydrocarbons (PAHs) are procarcinogens that require activation by host metabolism. Aldo-Keto-Reductases (AKRs) activate PAHs to reactive and redox active *o*-quinones, which can cause oxidative DNA damage. Spectrophotometric analysis showed that NADPH promoted PAH *o*-quinones to enter futile redox-cycles, which result in the depletion of excess cofactor. Copper (II) amplified NADPH-dependent redox-cycling of the quinones, whereas rat liver S9 did not stimulate redox-cycling except for naphthalene-1, 2-dione. Concurrent with NADPH oxidation, molecular oxygen was consumed, indicating the production of reactive oxygen species (ROS). To measure 8-oxo-dG formation in salmon testis DNA by PAH *o*-quinones, three pre-requisite experimental conditions were satisfied. Quantitative complete enzymatic hydrolysis of DNA was achieved, adventitious oxidation of dG was eliminated by the use of Chelex and Desferal, and basal levels of 8-oxo-dG were lower than 2 adducts/10^5 dG. This method was validated by spiking the DNA with standard 8-oxo-dG and demonstrating quantitative recovery by EC-HPLC. EC-HPLC analysis revealed that in the presence of NADPH and Copper (II), submicromolar concentrations of PAH *o*-quinones generated $\rangle$ 60 8-oxo-dG adducts/10^5 dG. The dependency on Copper (II) suggests that Copper (I) fenton-chemistry may be responsible. The rank order of 8-oxo-dG generated *in vitro* was naphthalene-1, 2-dione $\rangle$ benz[a]anthracene-3, 4-dione $\rangle$ 7, 12-dimethylbenz[a]anthracene-3, 4-dione $\rangle$ benzo[a]pyrene-7, 8-dione. Experiments are being performed to detect whether ROS or Cu(I)OOH is the DNA damaging species. (Supported by P01-CA-092537 & R01-CA-39504 awarded to Trevor M. Penning)

1935 PYRENE ALTERS GENE EXPRESSION AND FATTY ACIDS IN CD-1 MICE.

L. Y. Hernandez and L. J. Bain. *Biological Sciences, University of Texas at El Paso, El Paso, TX.*

Pyrene is a polycyclic aromatic hydrocarbon that is ubiquitous in the environment as a product of the incomplete combustion of fossil fuels. A large amount of literature exists for the toxicity and carcinogenicity of PAHs, but data for pyrene is very limited. In order to further understand the toxic effects of pyrene at nonlethal doses, CD-1 adult male mice were fed pyrene in honey for 7 days. Six mice were assigned to a control group, 5 mice were assigned to the 3 mg/kg pyrene group, and 5 mice were in the 30 mg/kg pyrene group. The differential expression of the CYP1A2, AhR and ARNT genes, which have been shown to be differentially expressed as a result of PAH exposure, was also determined by real-time PCR. There was no significant difference in levels of expression of CYP1A2. There was a significant 2.3-fold increase in expression of AhR in 3 mg/kg mice compared to controls. A significant 1.5-fold decrease in expression of ARNT in the 30 mg/kg group was observed. This decrease might be the organism's mechanism for compensating with the increased levels of pyrene in order to avoid overexpression of enzymes that could lead to inflammation. To determine whether liver damage or fatty acid class alteration occurred, total levels of serum triglyceride, cholesterol, and bilirubin were measured. Serum triglycerides decreased by 1.5-fold in the 30 mg/kg group, which may be due to alteration of fatty acid cycle. Serum bilirubin decreased by 1.9-fold in 3 mg/kg group and 2.2-fold in 30 mg/kg group. Lipids were then extracted from the livers of control and treated mice to determine if fatty acid class alteration occurred using thin layer chromatography. There was no significant change in concentration of monoglycerol, monooleic acid or triolein in control compared to treated mice. However, the diolein concentration was 2.1-fold lower in the 3 mg/kg group and 3.9-fold lower in the 30 mg/kg group. These results suggest that pyrene exposure causes differential expression of genes and alters the expression of fatty acids.

1936 DEVELOPING A CARCINOGENIC BIOASSAY FOR COMPLEX MIXTURES.

D. Warshawsky, K. LaDow, R. Albert, M. Anderson, W. Xue, S. Spalding, G. Boivin, D. Ginsburg and P. Succop. *Environmental Health, University of Cincinnati, Cincinnati, OH.*

Complex mixtures of combustion and related products contain many carcinogens such as polycyclic aromatic hydrocarbons as well as anti-carcinogens that interact in theoretically unpredictable ways. The conventional mouse skin tumor carcinogenesis assay is time-consuming and expensive, and skin tumor assays based on initiation models alone are of uncertain accuracy in the prediction of cancer outcomes and are also not rapid. We are evaluating a more rapid approach for assessing car-

cinogenic potency with carcinoma production as the endpoint. Using a dominant-negative p53 mutant transgenic mouse (VAL p53) skin model, we are studying the potency of complex mixtures in an initiation-progression assay, with concurrent exposure to a topical application of the test mixture and a strong promoter, 12-O-tetra-decanoylphorbol-13-acetate (TPA). As a positive control comparison, the mixtures are also tested in a complete carcinogenesis assay. Three dose levels of Benzo(a)pyrene (BaP) were tested to confirm the sensitivity of the model. At 16 and 32 ug of BaP, tumors start appearing at 7 weeks following the start of exposure using the initiation-progression assay. However, tumors start appearing at 15 weeks at 32 ug using the complete carcinogenesis assay while no tumors have appeared following 22 weeks of exposure with 16 ug. The resulting tumors were determined to be carcinomas in situ and the number of mice with tumors indicates a dose related response. Three mixtures studies are underway using an NIST Coal Tar standard (CT), a 7H-Dibenzo[c, g]carbazole (DBC) and BaP mixture, or a DBC, BaP and CT mixture. Of the 9 mice that have completed the entire 26 week period of CT treatment, 4 have tumors; 6 of 14 additional mice now in the 19th week of treatment have tumors. Use of the transgene model using either CC or initiation-promotion shortens the latency period of the assay, thereby accelerating the appearance of tumors compared to conventional assays. (Funded by USEPA Star Grant)

1937 METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBON *trans*-DIHYDRODIOLS BY CYTOCHROME P450 AND ALDO-KETO REDUCTASE ENZYMES.

A. M. Quinn, H. Jiang and T. M. Penning. *Pharmacology, University of Pennsylvania, Philadelphia, PA.* Sponsor: S. Burchiel.

Polycyclic aromatic hydrocarbons (PAH) are environmental pollutants and human carcinogens that require metabolic activation to exert their deleterious effects. Activation of PAH in humans has previously been assigned primarily to the cytochrome P450 (CYP) enzymes, including CYP1A1, CYP1A2, and CYP1B1. However, members of the aldo-keto reductase superfamily (AKR) can oxidize the *trans*-dihydrodiols of PAH to generate cytotoxic and mutagenic *o*-quinones. *In vitro* reconstituted systems and *in vivo* culture methods have been established to measure (±)-*trans*-7, 8-dihydroxy-7, 8-dihydrobenzo[*a*]pyrene (BP-7, 8-diol) metabolism by both the CYP and AKR pathways using quantitative RP-HPLC methods. Rates of BP-7, 8-diol metabolism were measured in *in vitro* systems containing CYP1A1 and CYP1B1 Supersomes or bis-cistronic constructs expressing NADPH cytochrome P450-reductase with either CYP1A1 or CYP1B1 in bacterial membranes. Purified recombinant AKR1C1-AKR1C3 enzymes were also used to determine rates of BP-7, 8-diol metabolism. In human cell lines, AKR1C expression was identified by isoform-specific RT-PCR, Western blot analysis, and by functional assay of 1-acenaphthenol oxidation. Treatment of cells with 10 nM 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin induced expression of CYP1A1 and CYP1B1, confirmed by RT-PCR, Northern analysis, Western blot, and ethoxyresorufin *O*-deethylation. These methods have been used to screen for human cell lines that possess both AKR and CYP activities, so that the relative contribution of each pathway to PAH *trans*-dihydrodiol metabolism may be established. *(Supported by Grants R01 CA39504 and P01 CA92537 awarded to T.M.P.)*

1938 QUANTITATIVE ANALYSIS OF PHOSPHOLIPID PEROXIDATION: APPEARANCE OF OXIDIZED PHOSPHATYDYLSERINE ON THE SURFACE OF HL-60 CELLS DURING INTRINSIC APOTOSIS.

V. B. Ritov[2], V. A. Tyurin[1], Y. Y. Tyurina[1] and V. E. Kagan[1]. *[1]Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA and [2]Medicine, University of Pittsburgh, Pittsburgh, PA.*

We have demonstrated that oxidation of phosphatidylserine (PS) is required for its externalization during apoptosis. Since PS and oxidized PS (PSox) act as ligands for macrophage cognant receptor(s) required for effective phagocytosis of apoptotic cells we hypothesized that PSox appears, along with PS, on the cell surface during apoptosis. To test the hypothesis, we developed a new protocol for assessment of aminophospholipid (APL) oxidation on cell surface. The protocol is based on covalent labeling of APLs with cell impermeable reagent fluorescamine, (FC), followed by separation of phospholipids (PLs) using 2D-HPTLC. PLs spots are subjected to hydrolysis using a phospholipase A_2 (PLA_2)/melittin. The fatty acid hyroperoxides (FA-OOH) released are quantified by a fluorometric assay using Amplex Red reagent (10-acetyl-3, 7-dihydroxyphenoxazine) and horseradish peroxidase (HRP) or microperoxidase (MP). In this system, FA-OOH react with Amplex Red reagent at a stoichiometry of 1:1 to generate a fluorescent product, resorufin (ex=563 nm, em=587 nm) that was resolved by HPLC. Before exposure FA-OOH to HRP/Amplex Red all PLs were hydrolyzed by PLA_2. MP was able to interact with PLs hydroperoxides without its pretreatment with PLA_2. Using the protocol, we assessed PS oxidation in two models of apoptosis triggered in HL-60 cells by an oxidant (H_2O_2) or non-oxidant (staurosporine). In both cases, apoptosis was effectively induced as evidenced by caspase-3 activation and PS externalization. Analysis of PSox on the cell surface demonstrated that all PSox underwent externalization and became available to FC during apoptosis. Our results demonstrate that Amplex Red/MP-based assay can be used as a reliable and specific tool for quantification of PL hydroperoxides. We conclude that PS oxidation during apoptosis results in externalization of PSox where it can act as an important "eat-me" signal for macrophages. Supported by NIH HL70755.

1939 ENHANCMENT OF TRANSBILYER DIFFUSION OF PHOSPHATIDYLSERINE BY ITS OXIDATION PRODUCTS: MECHANISM OF PHOSPHATIDYLSERINE EXTERNALIZATION DURING APOPTOSIS.

Y. Y. Tyurina[1], Q. Zhao[1], V. A. Tyurin[1], M. Djukic[1] and V. E. Kagan[1, 2, 3]. *[1]Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, [2]Pharmacology, University of Pittsburgh, Pittsburgh, PA and [3]Cancer Institute, University of Pittsburgh, Pittsburgh, PA.*

Our previous work established that intrinsic apoptosis is associated with selective PS oxidation preceding its externalization. We hypothesized that PS oxidation enhances its externalization by one of the following mechanisms: (i) aminophospholipid translocase (APT) fails to recognize oxidized PS (PSox), (ii) reactive PS oxidation product poisons the enzyme, and (iii) PSox facilitates transbilayer diffusion of PS and/or PSox. To experimentally address these hypotheses we utilized HL-60 cells and Raji cells. PS or PS PSox were integrated into plasma membrane by incubation of cells in the presence of PC liposomes containing different amounts of PS or PSox. This resulted in a significant enrichment of plasma membrane with PS or PSox as evidenced by HPTLC analysis of phospholipids. PSox was effectively recognized and internalized by APT. Neither PS nor PSox had any significant effect on APT activity in both cell lines as monitored with a fluorescently-labeled PS homolog, NBD-PS. We further utilized Raji cells with a very low endogenous scramblase activity, to study diffusion of internalized PS to the outer leaflet of the membrane. APT was inhibited by depletion of ATP. To quantify PS exposure we used two different assays: (i) flow cytometric analysis of fluorescently labeled annexin V to evaluate the number of cells with externalized PS, and (ii) derivatization of externalized aminophospholipids by a non-permeable reagent for primary amines, fluorescamine, to determine the amount of PS on the cell surface. We found that PSox increased both the number of cells with externalized PS as well as the content of externalized PS. These results suggest that oxidation of PS stimulates its externalization through increased rate of PS and/or PSox transmembrane diffusion thus contributing to regulation of recognition and phagocytosis of apoptotic cells. Supported by NIH HL70755.

1940 CYTOCHROME *C* -/- CELLS FAIL TO TRIGGER PHOSPHATIDYLSERINE SIGNALING DURING APOPTOSIS.

J. Jiang[1], V. Kini[1], Y. Y. Tyurina[1], G. G. Borisenko[1], A. J. Schroit[2] and V. E. Kagan[1]. *[1]Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA and [2]Cancer Biology, The University of Texas M.D. Anderson Cancer Center, Huston, TX.*

Cytochrome *c* (cyt *c*) has been demonstrated to participate in phosphatidylserine (PS) signaling through catalysis of PS oxidation. We hypothesized that cyt *c* catalysis of selective PS oxidation is central to its subsequent externalization and recognition of apoptotic cells by macrophages. To address the hypothesis, we examined the PS oxidation and externalization in cyt *c* deficient mouse embryonic Cyt *c* -/- cells. Upon cycloheximide (CHX)/TNF-α treatment, caspase-3 was activated in Cyt *c* -/- cells as determined by cleavage of z-DEVD-AMC. Double staining with Annexin V-FITC and Hoechst 33342 showed that induction of apoptosis was not accompanied by PS externalization suggesting that caspase-3 activation was insufficient for PS externalization in Cyt *c* -/- cells. No lipid peroxidation was detectable in apoptotic cells by *cis*-parinaric acid assay. We next attempted to use N-ethylmaleimide (NEM), that was able to release cyt *c* in Raji cells and overcome its inability to express PS during apoptosis. However, exposure of Cyt *c* -/- cells to NEM failed to externalize PS. Further, we reintroduced cyt *c* into Cyt *c* -/- cells by mild sonication. Intracellular concentration of cyt *c* was dramatically increased as evaluated by Western blot. In the presence of H_2O_2, cyt *c*-enriched cells responded by a more than 2.5-fold greater PS oxidation than control. Importantly, integration of cyt *c* potentiated CHX/TNF-α triggered apoptosis and re-enacted PS externalization. Finally, we found that peroxidase activity of cyt *c* was enhanced in the presence of PS-containing (DOPC/DOPS (1:4)) liposomes as compared with cyt *c*/DOPC liposomes or cyt *c* in the absence of liposomes as monitored by oxidation of dichlorofluoresceine ($DCFH_2$). We suggest that once released into the cytosol, cyt *c* interacts with PS on inner leaflet of plasma membrane, catalyzes (in the presence of H_2O_2) PS oxidation, and facilitates its externalization during intrinsic apoptosis. Supported by NIH grant HL70755

1941 METALLOTHIONEIN MEDIATES GLUCOCORTICOID HORMONE RESPONSIVENESS IN IMMORTALIZED MOUSE FIBROBLASTS AND PRIMARY THYMOCYTES.

F. Haq[1], R. K. Zalups[2] and J. Koropatnick[3]. *[1]Department of Microbiology & Immunology, University of Western Ontario & London Regional Cancer Center, Ontario, ON, Canada, [2]Basic Sciences, Mercer University, Macon, GA and [3]Departments of Physiology & Pharmacology, Oncology, & Pathology, University of Western Ontario, & London Regional Cancer Center, Ontario, ON, Canada.*

We have previously reported that the amount of zinc-associated metallothionein (MT) in mammalian cells correlates with glucocorticoid receptor (GR) activity *in vitro*. To assess the role of MT on immune function *in vivo*, we measured the capacity of immortalized fibroblasts from MT-null and wild type mice to support GR-mediated gene transcription, and primary thymocytes to undergo apoptosis in response to a synthetic glucocorticoid (dexamethasone, DEX). We report that in MT-null fibroblasts, there is a reduction in the response to DEX (to mediate transcription from a GR-responsive luciferase reporter gene), indicating a role for MT in GR-induced gene transcription. MT-null and wild type mice were maintained on either a zinc-deficient or control diet for a period of 1 month. Thymocytes were isolated and treated *ex vivo* for 4 hours with concentrations of DEX ranging from 10^{-6}-10^{-9} M. DEX-induced apoptosis was measured flow cytometry. Cells from male and female mice showed a concentration-dependent increase in apoptosis in response to DEX, indicating that DEX was active and the cells responded to signaling. DEX-induced apoptosis was lower in thymocytes from male MT-null mice than in those from male wild type mice under both zinc-conditions. The opposite was observed in females: a higher fraction of thymocytes from MT-null mice underwent DEX-induced apoptosis under both zinc-conditions than thymocytes from MT-WT mice. These data indicate that the absence of MT in knockout mice affects responsiveness of primary thymocytes to glucocorticoid-induced apoptosis, mediated through the zinc-dependent GR. Furthermore, the effect of loss of MT on GR activity is sex-related. *Supported by grants from the Canadian Institutes of Health Research to JK, and from the NIEHS to RZK (ES05157 and ES05980) and to RZK and JK (ES11288).*

1942 ACUTE ETHANOL PRE-EXPOSURE SENSITIZES LIVER AND KIDNEYS TO FUROSEMIDE-INDUCED APOPTOTIC AND NECROTIC CELL DEATHS BY SELECTIVELY INFLUENCING OXIDATIVE STRESS AND GENOMIC DNA FRAGMENTATION *IN VIVO*.

C. Patel, R. R. Raje and S. D. Ray. *Mol. Toxicology. Program, Division of Pharmacology & Toxicol., Long Island University, Brooklyn, NY.*

Ethanol (EtOH) and furosemide (FUR) both are well-known apoptogenic hepato- and nephrotoxins. FUR, a loop diuretic, is frequently used to treat left ventricular failure, high blood pressure, and associated edema. Although alcoholics are frequently associated with cardiovascular malfunctions, it is not known if FUR has any adverse effects in such patients. Since, it is also known that EtOH does not induce any form of FUR-metabolizing CYP-450, this study was designed to investigate whether acute EtOH pre-exposure:(i) potentiates FUR-induced hepato- and nephro toxicities, (ii) influences FUR-induced bcl-XL expression in the liver, (iii) alters oxidative stress in the liver and kidneys, and (iv) modulates FUR-induced apoptotic and necrotic cell deaths in the liver and kidneys. Adult male ICR mice (8-10 wks old) were divided into four groups: Control, EtOH, FUR and EtOH+FUR. EtOH was (7.6 ml/Kg, 10% solution) gavaged 48 hours prior to FUR exposure, and sub-toxic doses of FUR (300 mg/kg, i.p.) were administered to study this interaction. Mice were sacrificed 24h after FUR administration, and their blood, liver and kidneys were collected for histopathology and biochemical analysis. Analysis of serum chemistry revealed a substantial increase in hepatotoxicty (ALT University/L: FUR-1377, EtOH+FUR-2021) and nephrotoxicity (BUN mg/dl:FUR-34, EtOH+FUR-56). Organ toxicities were amplified at higher doses of FUR. Quantitations of various forms of cell deaths (VIDEO microscopy) disclosed a dramatic increase in apoptotic death in EtOH+FUR-exposed liver and kidney sections. Qualitative analysis of DNA damage showed a greater induction of Caspase activated DNAse in EtOH+FUR compared to FUR alone, and mirrored the pattern of oxidative stress. Analysis of bcl-XL expression in the liver closely reflected the changes observed in biochemical parameters. Collectively, these data suggest that EtOH may accelerate FUR-induced liver and kidney injuries *in vivo*. [Supp. By AMS Coll. of Pharmacology]

1943 HYPOXIA-MEDIATED NFκB ACTIVATION IS INHIBITED BY N-ACETYLCYSTEINE IN TUMOR CELLS.

S. Qanungo and A. Nieminen. *Case Western Reserve University, Cleveland, OH.*

Hypoxic cancer cells resist the oxygen-dependent toxicity of ionizing radiation and chemotherapy. The transcription factor NFκB is an important regulator in oncogenesis. The aim of this study was to examine the role of NFκB in hypoxia-mediated apoptosis in cancer cells. p53+/+ and p53-/- mouse embryonic fibroblasts (MEFs) transformed with proto-oncogenes Ras and E1A were exposed to hypoxia (~0.2% O2) for 0-14 h. Hypoxia induced 12% apoptosis in p53+/+ MEFs compared to 2% during normoxia. N-acetylcysteine (NAC) enhanced hypoxia-mediated apoptosis in a dose-dependent manner, 20 mM NAC having a maximum effect and causing 46% apoptosis. p53-/- MEFs were completely resistant to hypoxia and NAC-mediated apoptosis. NAC also induced cytochrome c release into the cytosol during hypoxia. Hypoxia alone induced 67% increase in reactive oxygen species (ROS) formation measured by DCF fluorescence and this increase of ROS was inhibited by NAC as well as other potent antioxidants like epigallocatechin gallate and Trolox (vitamin E analog). Furthermore, hypoxia-mediated ROS generation induced NF B activation which was blocked by NAC as measured by p65 specific ELISA using nuclear extracts. Confocal microscopy also showed that hypoxia induced translocation of the active subunit of NFκB (p65) to the nucleus whereas this translocation was inhibited by NAC. To identify the downstream targets of NFκB signaling during hypoxia we determined the expression of the anti-apoptotic protein Bcl-xL but found no changes in Bcl-xL protein levels following hypoxia or NAC treatment. In conclusion, our results suggest that hypoxia-mediated ROS formation results in NFκB activation providing survival pathway. Inhibition of ROS formation by potent antioxidants results in inhibition of NFκB activation and enhancement of apoptosis. Utilizing the NFκB inhibition as a therapeutic strategy to sensitize cancer cells to hypoxic apoptosis may be useful. Supported by NS39469 and CA43703

1944 PHAGOCYTOSIS OF APOPTOTIC JURKAT CELLS BLOCKS ZYMOSAN-INDUCED INTRACELLULAR PRODUCTION OF REACTIVE OXYGEN SPECIES AND NITRIC OXIDE IN RAW 264.7 MACROPHAGES.

B. F. Serinkan, H. N. Babu, A. I. Potapovich, F. Gambelli, L. A. Ortiz and V. E. Kagan. *Environmental and Ocupational Health, University of Pittsburg, Pittsburgh, PA.*

Apoptosis is an effective way for clearance of injured cells without damaging surrounding tissues and massive inflammatory response. Proper removal of apoptotic cells is achieved by different "eat-me" signals on the cell surface, such as phosphatidylserine (PS), recognizable by phagocytes. Interactions of macrophages with apoptotic cells trigger release of anti-inflammatory cytokines and block the production of pro-inflammatory cytokines. Because activated macrophages generate cytotoxic reactive oxygen species (ROS) and nitric oxide (NO·), we hypothesized that limitation of inflammatory damage by apoptotic cells should involve inhibition of ROS/NO· production. To test the hypothesis, we prepared anti-Fas-induced apoptotic Jurkat T cells and co-cultured them with murine macrophage RAW 264.7 cells stimulated by zymosan (0.25 mg/ml). We used dihydroethidium (DHE) and diaminofluorescein-2 diacetyl (DAF2-DA) as probes for intracellular production of superoxide and NO·, respectively. We found that phagocytosis of apoptotic cells blocked zymosan-induced intracellular ROS and NO· production in macrophages. Flow cytometric measurements revealed that 38±1% and 57±16% of macrophages were DHE- and DAF2-positive cells, respectively, after phagocytosis of apoptotic cells as compared with the maximal effect elicited by zymosan alone (100%). Notably, similar effect was observed when non-apoptotic target cells with integrated PS-containing liposomes were used in place of apoptotic cells. In this case, 60±9% of DHE-positive cells was observed as compared with the maximal effect produced by zymosan alone. We confirmed that PS-dependent apoptotic signaling was important for regulation of the release of pro- and anti-inflammatory cytokines by RAW 264.7 macrophages. We conclude that PS signaling may be a promising approach to control the magnitude of inflammatory response *in vivo*. Supported by NIH HL 70755.

1945 NITRIC OXIDE INDUCES P53-DEPENDENT APOTOSIS IN RAT NEURAL CELL LINES.

C. Brynczka[1], B. A. Wetmore[1], C. McNeil-Blue[1], W. A. Freed[2] and B. A. Merrick[1]. *[1]NCT, NIEHS, NIH DHHS, Research Triangle Pk, NC and [2]Cell Neurobiol Rsch Branch, NIDA, NIH, DHHS, Baltimore, MD.*

The involvement of nitric oxide in neural cell death was investigated in the well-characterized rat PC12 pheochromocytoma cell line and the AF5 undifferentiated rat mesencephalic cell line using sodium nitroprusside (SNP). We hypothesized that the nitric oxide donor, SNP, may produce p53 pathway-mediated apoptosis in exposed cultured cells. In PC12 cells, SNP causes accumulation of p53 and its transcriptional target gene, p21-waf1, in a dose and time-dependent manner. AF5 cells, however, accumulate p53 and p21-waf1 in only modest amounts compared with PC12 cells. Contrary to evidence in other cell types, NFκB does not appear to be involved in the accumulation of p53 in PC12 cells as evidenced by lack of IκB phosphorylation and protein degradation across a variety of SNP dose ranges. Evidence of p53 activation was shown by phosphorylation at serine 15 in a dose and time-dependent manner in both PC12 and AF5 cells. Increases in phospho-

p53 levels occurred over a range of 0.1 to 1.0 mM of SNP and attained a maximal level of phosphorylation at 12 hours after 1mM SNP. Treated PC12 cells display cytochrome c release into the cytosol and Bax translocation to the mitochondria at 24 hours post 0.5mM SNP treatment. These trademark signs of apoptosis are significantly diminished by the addition of the p38 MAPK inhibitor SB202190, implicating p38 MAPK as an upstream regulator of both p53 and the apoptotic response. AF5 cells do not show marked Bax translocation to the mitochondria or in cytochrome c release into the cytosol. Pretreatment of PC12 cells with 20mM SB202190 attenuates cell death in SNP-treated cells as determined by trypan blue exclusion, with a greater effect seen in 1mM versus 0.5mM or less doses of SNP. Pretreatment with 20mM SB202190 does not significantly decrease cell death in nitric oxide exposed AF5 cells as determined by trypan blue exclusion. These results support our hypothesis that nitric oxide induces a p53-mediated apoptotic response in PC12 cells, with p38 MAPK involvement in this apoptotic pathway.

1946 SIMILAR APOPTOTIC ULTRASTRUCTURAL DAMAGE BUT DIFFERENT BIOCHEMICAL PATHWAYS INDUCED BY ROTENONE AND CAMPTOTHECIN IN HUMAN NEUROSPHERES.

J. Li, M. L. Spletter and J. A. Johnson. *School of Pharmacy, University of Wisconsin at Madison, Madison, WI.*

We used rotenone (RO) and camptothecin (CA) to investigate ultrastructural damage and apoptotic pathways. Neurospheres treated with RO (0.1-16 μM), CA (0.1-20 μM), or vehicle for 2-24 hours were harvested and prepared for the subsequent experiments. TUNEL staining showed a dose-dependent increase in TUNEL positive cells after treatment with RO and CA. TEM observation showed that RO and CA treated cells have characteristic hallmarks of apoptotic morphology including nuclear condensation, chromatin margination, and intact cellular membrane. CA treatment results in the cleavage of PARP and caspase-3, depolarization of the mitochondrial membrane, release of cytochrome c, and increased caspase activity in both time-course and dose response studies, indicating that CA induced apoptosis is mediated by a caspase–dependent pathway. The cell death also was attenuated by pretreatment with a pan-caspase inhibitor, zVAD (50μM). RO treated cells, in contrast, show low caspase activity and little PARP or caspase-3 cleavage. However, RO treatment results in depolarization of the mitochondrial membrane, release of AIF, and subsequent translocation of AIF to the nucleus. Combined with apoptotic-like morphological characteristics, this suggests that RO induced apoptosis is mediated by a caspase-independent pathway. Pretreatment with zVAD (50μM) does not prevent RO induced cytotoxicity. In contrast to CA, RO promptly decreased the ATP level, and fluorometry assay indicated the addition of ATP to the cell lysates obtained from RO-treated samples was sufficient to activate both caspase 9 and caspase 3. These results present a unique ultrastructural and molecular characterization of RO and CA induced cytotoxicity in human neurospheres. Mitochondria are integrated into both caspase dependent (CA) and independent (RO) apoptotic pathways. Intracellular ATP level may contribute an important role to determine whether neuronal progenitors undergo a caspase -dependent or -independent pathway after acute insults from neural toxicants.

1947 ANTI-APOPTOTIC MECHANISM OF MOUSE LEYDIG CELL LINE TM3 AFTER BENZO(A)PYRENE EXPOSURE IS ASSOCIATED WITH THE UP-REGULATION OF X-CHROMOSOME LINKED INHIBITOR OF APOPTOSIS PROTEIN.

J. Chung, J. Kim, J. Kim, J. Kim, K. Yoo, Y. Yoo and J. Kim. *Department Anatomy & Cell Biology, Dong-A University, Busan, South Korea.* Sponsor: K. Yang.

Benzo[a]pyrene (BaP) belongs to one of the polycyclic aromatic hydrocarbons affects cellular signal transduction mechanism and induces apoptosis. In the present study we investigated the anti-apoptotic cellular mechanism of testicular Leydig cell line TM3 after BaP treatment. Up to 100 uM concentration of BaP has been challenged to the cells for 72 hours, however, the apoptosis rate was not changed significantly compared to that of control. Meanwhile, cell cycle analysis showed that the treatment of BaP (10 uM) significantly results in increase of S phase. BaP treatment in TM3 increased both Ah receptor and Arnt protein contents. Both transcriptionally and translationally the CYP1A1 levels were elevated significantly by the BaP treatment, indicating that BaP metabolizing activity is present in the cells. Therefore, we next asked question how the TM3 cells can remain survived in the presence of a high CYP1A1 activity. Caspase-3, a central and effector protease in the apoptotic cellular pathway, was then first studied in this system. Procaspase-3 protein content obviously increased after BaP treatment, but its activation was not occurred as evident by absence of cleavage products of procaspase-3 in the Western blot analysis. This indicates that while procaspase-3 protein expression can be induced by BaP, its activation is somehow strongly suppressed by certain upstream molecules that involve anti-apoptotic mechanism. X-chromosome linked inhibitor of apoptosis protein (XIAP) has been identified as a potent cellular inhibitory protein against caspase-3 activation. Interestingly, XIAP protein content increased and maintained coincidental with those of procaspase-3 and CYP1A1 after BaP exposure. Finally the BaP treatment to TM3 cells did not evoke decrease of mitochondrial membrane potential as well as cytochrome c release. In conclusion, the anti-apoptotic cellular mechanism in TM3 cells after BaP might be strongly related to the role of XIAP and cellular specific mitochondrial integrity.

1948 CASPASE 2 AND THE MITOCHONDRION IN PAH-INDUCED PRO/PRE-B CELL APOPTOSIS.

H. Ryu[1], J. K. Emberley[2], J. J. Schlezinger[1], L. L. Allan[2] and D. H. Sherr[1, 2]. [1]*Envrionmental Health, Boston University Sch. of Medicine, Boston, MA and* [2]*Microbiology, Boston University Sch. of Medicine, Boston, MA.*

PAH are common environmental pollutants which suppresses the immune system in part by inducing pro/pre-B cell apoptosis. Previously, we demonstrated that DMBA, a prototypic PAH, induces apoptosis in pro/pre-B cells co-cultured with bone marrow stromal cells (BMS2) and that caspase 8 plays an important role in early apoptosis signaling. The purpose of this study was to define whether a second apoptosis pathway involving caspase 2, mitochondrial activation, and caspase 9 is also likely to be invoked. Western blotting indicated cleavage of pro-caspases 2, 3, and 9 within 8, 8, and 18 hrs of DMBA exposure respectively. Inhibitors specific for each of these three caspases protected pro/pre-B cells from DMBA-induced death. Primary pro-B cells from caspase $2^{-/-}$ mice were relatively resistant to DMBA-induced apoptosis, confirming a role for that caspase in DMBA-induced apoptosis. Markers for mitochondrial activation such as cytochrome C release and membrane potential loss (MPL), were also observed in apoptotic BU-11 cells. Interestingly, cytochrome C was released into the cytosol within 4 hrs of DMBA exposure while MPL occurred concurrent with caspase 2 activation at 8 hrs. However, MPL loss was not blocked by caspase 2-, 3-, 8-, or 9-specific inhibitors, suggesting that membrane potential loss was not dependent on any of these caspases. That the mitochondrion was involved in apoptosis signaling was further supported by the appearance of the Bid cleavage product, tBid, after DMBA exposure and by the demonstration that $Bax^{-/-}$ pro-B cells were relatively resistant to PAH-induced apoptosis signals. These results support the hypothesis that PAH-induced bone marrow B cell death is mediated by two pathways. One pathway is initiated by caspase 8 while the other involves caspase 2, the mitochondria, and caspase 9.

1949 TESTICULAR SERTOLI CELLS SURVIVE DESPITE CISPLATIN-INDUCED INJURY DUE TO THE EXPRESSION OF INHIBITOR OF APOPTOSIS PROTEINS THAT DISRUPT MITOCHONDRIAL-MEDIATED APOPTOTIC SIGNALING.

P. Sawhney and J. H. Richburg. *College of Pharmacy, The University of Texas at Austin, Austin, TX.*

Functional spermatogenesis and male fertility are critically dependent on the long-term survival of testicular Sertoli cells. Systemic exposure to a variety of toxicants results in the initiation of apoptosis in testicular germ cells. However, very few reports exist regarding apoptosis of Sertoli cells, and those that do, describe its occurrence during embryonic and neonatal testicular development, a time period where Sertoli cells are still dividing. Differentiated Sertoli cells appear to be resistant to apoptosis but, little is known about the mechanisms by which these cells are sustained throughout adult life. Here, we evaluate effects of cisplatin exposure in testes of 11 wk old C57/Bl/6J mice that have been exposed to either one, two or four cycles (one injection daily for 5 days with a recovery phase of 21 days between cycles) of 2.5 mg/kg cisplatin. Our observations indicate that Sertoli cells (1) demonstrate cleavage of caspase-9 (2) express the prototypical IAP family member XIAP (3) activate the transcription factor NF-κB after exposure to cisplatin, which has been implicated in activation of IAPs and (4) activate kinase dependent survival pathways which have been implicated in both activation of NF-κB and regulation of pro-apoptotic members of the Bcl-2 family. These data indicate that Sertoli cells are resistant to toxicant-induced apoptosis, despite the cleavage if caspase-9, due to the expression of XIAP and its ability to disrupt mitochondrial mediated apoptotic signaling. We will also present data to demonstrate that multiple parallel pathways for cellular survival are activated in Sertoli cells in order to ensure survival of this non-renewable cell population in the presence of toxicant exposure, in order to preserve fertility of the animal. Which of these multiple pathways plays the predominant role in ensuring survival of Sertoli cells remains to be tested (Supported, in part, by grants from NIH/NIEHS ES09145 & ES07784 and from the Lance Armstrong Foundation).

1950 EVIDENCE THAT 1, 1-DICHLOROETHYLENE INDUCES APOPTOTIC CELL DEATH IN MURINE LIVER.

E. J. Martin and P. Forkert. *Anatomy and Cell Biology, Queen's University, Kingston, ON, Canada.*

1, 1-Dichloroethylene (DCE) causes hepatotoxicity in mice. Our studies suggested that functional alterations in mitochondria initiated the toxic response. Mitochondria have been implicated in apoptotic cell death through transient opening of the permeability transition pore (PTP). This event may cause mitochondrial swelling and rupture of the outer membrane to release cytochrome *c*, which then activates the caspase cascade and sets apoptosis in motion. Here, we have undertaken studies to test the hypothesis that DCE induces apoptosis in murine liver. Female CD-1 mice were treated with DCE (125 mg/kg, i.p.). A decrease in hepatic mitochondrial membrane potential was detected at 2 h using the fluorescent dye JC-1. Western blotting of liver cytosolic proteins showed greater immunoreactivity for cytochrome *c* in fractions from mice treated with DCE for 4 h than in those from control animals. Furthermore, caspase-9 activity was significantly increased at 4 h after DCE exposure. These alterations were inhibited by pretreatment with cyclosporin A (CsA; 50 mg/kg), a specific inhibitor of the mitochondrial PTP. Immunohistochemical studies with an antibody to active caspase-3 and TUNEL staining were used to detect apoptotic cells. In both experiments, positive reactivity was observed at 12 and 24 h after DCE in centrilobular hepatocytes, although a few individual hepatocytes in other areas of the parenchyma were also stained. No reactivity was observed in liver sections from control animals. Additionally, centrilobular hepatocytes showing morphological criteria of apoptosis (chromatin condensation and margination, cell shrinkage, apoptotic bodies) were observed at 24 h. Liver toxicity, as assessed by serum alanine aminotransferase (ALT) activity, was also evaluated. ALT levels were significantly elevated at 2 to 24 h after DCE or DCE and CsA treatment. These data suggested that DCE produces apoptotic cell death by inducing a mitochondrial permeability transition that causes release of cytochrome *c* into the cytosol and caspase activation. Supported by Grant No. MOP 11706 from CIHR.

1951 BCL-X_L AND CYCLOSPORIN A (CsA) INHIBIT LEAD-INDUCED ROD PHOTORECEPTOR APOPTOSIS AND DECREASED MITOCHONDRIAL RESPIRATION BY BLOCKING CYTOCHROME C RELEASE.

D. A. Fox[1], L. He[2], A. T. Poblenz[3] and C. J. Medrano[1]. [1]*University of Houston, Houston, TX,* [2]*UNC, Chapel Hill, NC and* [3]*UT MDACC, Houston, TX.*

Photoreceptor apoptosis and visual deficits occur in man and animals with inherited and chemical-induced retinal degenerations, retinal diseases and injuries, and aging. Rod-selective apoptosis, mediated by cytochrome c-caspase, is produced by developmental lead exposure in mice and rats, and by Pb^{2+} and/or Ca^{2+} in isolated rat retinas (EER, 1988; JBC, 2000; PNAS, 2003). This study used isolated mice retinas and rat retinal mitochondria: 1) to compare *in vivo* and *ex vivo* lead studies in Bcl-x_L transgenic mice, 2) to examine species differences and 3) to investigate the mechanism of Pb^{2+}-induced alterations in mitochondrial bioenergetics. Adult retinas from wild-type and transgenic mice overexpressing Bcl-x_L only in photoreceptors were briefly exposed to 1 μM Pb^{2+} and/or 0.5 mM Ca^{2+}. Isolated adult rat retinal mitochondria, in the presence of different site-specific substrates, were exposed to 10 pM-30 nM Pb^{2+}. In adult mice, Pb^{2+} and/or Ca^{2+} induced rod-selective apoptosis characterized by genomic DNA fragmentation, decreases in rod mitochondrial respiration and membrane potential (depolarization), cytochrome c release and caspase activation. Bcl-x_L overexpression or CsA completely blocked all the Pb^{2+} and/or Ca^{2+} effects. Pb^{2+} produced concentration-dependent decreases in mitochondrial respiration at sites I, II and III: largest effects were with NAD-linked substrates (site I). Nevertheless, CsA completely blocked the Pb^{2+}-induced inhibition of mitochondrial respiration. These results suggest that Bcl-x_L and CsA prevented Pb^{2+}-induced mitochondrial depolarization, inhibition of the electron transport chain and rod apoptosis - in rats and mice as well as *in vivo* and *in vitro* - by blocking the release of cytochrome c through the permeability transition pore. These findings have relevance for therapies in human and animal retinal and neuronal degenerations where lead exposure, calcium overload and/or mitochondrial dysfunction occur. Supported by NIH Grants ES03183, ES012482, EY07024.

1952 CASPASE-2 DIRECTLY IMPAIRS MITOCHONDRIAL FUNCTION AND STIMULATES CYTOCHROME C RELEASE.

J. D. Robertson[2,1], V. Gogvadze[1], A. Kropotov[1], H. Vakifahmetoglu[1], B. Zhivotovsky[1] and S. Orrenius[1]. [1]*Toxicology, Karolinska Institutet, Stockholm, Sweden and* [2]*Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Although caspase-2 has been implicated in a variety of cell death paradigms, the mechanism by which this protease executes apoptosis remains obscure. Recent findings indicate that caspase-2 is activated early in response to DNA-damaging antineoplastic agents and may be important for the engagement of the mitochondrial apoptotic pathway. We demonstrate here the ability of caspase-2 to stimulate cytochrome c release and cause mitochondrial dysfunction directly. In particular, purified recombinant human caspase-2, but not caspase-3, triggers a concentration- and time-dependent release of cytochrome c from mitochondria in permeabilized Jurkat T-lymphocytes. This effect depends on the catalytic activity of caspase-2 as evidenced by the fact that inactivating the protease with an irreversible caspase-2 inhibitor, benzyloxycarbonyl-Val-Asp-Val-Ala-Asp-fluoromethyl ketone (z-VDVAD-fmk), or by introducing an active site mutation results in a significant attenuation of cytochrome c release. Further, experiments performed with isolated mitochondria indicate that caspase-2-induced cytochrome c release does not depend on the presence or cleavage of a cytosolic substrate, such as Bid, suggesting that a caspase-2 death substrate likely resides at the level of mitochondria. Finally, functional studies of mitochondria revealed that caspase-2 markedly stimulates state 4 respiration and decreases the respiratory control ratio due, in large part, to an uncoupling effect. Taken together, our data indicate that caspase-2 retains a unique ability to stimulate cytochrome c release by damaging mitochondria directly and thus may act as the initiator caspase in response to certain death stimuli.

1953 THE MITOCHONDRIAL PERMEABILITY TRANSITION (MPT) IS A KEY FACTOR IN ACETAMINOPHEN KILLING OF HEPATOCYTES.

K. Kon[1], J. Kim[1], E. A. Doyal[1], H. Jaeschke[2] and J. J. Lemasters[1]. [1]*University of North Carolina, Chapel Hill, NC and* [2]*University of Arizona, Tucson, AZ.*

BACKGROUND. Acetaminophen (AAP) overdose causes massive hepatic failure by mechanisms involving glutathione depletion, oxidative stress and sensitization to inflammatory cytokines. The ultimate target causing cell death remains uncertain. Our AIM was to characterize the MPT in AAP-induced hepatocyte killing. METHODS. Hepatocytes were isolated from fasted C3Heb/FeJ male mice and cultured in RPMI 1640 supplemented with insulin, transferrin, selenium and fatty acids. Necrotic cell killing was determined by propidium iodide fluorometry. Apoptosis was assessed by TUNEL. Mitochondrial polarization and permeabilization were visualized by confocal microsopy of TMRM and calcein. RESULTS. AAP (10 mM) caused 81±3% necrotic killing after 16 h. Fructose (20 mM) plus glycine (5 mM) (F+G) decreased this killing to 33±1%. Cyclosporin A (CsA) and NIM 811 (2 μM), MPT blockers, decreased killing after 6 h by 40%, but cytoprotection was lost after 16 h. 2-Aminoethoxydiphenyl borate (APB, 1-30 μM), a blocker of the CsA-insensitive MPT, protected cells dose-dependently: 30 μM APB decreased killing to 25±1% after 16 h. Ru360 (mitochondrial calcium uniporter blocker) did not prevent killing. After 16 h, 45±4% of hepatocytes exposed to AAP were TUNEL positive. F+G increased TUNEL labeling to 62±8%, and CsA decreased this apoptosis by half. Mitochondrial depolarization and inner membrane permeabilization preceded AAP killing and were blocked by CsA. CONCLUSIONS. CsA and NIM 811 protect early but not late against AAP toxicity, whereas APB protects both early and late. F+G protects against oncotic necrosis but paradoxically increases CsA-sensitive apoptosis. These findings suggest that AAP promotes a CsA-sensitive MPT early and a CsA-insensitive MPT late in toxicity, which induces either apoptosis or necrosis depending, in part, on ATP availability by glycolysis.

1954 LIVER CELL DEATH AFTER ACETAMINOPHEN (AP) OVERDOSE: APOPTOSIS OR ONCOTIC NECROSIS.

S. Phadke, C. Patel, R. Raje and S. D. Ray. *Mol. Toxicology. Program/Pharmacology & Toxicol., Long Island University, Brooklyn, NY.*

Although, criteria to distinguish NECROSIS from ONCOTIC NECROSIS are lacking, results from several laboratories have provided consistent biochemical, histopathological, ultrastructural and molecular evidence in favor of APs apoptogenic potential (Liu et al, Hepatology, 2003; Ishida, et al, FASEB J, 2002; Antonelli, Br.J.Pharmacology:2002; Ferret, et al, Hepatology:2001; Zhang, et al, Nature Biotech: 2000; Ray, et al, JPET:1996). However, at least one study has proposed that alternatively, AP might stimulate oncotic necrosis (Gujral, et al., Toxicology. Sciences. 67:322, 2001). To revisit the question of APs apoptogenic/necrogenic potentials, male ICR (fed) and B6C3F1 (fed), and female B6C3F1 (starved) mice were treated with AP (300-500 mg/kg, ip) and sacrificed 0, 6, 18 and 24 hr later. AP increased serum ALT and liver-DNA fragmentation over time in all strains. Although apoptotic cell quantitation in *in vivo* models is difficult (due to their rapid clearance), VIDEO-microscopic quantitations revealed that the highest dose of AP caused liver-injury maximally up to 60-70% in all the species and over 50% of cells showed characteristic features of apoptosis (12 to 24h). Lowest AP dose (300 mg/kg) at 6h showed maximum variability in all the parameters tested (ALT increased 5- to 10-fold; glycogen depletion was minimal; low injury with weak apoptogenicity). Apoptotic cells were not detectable at 0h, only a few were detected at 6h, and were maximal between 12-24h. Thus these results are consistent with apoptosis rather than oncotic necrosis as the mechanism for AP toxicity. Potential differences between this study and those proposing oncotic necrosis as a mechanism of AP toxicity might include: 1) differences in AP treatment doses;

2) different methods employed for apoptotic quantification; 3) lack of corrections for phagocytic dead cell clearance and caspase-independent pathways; and 4) different magnifications utilized for ultrastructural determinations of apoptotic markers.

1955 ALTERED GENE PROFILES IN RAT TESTES AFTER INHALATION EXPOSURE TO 1-BROMOPROPANE AND 2-BROMOPROPANE.

W. Li[1], E. Kitagawa[2], H. Iwahashi[2], H. Wang[1], S. Ichihara[1] and G. Ichihara[1]. *[1]Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan and [2]Human Stress Signal Research Center, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaragi, Japan.*

As alternatives for chlorofluorocarbons, 1-Bromopropane (1-BP) and 2-Bromopropane (2-BP) have been recently introduced to the workplace. Our previous studies have shown that 2-BP targets spermatogonia, while its isomer 1-BP causes spermiation failure in rats. The present study was performed to compare the mechanism of male reproductive toxicity between 1-BP and 2-BP at gene expression levels. Eighteen Male F344/NSlc rats were divided randomly into 3 groups of six each. The three groups were exposed to 1-BP at 1000 ppm, 2-BP at 1000 ppm, or fresh air for 8 hours in inhalation chambers. Sixteen hours after the end of exposure, rats were sacrificed by decapitation and testes were immediately removed. Global changes in gene expression were determined by cDNA microarray analysis using rat genital chips (DNA Chip Research Inc., Yokohama, Kanagawa, Japan). In each group, three chips were repeated using different testis samples. Results showed rather high repeatability of the altered gene profile. Among the 5082 genes in the genital chip, 351 genes were suppressed by both 1-BP and 2-BP, which includes S100, creatinine kinase and gluthatione S-transferase genes; 37 genes were suppressed by 1-BP only, which includes sex hormone synthesis genes; 119 genes were suppressed by 2-BP only, which includes proto-oncogenes or cell cycle checkpoint genes. This difference of altered gene profiles might reflect a difference in toxic mechanism between 1-BP and 2-BP, when taken together with previous studies suggesting that 1-BP disrupts the endocrine balance while 2-BP disrupts undifferentiated cells.

1956 GENE EXPRESSION IN MOUSE BRAIN FOLLOWING SUBCUTANEOUS INJECTIONS OF SARIN.

C. M. Garrett, S. J. Paton, D. R. Cool, R. D. Grubbs, W. R. Price and M. Morris. *Pharmacology/Toxicology, Wright State, Dayton, OH.* Sponsor: J. McDougal.

Sarin is an organophosphate chemical warfare agent, which acts non-competitively to inhibit acetylcholinesterase (AChE). It has been linked to Gulf War Syndrome because of its effects on central nervous system function. Understanding the transcriptional response of the brain to Sarin will increase our understanding of the mechanisms and symptoms of toxicity. Male C57Bl mice were injected subcutaneously on two consecutive days with Sarin (0.05 or 0.4 LD50). Control mice were injected with isotonic saline. Animals were sacrificed 4 hr after the second injection. Blood and brain were rapidly collected and assayed for ChE activity and mRNA expression. Total RNA was immediately isolated from pre-frontal cortex and hypothalamus. Biotin labeled cRNA targets were prepared according to Affymetrix standard protocols, hybridized to Affymetrix MGU74Av2 mouse arrays. Our results indicate that cholinesterase activity in the blood, hypothalamus and cortex were reduced at the higher sarin dose but not the lower one. Exposure to Sarin produced changes in gene expression in the cortex and hypothalamus. mRNA changes in the cortex were greatest with the lower dose and greatest in the hypothalamus at the higher dose In the cortex at the low dose, genes encoding protein kinases, structural proteins and prostaglandin synthesis were up regulated, while genes encoding receptor kinases, intracellular signaling proteins, GTPases, and synaptic vesicle membranes were all down regulated. At the high dose, calcium binding proteins and transcription factors were increased and tachykinins were decreased. In the hypothalamus, no genes were significantly increased in either dose but prostaglandin synthesis related genes were down regulated at both doses.These results document the effect of Sarin exposure on gene expression in mice brain. They suggest that the cortex is more sensitive to sarin's effects than the hypothalamus as seen by changes in gene expression without concomitant changes in AChE activity. (supported by the Department of Defense contract #DAMD 17-00-C-0020)

1957 GLOBAL GENE EXPRESSION CHANGES UNDERLYING TOXICOLOGICAL INTERACTIONS OF *STACHYBOTRYS CHARTARUM* TOXINS AND MURINE ALVEOLAR MACROPHAGES.

H. Wang and J. S. Yadav. *Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH.* Sponsor: D. Warshawsky.

Inhalation of toxin-containing spores of the toxic indoor mold *Stachybotrys chartarum* (SC) has been associated with severe respiratory problems, particularly acute pulmonary hemorrhage in infants. This SC toxicity has been ascribed mainly to its trichothecene mycotoxins that are potent protein synthesis inhibitors. Alveolar macrophages play an important role in phagocytosis and clearance of inhaled fungal spores and toxins. Our recent studies using murine alveolar macrophages showed that SC toxins induced inhibited cell proliferation, DNA damage, and caused apoptosis and cytotoxicity, but the mechanism underlying this toxicity is not yet clear. To understand molecular interactions between SC toxins and host alveolar macrophages, we monitored the genome-wide gene expression changes using oligonucleotide microarray (13, 443 genes) and real-time quantitative RT-PCR analyses. Macrophages treated with SC toxins showed 546 genes upregulated (2- to 24-fold) and 538 genes downregulated (2- to 64-fold). Among the 80 most upregulated genes (> 4-fold), 27 genes code for proteins with unknown function. The toxin regulated genes with known function ranged from those involved in apoptosis, cell cycle regulation, cytoskeleton, and cell adhesion to DNA repair, signal transduction, immune response, transcription factors, and metabolism. Pro-apoptotic genes were upregulated and anti-apoptotic genes were down regulated, indicating the basis of the observed apoptosis induction in macrophages. Some genes for MAPK and NF-kB pathways were upregulated indicating toxin induction of stress signaling. Genes encoding members of tumor necrosis factor ligand/receptor superfamily and selected chemokine and growth factors were downregulated indicating a decreased inflammatory and immune response of the host alveolar macrophages. In conclusion, both *de novo* synthesis and synthesis inhibition reactions are associated with the observed toxicity during SC toxin-macrophage interactions.

1958 GENETIC TOXICITY AND GENE EXPRESSION PROFILES IN TK6 CELLS EXPOSED TO IONIZING RADIATION.

G. Akerman[1], O. E. Domon[1], B. Rosenzweig[3], M. E. Bishop[1], L. J. McGarrity[1], C. A. Tsai[1], P. S. Pine[3], J. T. MacGregor[2], F. D. Sistare[3], J. J. Chen[1] and S. M. Morris[1]. *[1]NCTR, Jefferson, AR, [2]NCTR, Washington, DC and [3]USFDA, Laurel, MD.*

Human p53-proficient TK6 cells were treated with 0.5, 1, 5, 10 and 20 gray (Gy) ionizing radiation (IR). Following exposure, the cells were cultured for either 4 or 24 h and aliquots of the cells were then used to measure genetic toxicity endpoints and develop gene expression profiles. Significant decreases in cloning efficiency were detected at all doses, whereas decreases in cell *via*bility were observed only at the 5, 10 and 20 Gy doses. The micronucleus frequency was increased at the low doses (0.5 and 1 Gy), but insufficient binucleated cells were available for scoring at the 5, 10 and 20 Gy doses. Microarray analysis was performed on RNA isolated from the 5, 10 and 20 Gy samples using a 350-gene human cDNA array. Fewer genes were up-regulated (>1.5- fold) or down-regulated (<0.67-fold) at 4 h than at 24 h. Genes differentially expressed at 4 h included those primarily associated with cell proliferation, DNA repair and the initiation of apoptosis. At 24 h, there was an up-regulation of glutathione-associated genes in addition to genes involved in apoptosis. Additional genes involved in cell cycle progression and mitosis were down-regulated at 24 h. When the gene expression profiles from IR-treated cells were compared to BPDE-treated TK6 cells at a dose with similar survival levels (<10%), genes unique to IR and to BPDE were identified. We also identified a number of "genes in common" that were differentially expressed with both genotoxic agents. Real-time quantitative RT-PCR (SYBR Green) was used to confirm the differential expression of selected genes and to evaluate expression changes at the lower IR exposures. Gene expression changes were observed at the lower doses which yielded significant alterations in micronucleus frequency.

1959 TRANSCPRIPTIONAL RESPONSES OF MOUSE EMBRYO CULTURES EXPOSED TO BROMOCHLOROACETIC ACID.

E. D. Karoly[1, 2], J. E. Schmid[2] and S. Hunter[2]. *[1]Curriculum in Toxicology, UNC Chapel Hill, Chapel Hill, NC and [2]Reproductive Toxicology Division, USEPA, NHEERL, ORD, Research Triangle Park, NC.*

A major class of disinfection by-products (DBPs) found in municipal drinking water are the haloacetic acids. Bromochloroacetic acid (BCA), a common and abundantly found DBP included within the family of haloacetic acids, is a reproductive toxicant in animal models. The mechanism of haloacetic acid embryotoxicity has not been defined, but may be mediated in part through the inhibition of protein kinases. In order to examine altered gene expression in mouse embryos following exposure to BCA, CD-1 mouse embryos (3 to 6 somite stage) were cultured in 300µM BCA for 0, 1, 3 and 6h. Additionally, embryos were exposed to 30 and 3µM BCA for 6h. Following exposure, embryos were terminated and a total of 30 heads per treatment were pooled for RNA isolation. Total RNA was reverse transcribed with amino allyl-dUTP and indirectly labeled with Cy3 and Cy5. Fluorescently labeled cDNA from treated and untreated samples were labeled with

Cy5 and hybridized against a Cy3 labeled standard reference sample onto custom glass slide arrays. Arrays were spotted with 17, 388 70mer oligonucleotide probes from the Operon Array-Ready Oligo Set for the mouse genome version 2 (Qiagen Operon, Valencia, CA). The data were normalized by slide using an intensity dependent local regression (SAS Proc Loess). Two-way ANOVA identified 276 genes as being differentially expressed across dose and/or time. Cluster analysis and linear models were used to identify the transcriptional responses in mouse embryo culture exposed to BCA and their association with mouse embryotoxicity. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy. Funded by EPA/UNC Toxicology research program, training agreement CT 827206 with the Curriculum of Toxicology, University of North Carolina at Chapel Hill, North Carolina.*

1960 *IN VITRO* GENE EXPRESSION PROFILING OF HEPATOTOXINS USING DNA MICROARRAYS.

C. M. Glatt[1], K. A. Ewane[2], D. L. Nabb[1] and R. A. Kemper[1]. *[1]Haskell Laboratory, DuPont, Newark, DE and [2]Biological Sciences, University of Delaware, Newark, DE.*

A major effort has been made to identify potential hazards associated with new compounds as early as possible and on as many potential chemical leads as possible in order to guide discovery programs towards the development of safer products. Consequently, there is an increasing need to develop assays for toxicity that use small amounts of experimental compounds while providing an accurate prediction of toxicity. It has been proposed that toxicogenomic biomarkers be used in a discovery toxicology screening mode to reduce overall animal usage and time by enabling one to discover and eliminate toxic compounds from the pipeline earlier in the development process. The objective of this study was to determine if compounds with similar mechanisms of toxicity produce similar changes in gene expression *in vitro*. Freshly isolated primary rat hepatocytes were cultured on a hepatocyte Matrigel? matrix and treated for 72 hours with a set of known hepatotoxins including indomethacin, 2-thiouracil, carbon tetrachloride, vinyl acetate, and dicumarol. The compounds used are known to cause a variety of hepatocellular injuries including hepatic carcinoma, necrosis, and hypertrophy. Doses were based on published *in vitro* IC50 values as well as our own cytotoxicity (MTT) data. Microarray analysis was done on total RNA isolated from the hepatocytes and several gene expression changes were revealed for each treatment compared to control. Our results suggest that hepatotoxins may be characterized and classified based on gene expression. While the analysis of gene expression profiles may contribute to our capability to predict adverse toxicity, toxicogenomics also has great capacity to further our mechanistic understanding of toxicant action and increase the fidelity of comparing toxicity across species or extrapolating results to humans. The technological advances promise to improve our ability to characterize hazard and eventually to provide the data to apply to mechanistic-based risk assessment.

1961 MICROARRAY ANALYSIS OF LUNG GENE EXPRESSION IN MOUSE PUPS AFTER FETAL DRINKING WATER EXPOSURE TO URANYL-NITRATE.

J. M. Orozco[1], S. L. Marion[3], C. Begay[3], P. Hoyer[3], C. Dyer[4] and R. C. Lantz[2, 1]. *[1]Pharmacology and Toxicology, University of Arizona, Tucson, AZ, [2]Cell Biology and Anatomy, University of Arizona, Tucson, AZ, [3]Physiology, University of Arizona, Tucson, AZ and [4]Biological Sciences, Northern Arizona University, Flagstaff, AZ.*

In some areas of the US, including the southwestern states, ground water concentrations of uranium are at or above the EPA standard of 30 ppb. The effects of uranium in drinking water have not been well studied but some recent studies indicate an association with chemically mediated genotoxicity in addition to radiological effects. The present study replicates uranium concentrations that are representative of human exposures in some locations of the US. Female C57BL/6 mice were exposed to 0.5, 2.5, 12.5, and 60 ppb uranyl-nitrate in the drinking water throughout the entire pregnancy. Lungs from 1-day-old mouse pups were harvested and tissue from litters consisting of 9 to 12 pups was pooled for isolation of RNA. No changes in litter size or pup weight were observed. Three litters were analyzed from each condition. Total RNA was used for Affymetrix microarray analysis using Mouse 430A gene chips. Two-fold or greater changes in gene expression were seen at all exposures. At the highest dosage of uranyl-nitrate, 211 genes were upregulated greater than 2 fold and 124 were downregulated greater than 2 fold. Expression changes were seen involving regulation of stress response, apoptosis, cell differentiation and proliferation, and immune responsive genes. This study shows that environmentally relevant uranium exposures produce gene expression changes that may modulate lung physiology. (Supported by NIH grant #CA96281, CA96320 and ES06694)

1962 EFFECT OF VALPROIC ACID ON THE EXPRESSION OF GENES IN THE LIVERS OF PREGNANT MICE.

J. Fuscoe[1, 2], C. D. Melvin[1, 2], C. L. Moland[1, 2], W. S. Branham[1, 2], L. Shi[3] and D. K. Hansen[2]. *[1]Center for Functional Genomics, NCTR, Jefferson, AR, [2]Division of Genetic and Reproductive Toxicology, NCTR, Jefferson, AR and [3]Division of Biometry and Risk Assessment, NCTR, Jefferson, AR.*

Valproic acid (VPA) is used extensively in the therapy of epilepsy and neurological disorders. However, it produces developmental abnormalities in laboratory animals and humans, especially defects of the neural tube. In addition, VPA is a hepatotoxin. The mechanisms underlying VPA's teratogenic and hepatotoxic effects are unclear. It has been suggested that VPA may interact with folic acid metabolism, and it is known to be a histone deacetylase inhibitor *in vitro*. To better understand the mechanisms involved in hepatotoxicity, gene expression profiles were examined in liver of mice exposed to VPA. Pregnant CD-1 mice were injected with either the vehicle (saline) or VPA at 600 mg/kg on gestation day 8. This dose of VPA produces neural tube defects in over 70% of embryos. Six hours later, the mice were sacrificed. The RNAs from the livers of 5 control and 5 VPA-exposed mice were isolated, reverse transcribed, labeled with Cy5 and hybridized with Cy3 labeled reference RNA to microarrays consisting of 5000 mouse genes on glass slides. Reverse labeling was also performed to reduce dye-specific bias effects. After normalizing and combining the data, Statistical Analysis of Microarrays (SAM; PNAS 98: 5116, 2001) showed approximately 65 genes were differentially expressed with a false discovery rate of <5%. Genes involved in xenobiotic biodegradation, and amino acid and lipid metabolism were significantly altered.

1963 TOXICOGENOMIC STUDIES ON THE MECHANISMS OF SARIN-INDUCED NEURODEGENERATION IN RATS.

T. V. Damodaran, S. T. Greenfield, A. G. Patel and M. B. Abou-Donia. *Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.*

Sarin induced global gene expression patterns for an early time point (2 hrs: 0.5 X LD50) and late time point (3-months:1 X LD50) were studied using affymetrix : Rat Neurobiology U34 chips in Dawley-Sprague rats. A total of 46 and 38 genes were identified, showing statistically significant alteration from control levels at 2 hrs and 3-months respectively. At 2 hrs, genes that are classified as ion channels (8), calcium channels and binding proteins (6) were more in number than any other groups. Other genes that showed changes included: ATPases and ATP-based transporters (4), growth factors (4), G-protein coupled receptor pathway related molecules (3), neurotransmission and neurotransmitter transporters (3), cytoskeletal and cell adhesion molecules (2), hormones (2), mitochondrial associated proteins (2), myelin proteins (2), stress activated molecules (2), cytokines (1), capsases (1), GABAnergic (1), glutamergic (1), immediate early gene (1), prostaglandins (1), transcription factors(1), and tyrosine phosphorylation molecules(1). At three-month, genes that are classified as calcium channels and binding proteins, cytoskeletal and cell adhesion molecules, GABAnergic signaling molecules were more in number than any other group. The microarray data showed persistent altered expression in 8 genes at all time points. Selected genes from each of these time points were further validated using RT-PCR. Principal component analysis (PCA) of the expression data of the time points was used for comparative analysis of the gene expression, which indicated that the changes in gene expression is a function of dose, time of euthanasia after the treatment and age of the animal. Our model also predicts that both degenerative and regenerative pathways activated early, contribute to the level of neurodegeneration at later time, leading to neuropathological alterations. (Supported in part by DOD contract: DAMD 17-98-8027).

1964 INVESTIGATION OF A COMPOUND-INDUCED VASCULAR INFLAMMATORY SYNDROME IN DOGS USING GENE EXPRESSION ANALYSES.

Q. Huang, O. DiSorbo, Y. Yu, S. Jayadev and K. Blanchard. *Toxicology and Safety Assessment, Boehringer Ingelheim, Ridgefield, CT.*

Safety assessment of a proprietary compound, compound X, identified a compound-induced vascular inflammatory syndrome in dogs but not in rats or monkeys. The occurrence of vascular lesions in dogs was usually accompanied by an elevation in peripheral blood neutrophil counts suggesting that neutrophils may play a role in the vascular inflammatory response. In the current study, compound X-induced gene expression changes in human and canine polymorphnuclear cells (PMNs) were investigated. The goals of the study were two-fold: 1) to define the role of neutrophils in vascular inflammation, and, 2) to identify canine-specific gene changes which could delineate the dog-specific vascular inflammation. PMNs isolated from the periphery blood of a male human or a male dog were cultured in the presence of compound X or zymosan A at 37oC for 6 hrs. Zymosan A, a strong activator of neutrophils, was used as a positive control. Gene expression was ana-

lyzed using Affymetrix U133A human chips (~22, 000 fragments) or gITOX-CF1a canine chips (~12, 000 fragments). Zymosan A affected 836 genes and 1, 784 genes by greater than or equal to two-fold in canine and human PMNs respectively. Compound X affected expression of 140 genes and 230 genes by greater than or equal to two-fold in canine and human PMNs, respectively. Inflammatory mediators such as ICAM-1 (13.5 fold), complement component 3 (9.9 fold), CD69 (4.8 fold) and TNFAIP3 (4.4 fold) were found to be induced exclusively in canine PMNs in response to compound X treatment. The current study begins to provide mechanistic understanding of compound X-induced vascular inflammation. Additionally, these studies are identifying differences in the response of dog and human PMNs to compound X treatment. On-going studies using larger sets of donor PMNs will serve to validate the findings from the current pilot study and should help us identify more subtle changes elicited by compound X.

1965 THE EFFECT OF ANTI-HEPATITIS B PRODRUG HEPAVIR B ON HEPATIC TOXICOLOGICAL GENE EXPRESSION IN RATS.

R. Yan, C. Fang, C. Lim, D. Vitarella, P. Srivastava, Y. Liu, L. Yeh and C. Lin. *R&D Department, ICN Pharmaceuticals Inc., Costa Mesa, CA.*

Background: PMEA is an acyclic nucleoside phosphonate analog with potent anti-hepatitis B virus activity. However it has low oral bioavailability and accumulated at high levels in kidney. Hepavir B is an orally bioavailable prodrug of PMEA and metabolized primarily by cytochrome P450 (CYP) 3A4 in liver, resulting in preferential distribution of PMEA in the liver over kidney. Aim: To detect if there are any hepatic damage and toxicological gene response to prolonged treatment of Hepavir B. Methods: Five male rats per group were dosed orally with Hepavir B (300 mg/kg/day) for 8 days and 28 days. RNA samples were isolated from the liver tissue and Affymetrix Rat Toxicology Arrays were employed. Results: A greater than 3-fold up-regulation of detoxification enzymes (i.e. GSTA5, Mdr1b, carboxylesterase and dUTPase) and cell proliferation regulators (Cdc2 and cyclin G) could be detected by microarrays as early as on the 8th days of Hepavir B treatment, before the appearance of any significant pathology in the liver. Twenty-eight-day dosing of Hepavir B increased the mRNA level of p21 protein, UGTR-2, Aflatoxin B1 aldehyde reductase, NAD(P)H-menadione oxidoreductase, CYP2C12 and CYP51, in addition to the above mentioned genes. The expression of GSTA5, Mdr1b, Cyclin G and p21 was further confirmed by RT-competitive PCR. Histological examination demonstrated no effects after 8 days of Hepavir B dosing, only slight to moderate hepatocytic hypertrophy and diffuse hyperplasia of the bile duct were found after 28 days of Hepavir B treatment. LC/MS analysis showed that 8 or 28-day dosing of Hepavir B significantly increased liver PMEA up to 23.9 ± 9.4 or 25.7 ± 13.5 μg/g tissue. Conclusions: (1) Microarrays can be a useful tool for early predictive toxicology. (2) Hepavir B (at 300 mg/kg/day up to 28 days) did not cause significant liver damage in rats. (3) Up-regulation of detoxification and proliferation genes may contribute to liver tolerance to the Hepavir B-converted high level of PMEA.

1966 IDENTIFICATION OF GENE EXPRESSION IN THE UROTHELIUM OF RATS FED DIMETHYLARSINIC ACID USING MICROARRAY ANALYSIS.

S. M. Cohen, M. Wei, L. L. Arnold and J. D. Eudy. *Path/Micro, University of Nebraska Med. Ctr, Omaha, NE.*

Dimethylarsinic acid (DMA), a major organic metabolite of arsenic in most mammals including humans, has been shown to be a bladder carcinogen in rats when administered in the diet or in the drinking water. However, the molecular mechanisms underlying DMA bladder carcinogenesis remain unclear. The purpose of the present study is to identify the DMA-responsive genes in the urinary bladder epithelial cells of rats. Female F344 rats were fed 100 ppm DMA or control diet. Five rats from each group were sacrificed 24 hours and 2 weeks after the start of treatment and RNA was isolated from urinary bladder epithelial cells using the Qiagen method. RNA of DMA-treated and control rats was labeled with Cy3 and Cy5 fluorescent tags respectively and simultaneously hybridized to a Rat 10K chip (MWG Biotech) containing 10, 000 genes. Genes in which the expression ratio of paired DMA-treated to control tissue showed a greater than 2-fold difference and was observed in at least 4 rats were considered to be DMA-responsive genes. Seventeen genes were down-regulated after 24 hours of DMA treatment and no up-regulated genes were observed. After 2 weeks of DMA treatment, an expected increase in the number of DMA-responsive genes was found (32 genes). Seven of these genes were up-regulated by DMA, while 25 genes were down-regulated by DMA. Furthermore, the expression of 12 genes was suppressed both after 24 hours and 2 weeks. These findings suggest that analysis of expression profiles may provide clues for elucidation of DMA-induced bladder carcinogenesis. Further investigation of gene expression after long-term DMA administration is underway.

1967 XENOBIOTIC-INDUCED CHANGES IN RAT HEART GENE EXPRESSION.

B. P. Hirakawa[1], B. Jessen[1], A. de Peyster[2] and G. Stevens[1]. *[1]Drug Safety Evaluation, Pfizer Global Research and Development, San Diego, CA and [2]Graduate School of Public Health, San Diego State University, San Diego, CA.*

Little is known about the changes in cardiac gene expression following xenobiotic-induced toxicity. Understanding the changes at the molecular level will provide insights into the toxicological mechanisms of injury and identify possible relevant biomarkers for xenobiotic-induced cardiotoxicity. In an effort to initially examine these changes, four xenobiotics known to affect the heart (digoxin, doxorubicin, isoproterenol and lipopolysaccharide) were studied. The toxicants were chosen on the basis of their diverse mechanisms of action. Adult male Sprague-Dawley rats were treated with acute doses of digoxin (20 mg/kg), doxorubicin (30 mg/kg), isoproterenol (70 mg/kg), or lipopolysaccharide (10 mg/kg) and sacrificed either 6 or 24 hours later. Doses were selected based on the maximum tolerated dose in preliminary studies, or were determined after a thorough review of the literature. Total RNA was extracted from cardiac tissue and gene expression measured using Affymetrix U34A rat GeneChips. Initial results indicate that these four xenobiotics elicit significant changes in expression levels for a number of genes when compared to untreated samples. Common genes induced by all four xenobiotics have the potential to become sensitive biomarkers for cardiotoxicity. These genes are associated with the immune response (complement protein, interferon gamma receptor), stress response (metallothionein, heme oxygenase), or cellular growth and repair (neuropilin, integrin, cyclin D2, grb2). Similar experiments on samples treated with additional xenobiotics and/or heart tissue undergoing various disease states, can reveal unique biochemical mechanisms of xenobiotic-induced cardiotoxicity and more sensitive measures of toxicity.

1968 HEPATIC GENE EXPRESSION PROFILES INDUCED BY PREGENOLONE 16α-CARBONITRILE.

V. Bhaskaran, D. M. Nelson, W. R. Foster, B. Gemzik, B. D. Car and L. D. Lehman-Mckeeman. *Discovery Toxicology, Bristol-Myers Squibb, Princeton, NJ.*

The goal of the present work was to determine global hepatic gene expression changes after exposure to pregenolone 16α-carbonitrile (PCN), a prototypical PXR agonist. Male and female SD rats were dosed with PCN (100 mg/kg) and gene expression patterns were analyzed at 6 and 24 hr after a single dosage or after 5 daily dosages using Affymetrix U34A gene arrays. Expression of known PXR-regulated genes, including induction of CYP3A1/2 and oatp-2 and repression of CYP7A1 were readily detected. Induction of other xenobiotic metabolizing enzymes was observed, including CYP2B1/2, UGT1A1 and glutathione-S-transferases, whereas PCN treatment decreased expression of CYP4B1 and sulfotransferase 1a. However, although the increase in hepatic CYP2B mRNA levels was confirmed by RT-PCR, there was no corresponding increase in O-dealkylation of 7-pentoxyresorufin. PCN treatment did not alter serum thyroid hormone levels after 5 days of dosing, but TSH levels were increased in female rats nearly 2.5-fold. Hepatic expression of thyroxine binding globulin was also decreased at 5 days, particularly in female rats. Several other major changes observed in PCN-treated liver were increased Mdm2 expression and decreased expression of S-adenosylhomocysteine hydrolase (SAHH) and glycine-N-methyltransferase (GNMT). Mdm2 induction was observed at all times, with maximal induction in male and female rats representing a 5- and 11-fold change, respectively. GNMT was decreased as early as the 6 hr time point, whereas SAHH expression was decreased at 24 hr and 5 days in female rats and at 5 days in male rats. For GNMT and SAHH, maximal repression determined by real time PCR was approximately 30 and 65% of control values, respectively. These results demonstrate altered hepatic expression of known PXR-dependent genes by PCN treatment and provide evidence to suggest additional genes regulated in a PXR-dependent manner. Furthermore PCN alters hepatic expression of genes that may contribute to alterations in thyroid hormone homeostasis, cell transformation and methyl group metabolism.

1969 THE SKELETAL MUSCLE INJURY INDUCED BY COMPOUND A WITH INHIBITORY EFFECTS ON HMG-COA REDUCTASE IS REVERSIBLE.

K. Tanaka[1], N. Kiyosawa[1], S. Sharyo[1], M. Teranishi[1], S. Manabe[1] and H. Ozaki[2]. *[1]Medicinal Safety Res. Labs., Sankyo Co., Ltd.,, Fukuroi-shi, Shizuoka, Japan and [2]Department of Vet. Pharmacology, University of Tokyo, Bunkyo-ku, Tokyo, Japan.*

When compound A (2-methyl-butyric acid 4-([1, 3]dioxan-5-ylmethoxyimino)-8-[2-(4-hydroxy-6-oxo-tetrahydro-pyran-2-yl)-ethyl]-7-methyl-6-oxo-1, 2, 3, 4, 6, 7, 8, 8a-octahydro-naphthalen-1-yl ester), which has inhibitory effects on HMG-CoA reductase, was given to rats, the rats showed clinical signs of skeletal muscle injury.

However, despite further treatment, the injury was no longer detected at the scheduled autopsy of animals. In this study, we investigated the mechanism by an exhaustive gene expression analysis. Male F344 rats (7-week-old; n=5) were fed a diet contained 0.13% compound A (100 mg/kg/day) for 28 days. Creatine kinase (CK) levels were measured periodically during the treatment. Microscopy of the skeletal muscle was conducted on Days 12 and 28. Gene expression using GeneChip microarrays was analyzed the skeletal muscle on Day 28. The plasma concentration of M1, a major active metabolite, was measured on Days 8 and 28. CK levels increased from Day 7 and showed an approximately 30-fold increase on Day 10. Microscopy demonstrated severe degeneration/necrosis of the muscle fiber on Day 12. CK values, however, declined after that, and returned to control levels from Day 18 onward. Regeneration in the muscle was just observed on Day 28. GeneChip analysis revealed that the expression levels of cardiac (9-fold) and vascular (2-fold) α-actins, IGF-II (5-fold) and kallikrein (18-fold) had increased. No considerable changes in exposure levels of M1 throughout the dosing period were observed. Markedly elevated CK levels and microscopic changes demonstrated that compound A had caused skeletal muscle injury. After repeated exposure, the injury by compound A was reversible and the muscle acquired tolerance to the toxicity. GeneChip analysis demonstrated that the expression of some genes related to muscle regeneration had specifically increased. Thus, regeneration leading to a unique gene expression profile may be attributed to the acquired tolerance of the muscle.

1970 GENE EXPRESSION STUDY OF ADRIAMYCIN INDUCED TOXICITY IN HEART OF MALE SPRAGUE-DAWLEY RATS.

M. Derbel, M. Cooper, D. Hutto, D. Enke, M. Subramanyam and J. Green. *Preclinical and Clinical Development Sciences Division, Biogen, Cambridge, MA.*

In the present study we evaluated the acute toxicity of doxorubicin following a single I.P. injection to male rats. Rats were assigned to one of three groups (10 animals/group). Each group received dose preparations containing the control article (50% PEG 400 in sterile water) or doxorubicin. Doses for doxorubicin were 2 and 20 mg/kg. Assessment of acute toxicity was based on mortality, clinical observations, and clinical, anatomic pathology evaluations and gene expression at two sacrifice intervals for each dose group (6 and 24 hours). The most notable clinical pathology effects of doxorubicin were consistent with an inflammatory response, stress or direct lymphoid tissue injury, and marked renal dysfunction. Sections of heart from control and doxorubicin-treated animals were evaluated microscopically. High dose doxorubicin animals exhibited focal to diffuse interstitial myocardial hemorrhage, as well as cleft-like spaces in the myocardium that contained granular basophilic material. Transcriptional profiling of the hearts of control and doxorubicin-treated animals was also evaluated. Gene expression analysis revealed several genes significantly regulated at the 2 and 20mg/kg reflecting drug activity and toxicity.

1971 PERSISTENT ALTERATIONS IN GENE EXPRESSION FOLLOWING CHRONIC DOXORUBICIN ADMINISTRATION IN RATS.

J. M. Berthiaume, J. A. Bjork and K. B. Wallace. *Biochem. & Mol. Biology, Toxicology Graduate Program, School of Medicine, University of Minnesota, Duluth, MN.*

Doxorubicin (DOX, Adriamycin®) is a potent antineoplastic agent shown to be effective in the treatment of a variety of cancers. Although efficacious, DOX has been associated with the development of a cumulative, irreversible cardiomyopathy which may appear years after therapy. Numerous studies have been conducted to elucidate the mechanism of cardiotoxicity. However, the mechanism responsible remains elusive despite evidence implicating oxidative stress as a major contributor. In addition, DOX has been shown to alter the transcription of several genes, particularly in heart. This investigation was designed to assess global gene expression in order to identify transcriptional targets of DOX toxicity. The experimental model used was chosen to define persistent alterations of gene expression following chronic treatment with DOX. RNA was isolated from heart tissue extracted from adult male rats treated s.c. with 6 weekly injections of 2mg/kg DOX dissolved in saline, after which 5 weeks of recovery was allowed (saline n=5, DOX n=4). cRNA was generated and hybridized to Affymetrix 230A chips. Raw intensity data from samples were adjusted for background and normalized using the robust multichip analysis (RMA) method. Normalized data was filtered to produce a list of probes detectable in at least one experimental sample. Cluster analysis of the data revealed distinct expression profiles between treatment groups. Analysis by t-test revealed 53 transcripts were significantly altered following chronic DOX treatment. Classification of genes revealed several pathways that were modulated to varying degrees by DOX including; glycolysis, fatty acid metabolism, immune response, and oxidative stress response, among others. This comprehensive transcript profile provides important insights into critical targets and molecular adaptations that are characteristic of the persistent cardiomyopathy associated with long-term exposure to doxorubicin. (Supported by HL-058016).

1972 GENE EXPRESSION PROFILING IN TESTIS AND LIVER OF MICE TO IDENTIFY MODES OF ACTION OF CONAZOLE TOXICITIES.

A. K. Goetz[2], W. Bao[1], J. E. Schmid[1], C. Wood[1], H. Ren[1], D. S. Best[1], R. N. Murrell[2], J. C. Rockett[1], M. G. Narotsky[1], D. C. Wolf[1], D. B. Tully[1] and D. J. Dix[1]. [1]*Reproductive Toxicology, USEPA, Research Triangle Park, NC and* [2]*Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.*

Conazoles are a class of azole fungicides used in both pharmaceutical and agricultural applications. This study focused on 4 conazoles that exhibit a range of carcinogenic and reproductive effects, in order to identify common and unique modes of action. Conazoles target cytochrome P450s (CYPs), and the inhibition and induction of various CYP activities may be part of the toxic modes of action in liver and testis. We used gene expression profiling to characterize a broader range of conazole effects and to identify additional modes of action. Adult male CD-1 mice were dosed by gavage for 14 days with fluconazole, propiconazole, myclobutanil or triadimefon (three doses each). Relative liver weight increased following fluconazole and propiconazole exposure, and histological analysis revealed centrilobular hepatocyte hypertrophy in response to all 4 conazoles. No weight or histological changes were observed in testis, and serum testosterone and luteinizing hormone were also unchanged. Microarrays queried expression of 16, 475 genes, and identified 2, 081 and 1, 424 differentially expressed genes in liver and testis, respectively, following conazole exposure. Of these genes, 118 in the liver and 94 in the testis were common to two or more conazoles. The majority of differentially expressed genes related to stress response, oxidative stress, xenobiotic metabolizing enzymes, steroidogenesis or carcinogenesis. Expression profiles between conazoles and between tissues affected similar biological pathways, suggesting the potential for common modes of action. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy. Funded by EPA Cooperative Training Agreement CT826512010 with North Carolina State University.

1973 GENE EXPRESSION CHANGES ASSOCIATED WITH ALTERED GROWTH AND DIFFERENTIATION IN BENZO(A)PYRENE OR ARSENIC EXPOSED NORMAL HUMAN EPIDERMAL KERATINOCYTES.

D. S. Perez, R. S. Yang and J. A. Campain. *Quantitative & Computational Toxicology Group, Center for Environmental Toxicology & Technology, Department of Environmental & Radiological Health Sciences, Colorado State University, Fort Collins, CO.*

Normal human epidermal keratinocytes (NHEK) have been chosen as an *in vitro* model for mechanistic studies into how altered regulation of differentiation may play a role in the transformation process in human cells. Our initial work demonstrated that two high priority skin carcinogens, arsenic and benzo[a]pyrene (BaP), inhibit terminal differentiation in NHEK *in vitro*. Arsenic (>2 μm) also decreases proliferation in a manner suggestive of a G2 block. In contrast, BaP increases proliferation rates and induces rapid progression through the cell cycle, possibly by a shortened G2 phase. Differentiation is much more sensitive to chemically-mediated perturbations than is proliferation, indicating that the former process may be the initial target at environmentally prevalent concentrations. To identify molecular alterations that are responsible for the observed chemical-specific effects, microarray analysis was carried out on NHEK treated with BaP and arsenic. From this analysis, chemically altered genes were selected that are known to be involved in growth regulation (Ras, IL-1 α and β, TGF), differentiation (RA/INF-inducible protein, YY1-associated factor 2), RNA synthesis (tRNA exportin, RNA cyclase homolog), cell structure/adhesion (Cadherin 5, α-integrin binding protein), apoptosis (CASP8, programmed cell death 10), and chromatin structure/function (RAD50, REV1). Alterations in expression of these genes induced by arsenic and/or BaP are currently being examined using highly quantitative Real-Time RT-PCR. Through extensive time-course and dose-response studies, we will aim to clarify whether similar, highly central, pathways are disrupted by these diverse skin carcinogens or whether only chemical-specific effects are evident. This research was supported by NIEHS Grant # RO1 ES09655 & Minority Supplement # RO1 ES09655-01S1.

1974 CHARACTERISTIC EXPRESSION PROFILES INDUCED BY GENOTOXIC AND NON-GENOTOXIC CARCINOGENS IN RAT LIVER ANALYZED ON AFFYMETRIX GENECHIPS.

H. J. Ahr[1], H. Ellinger-Ziegelbauer[1], B. P. Stuart[2] and B. S. Wahle[2]. [1]*Molecular and Genetic Toxicology, Bayer Health Care AG, Wuppertal, Germany and* [2]*Toxicology, Bayer Crop Science, Stilwell, KS.*

Application of the recently developed gene expression profiling techniques using microarrays in toxicological studies (toxicogenomics) facilitate the interpretation of a toxic compound's mode of action. In this study we investigated whether geno-

toxic and non-genotoxic carcinogens at doses known to induce liver tumors in the two year rat bioassay deregulate characteristic sets of genes in a short term *in vivo* study. Male Wistar Hanover rats were treated for up to 14 days with the genotoxic carcinogens Dimethylnitrosamine (DMN, 4 mg/kg/d), 2-Nitrofluorene (2-NF, 44 mg/kg/d), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK, 20 mg/kg/d), and Aflatoxin B1 (AB1, 0.24 mg/kg/d), and the nongenotoxic carcinogens Wy-14643 (Wy, 60 mg/kg/d), Methapyrilene HCl (MPy, 60 mg/kg/d), Diethylstilbestrol (DES, 10 mg/kg/d), and Piperonylbutoxid (PBO, 1200 mg/kg/d). After 1, 3, 7, and 14 days the livers were taken for histopathology and for analysis of the expression profiles. Using statistical and clustering tools, characteristically deregulated genes were extracted and then functionally annotated. For the genotoxic carcinogens commonly affected pathways were DNA damage response, a distinct detoxification response, and the activation of proliferative and survival signaling pathways. The nongenotoxic carcinogens showed more compound-specific effects with regard to the functional classes of deregulated genes, yet a common characteristic seemed to be the induction of an oxidative stress response as well as deregulation of genes reflecting DNA replication and cell cycle progression. Thus, with a short term *in vivo* study a common gene expression response could be identified for several genotoxic carcinogens. Although many of the gene deregulations by the nongenotoxic carcinogens implied more diverse mechanisms, there were also similarities in some pathways, which might then also allow to identify a nongenotoxic carcinogenic potential from short term studies.

1975 GENE EXPRESSION PROFILING OF MAMMARY TISSUE FOLLOWING NMU TREATED SENSITIVE FISHER 344 AND RESISTANT COPENHAGEN RAT STRAINS IN DIFFERENT TIME POINTS.

X. Ren[2, 1], R. C. Sullivan[1], A. S. Kim[1] and H. Zarbl[1, 2]. [1]*Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA and* [2]*Department of Environmental and Occupational Health, University of Washington, Seattle, WA.*

While human genetic linkage analyses have identified tumor suppressors that are responsible for familial breast cancer, many susceptibility genes are likely to be partially penetrant, due to the modifying effects of diverse genetic backgrounds and gene-environmental interactions. The study of inherited cancer susceptibility genes in inbred animal models can facilitate the identification of cancer suppressor mutations that have low penetrance in the human population, as these may present as highly penetrant phenotypes in experimental tumor models. Genetic mapping study indicated in the Fischer 344 rat, genetic susceptibility to nitrosomethylurea-(NMU) induced mammary cacinogenesis is a recessive trait controlled by a locus on rat chromosome 12. To gain insight into the putative tumor suppressor locus, we compared normal mammary cells gene expression profiles from the susceptible Fisher 344 and the resistant Copenhagen rat strains. We identified ~1000 genes whose expression levels are significantly different ($P < 0.001$) between the two rat strains, which implicated several biochemical pathways to differential sensitivities mammary carcinogenesis. In the present study we used Agilent Rat Genome GeneChips to compare the patterns of gene expression induced in mammary cells in response to NMU in the Fisher 344 and Copenhagen rat strains as well as F1 (F344 X COP). Briefly, NMU was administered to female rats at onset of puberty (55 days of age) in dose of 50mg/kg. The time points examined included 6 hours and 1, 7, 30 days. To further enrich for a subset of genes whose altered expression directly contribute to differential susceptibility we are also comparing these results with expression profiles in control and NMU treated N2 backcross progeny. Analyses of these data will provide insights into the underlying mechanisms and biochemical pathways that are associated with the differential sensitivities to mammary carcinogenesis.

1976 SERUM CYTOKINE ANALYSIS AND TRANSCRIPTIONAL PROFILING OF PBMCS IN CHIMPANZEES TREATED WITH A LYMPHOTOXIN BETA RECEPTOR AGONIST.

M. Cooper[1], E. Stanford[2], M. Wu[1], M. Derbel[1], J. Goyal[2], J. Green[2], M. Subramanyam[2] and D. Enke[1]. [1]*Biomarker Development and Validation, Biogen, Cambridge, MA and* [2]*Preclinical and Clinical Development Sciences, Biogen, Cambridge, MA.*

Described herein are the results of serum cytokine profiling and transcription profiling of PBMCs isolated from chimpanzees treated with LTBR mAb. The goal of this study was to identify potential biomarkers for LTBR mAb drug response. LTBR mAb is a monoclonal antibody that acts as an agonist to LTBR. The LTBR is expressed on both neoplastic and normal tissues. Activation of the LTBR is expected to induce cell death or differentiation of neoplasms. LTBR mAb is biologically active in humans and chimpanzees, but not macaques or other common laboratory species. Previous investigations have shown evidence of MIG (Monokine Induced by Gamma interferon; a ligand for the CXCR3 receptor), IP-10, and ITAC release in response to LTBR mAb treatment in *in vitro* and *in vivo* models. In this study, we observed a dose-dependent release of MIG into the serum of chimpanzees at 2-hours postdose in response to LTBR mAb treatment. This response was not observed with IP-10 or Th1 and Th2 cytokines. Additional studies with proper specificity controls are required to confirm and validate these findings *in vivo*. In addition, transcriptional profiling results suggested potential migration of certain lymphocyte cell types out of the free blood compartment, consistent with MIG-mediated PBMC activation *via* the CXCR3 receptor. Indeed, many transcripts associated with particular lymphocyte cell types were decreased in abundance in PBMC samples with high LTBR mAb and MIG levels. These transcripts included cathepsin W, killer cell immunoglobulin-like receptor, natural killer cell group 7, granulysin, granzyme B, and perforin. Additional studies including validation of the expressed transcripts using TaqMan PCR and additional *in vitro* or *in vivo* studies with appropriate controls will be required to confirm these findings. It was concluded that MIG and the MIG pathway should be further investigated as sources of potential biomarkers for LTBR mAb *in vivo*.

1977 BLOOD GENE EXPRESSION PROFILING: A BIOMARKER PILOT STUDY COMPARISON OF SMOKERS AND NONSMOKERS.

G. M. Hellmann[1], W. T. Morgan[2] and C. N. Overman[2]. [1]*Physiology and Pharmacology, Wake Forest University, Winston-Salem, NC and* [2]*R. J. Reynolds, Winston-Salem, NC.* Sponsor: D. Pence.

Transcriptional profiling of peripheral blood has attracted considerable attention within the field of toxicogenomics as a minimally invasive technique utilizing an easily accessible tissue representative of systemic physiological state. We hypothesized that the effects of cigarette smoking would be manifest in transcription profiles obtained from the peripheral blood of smoking subjects, and that those profiles could be distinguished from those of non-smoking subjects. To investigate this hypothesis, peripheral blood samples were obtained from adult smokers (n=8) and control never-smokers, stabilized by collection in PAXgene™ blood collection tubes, and the RNA subject to array analysis on a 1200-gene nylon membrane cDNA array. An extensive set of blood count parameters including total white count, differential leukocyte counts, and red blood cell and platelet counts was also determined from a second blood draw taken at the same time. Absolute levels of monocytes exhibited a mean increase of 56% in the smoking group. No other statistically significant differences in any of these metrics were noted between the smoking and non-smoking groups. However, above physiological variation and intrinsic interindividual variation, array analysis revealed 23 genes showing both statistically significant differences ($p<.05$) in the levels of the corresponding transcripts and fold-change greater than 2. Ignoring fold-change restrictions, a total of 133 statistically significant differences were noted. Differential regulatory responses included changes in relative mRNA levels for the following: upregulation of c-myc, GM-CSF, TNFα and the proinflammatory cytokines IL-6 and IL-13, and downregulation of MMP-9. The data support the hypothesis that there is a peripheral blood genomic response to smoking, and this response is associated with altered regulation of a set of mRNAs that can serve as important biomarker indicators of smoking-associated physiological status.

1978 ROLE OF INTESTINAL PERMEABILITY IN NSAID- AND LPS-INDUCED ACUTE PHASE RESPONSE IN RATS.

S. Tugendreich, S. Baumhueter, G. Day, S. Dunlea, B. Eynon, M. Fielden, S. Fujimoto, B. Ganter, R. Idury, K. Jarnagin, K. Kolaja, M. Lee, R. Nair, G. Natsoulis, S. Nicholson, C. Pearson, A. Roter, S. Thode and A. Tolley. *Iconix Pharmaceuticals, Mountain View, CA.*

To improve compound selection during drug discovery we have developed a large library of biomarker sets for important toxicities and mechanisms of action. These signatures derive from a comprehensive database that connects results derived from treating rats with 580 drugs at up to two doses and four time points in triplicate with traditional measurements of toxicity, pathology and pharmacology. The database contains ~13, 000 micorarray gene expression measurements. We have profiled ~50 immune-modulating compounds, and have found that high doses of most non-steroidal anti-inflammatory drugs (NSAIDs) induce an acute phase response expression pattern like that elicited by endotoxin (LPS). We hypothesize that NSAIDs increase intestinal permeability, allowing LPS to escape into the blood and stimulate an acute phase response in the hepatic macrophages. We conducted a study using indomethacin, ibuprofen, and several of the COX-2 selective "coxibs". Animals were necropsied after 1 and 3 daily doses and evaluated for intestinal histology and gene expression changes. Several drug treatments induced an acute phase response as indicated by blood inflammatory proteins and hepatic gene expression markers. Acute phase genes such as stat3 and lipocalin2 were induced by 2.5 to 30-fold. The mechanism of intestinal damage and associated hepatic changes is examined and discussed relative to various hypotheses related to COX selectivity.

1979 TOWARDS QUANTITATIVE PREDICTIONS OF HEPATOTOXICITY USING GENE EXPRESSION PROFILES.

Y. Yang, R. X. Ciurlionis and J. F. Waring. *Department of Cellular and Molecular Toxicology, Abbott Laboratories, Abbott Park, IL.*

Despite improved toxicology testing, hepatotoxicity associated with new drug candidates remains a major hurdle in drug discovery. Recent advances in microarray technology have provided a relatively high throughput tool to monitor toxicity responses on a genome scale. The purpose of this study was to develop a quantitative approach to predict hepatotoxicity based on gene expression profiles. To do so, a toxicogenomics database was constructed from three-day rat studies treated with a large number of hepatototoxins and non-hepatotoxins. A set of marker genes was identified that distinguished the hepatotoxins from the non-hepatotoxins using ANOVA analysis. Using neural networks coupled with principle component analysis for dimension reduction, a quantitative model was established to classify compounds according to a composite toxicity score. Our results showed that, using gene expression analysis, the neural network classified compounds based on their potential to cause hepatotoxicity with a high degree of sensitivity and specificity. Additional studies using compounds outside of the initial database were also correctly classified using the neural network. Our study demonstrates that a predictive gene expression based model has the potential to discriminate among different levels of toxicities. This type of assay would allow drug discovery teams to prioritize compounds for safety at the pre-investigational toxicology stage, resulting in a lower rate of attrition at later stages of drug development.

1980 BIOMARKER DEVELOPMENT INFORMATION MANAGEMENT SYSTEM (BMD-IMS): ELECTRONIC FORMS IN A 21 CFR PART 11 COMPLIANT LIMS SYSTEM.

D. Enke[1], M. Cooper[1], M. Derbel[1], M. Wu[1], Q. Duong[1], M. Subramanyam[1], S. Subramaniam[2], M. Means[2], M. Christow[2], M. Wang[2], Z. Xu[2], A. Siva[2], K. Natarajan[2], J. Calabro[2] and M. Rosenberg[2]. *[1]Biomarker Development, Biogen, Cambridge, MA and [2]Research Informatics, Biogen, Cambridge, MA.*

The application of new and complex technologies to drug development, including transcriptional profiling, has generated a need for a LIMS system capable of supporting multi-step workflows in a 21 CFR part 11 and GLP compliant environment. In an effort to create a system that can seamlessly support complex laboratory procedures such as the Affymetrix workflow as used to support regulatory filings, we have designed and implemented the Biomarker Development Information Management System (BMD IMS). This system is fully compliant and utilizes electronic forms for all data capture and witnessing functions. A Documentum document manager controls the laboratory workflow and audit trail. Data is input by the user into electronic forms *via* a PDF interface with tablet or desktop PCs, and all information is captured in an Oracle database. When a user completes a procedure, the system automatically notifies members of the group *via* email that the electronic forms are ready for witnessing, and witnessing functions are performed electronically through the PDF interface. Finally, data can be queried and reports generated through use of Business Objects. This system has been piloted to support the Affymetrix workflow, however, the system is highly flexible and can be used to support electronic forms for any laboratory procedure.

1981 HEPATIC GENE EXPRESSION SIGNATURES IDENTIFY MULTIPLE PATHWAYS RELATED TO AH RECEPTOR ACTIVATION FOLLOWING SHORT- AND LONG-TERM COMPOUND ADMINISTRATION IN RAT.

S. Nicholson, S. Baumhueter, G. Day, S. Dunlea, B. Eynon, M. Fielden, S. Fujimoto, B. Ganter, R. Idury, K. Jarnagin, K. Kolaja, M. Lee, R. Nair, G. Natsoulis, C. Pearson, A. Roter, S. Thode, A. Tolley and S. Tugendreich. *Iconix Pharmaceuticals, Mountain View, CA.*

To improve compound selection during drug discovery, we used several linear classification methods, SVM variants, to develop a large library of biomarkers, Drug Signatures™, highly interpretable short gene lists, for important toxicities and mechanisms of action. These signatures were derived from a comprehensive database that marries ~13, 000 micorarray gene expression measurements, obtained by treating rats with 580 drugs at two doses and four time points in triplicate, with traditional measurements of toxicity, pathology and pharmacology. Chronic aryl hydrocarbon receptor (AhR) activation can lead to hepatocarcinogenicity, immunomodulation, and drug-drug interactions, all of which are undesirable. To show that DrugSignatures™ predict the effects of AhR activation, male SD rats were orally administered AhR agonists, 3MC (300, 30, 12.5 mpk), beta-naphthoflavone (1500, 150, 80 mpk), and TCDD (0.02, 0.001, and 0.0007 mpk), and the weak agonist alpha-naphthoflavone (80 mpk), for 5, 28, and 91 consecutive days. Expression profiles of liver samples from control and treated rats were obtained and analyzed against our DrugSignature™ library. Examination of the AhR DrugSignature™ shows consistent induction of CYP1A1, CYP1A2, and glutathione-S-transferases, as well as genes associated with cell cycle regulation. A number of genes specific to TCDD-treatment were also affected. Long-term administration of AhR agonists modulates the expression of many additional genes, including those related to oxidative stress. These changes in gene expression occur in the absence of changes in classical endpoints (e.g. histopathology, clinical chemistry, and hematology) and highlight the powerful insight of toxicogenomic evaluation.

1982 A GENOMIC MODEL OF BENZO(A)PYRENE INDUCED ATHEROGENESIS.

C. D. Johnson[1, 2], T. L. Thomas[3] and K. S. Ramos[1, 2]. *[1]Center for Genetics and Molecular Medicine, University of Louisville, Louisville, KY, [2]Biochemistry and Molecular Biology, University of Louisville, Louisville, KY and [3]Biology, Texas A&M University, College Station, TX.*

Atherogenic stimuli trigger complex responses in vascular smooth muscle cells that culminate in activation/repression of overlapping signal transduction cascades involving oxidative stress. In the case of benzo(a)pyrene (BaP), a polycyclic aromatic hydrocarbon present in tobacco smoke, the atherogenic response involves interference with redox homeostasis by oxidative intermediates of BaP metabolism. In the present study, experiments were conducted to further define early global gene expression profiles in murine vascular smooth muscle cells challenged with BaP in the presence or absence of chemical antioxidants. Profiles of gene expression were defined using analysis of variance and clustering methodologies. Supervised and non-supervised analyses identified a number of gene clusters that were uniquely regulated by BaP, antioxidant, or the combined chemical treatments after acute (8 hr) challenge. NAD(P)H dehydrogenase, quinone 1 (Nqo1), cyclooxygenase 2 (Cox2), and TRNA-specific eukaryotic elongation factor (TREEF), were identified as redox-regulated genes in response to BaP challenge. Elongation factor TREEF is of particular interest given its role in the synthesis of thioredoxin reductase 2 (Txnrd2), an antioxidant enzyme regulated by heat shock factor 2 and linked to cytochrome p450 1b1. A number of previously unreported redox responsive genes, such as nuclear factor I/B and 1/X (Nfib, Nfix), and SRY-box containing gene 4 (Sox4) were also identified as putative targets of BaP in vascular smooth muscle cells. The latter genes are of interest in light of their involvement in Hmg signaling in mammalian cells. Collectively, these results indicate that oxidative stress plays a prominent role in the regulation of gene expression during the early stages of chemical atherogenesis. (Supported by NIH grants ES04849, ES09106, ES07273 to KSR, and ES012117 to CDJ).

1983 COMPOUND CLASSIFICATION USING TRANSCRIPT PROFILING.

S. ruepp[1], G. Steiner[2], F. Boess[1], R. Gasser[1], S. Evers[3], M. De Vera[1], S. Albertini[1] and L. Suter[1]. *[1]PRBN-S, Roche, Basel, Switzerland, [2]PRBI-B, Roche, Basel, Switzerland and [3]PRG, Roche, Basel, Switzerland.*

Toxicogenomics is expected to improve sensitivity, accuracy and speed of toxicological investigations. The main assumption of toxicogenomics is that toxicity is accompanied by changes in gene expression that are either causally linked to the toxicity or represent a response to toxic alterations. Toxicogenomics can be used for mechanistic investigations or for predictive tasks. The effect of various hepatotoxic compounds was studied in male Wistar and Sprague-Dawley rats. The compounds chosen for this study induced cholestasis, steatosis, peroxisomal proliferation or were "direct-acting". Histopathology and clinical chemistry were used as conventional methods to assess liver toxicity. Gene expression changes in the liver were evaluated using Affymetrix Gene-chip? microarrays (RG-U34A). Gene-chip analysis was performed on five individual animals within a treatment group. Different kinds of support vector machines (SVMs) were used for classification purposes. Compound profiles, not included in the training set for SVMs, could subsequently be assigned to the correct toxicity categories. Non-toxic pharmacologically active substances were correctly grouped with control gene expression profiles. The relationship of number of genes used for classification and accuracy of classification was studied by implementing recursive feature elimination (RFE). Results demonstrate that gene expression profiles can be used to discriminate toxicants with different modes of action from each other and from controls using only a small number of genes. Small sets of genes could potentially be used as sensitive surrogate markers for toxicity. Fingerprints derived from Wistar rats were successfully used to classify gene expression profiles derived from Sprague-Dawley rats. Furthermore, gene expression analysis successfully identified non-responding animals as defined by conventional endpoints. Toxicity of several substances could be detected at an earlier time than by conventional endpoints highlighting the potential of this approach.

1984 THE COMPARATIVE TOXICOGENOMICS DATABASE (CTD).

C. J. Mattingly[1], G. T. Colby[1], M. Rosenstein[1], J. N. Forrest[2, 1] and J. L. Boyer[2, 1]. [1]*Bioinformatics, MDI Biological Laboratory, Salisbury Cove, ME and* [2]*Medicine, Yale Univeristy, New Haven, CT.* Sponsor: W. Toscano.

Sequencing of the human genome has stimulated a new way of thinking in biology; however, our understanding of the sequence is limited by our ability to retrieve meaning from it. The human and other vertebrate sequencing projects have underscored the significance of comparative studies to reveal conserved sequences that may be functionally significant. The Comparative Toxicogenomics Database (CTD) will be the first publicly available, curated database devoted to genes and proteins of human toxicological significance. The major goals of this resource include: 1) curating and integrating sequence, reference and toxicant data for toxicologically important genes and proteins; 2) promoting comparative studies of these genes and proteins across evolutionarily diverse organisms; and 3) integrating information from existing molecular and toxicology resources. The infrastructure of data in CTD includes nucleotide and amino acid sequences, references from the published literature and controlled vocabularies for taxonomy and substances/toxicants. The current focus of curation and integration efforts in CTD is to create explicit associations between nucleotide and amino acid sequences, references and toxicants, as well as between sequences and genes through the construction of Gene Sets. Gene Sets place sequences in a comparative context by grouping all sequences from diverse species for a gene or related genes with associated toxicants, references and gene synonyms. CTD will provide focused access to data that is of toxicological interest and will facilitate interspecies comparisons of toxicological genes to promote understanding about their role in modulating human health.

1985 DISCOVERING SYSTEM-LEVEL FEATURES OF THE EARLY MOUSE EMBRYO USING MITOCHONDRIAL BENZODIAZEPINE RECEPTOR LIGANDS, MICROARRAY AND COMPUTATIONAL METHODS.

K. R. Nemeth, A. V. Singh, R. C. Craig, K. B. Knudsen, N. H. Owens, B. Nibbio, M. Green and T. B. Knudsen. *Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA.*

Exposure of early mouse embryos to some teratogens (MeHg, EtOH, 2CdA, hypoxia) can invoke a stress response involving the mitochondrial benzodiazepine receptor and downstream changes to at least two nuclear transcription factors: p53 tumor suppressor (Trp53) and nuclear respiratory factor (Nrf1). A working model proposes this receptor (Bzrp) is activated during mitochondrial dysfunction and generates a signal culminating in down-regulation of Nrf1 expression; furthermore, Bzrp signals induce p53 protein to accumulate in the nucleus. Since Nrf1 and Trp53 play key roles in respiratory and cellular differentiation, the mitochondrial localization of Bzrp may relay important information to the nucleus regarding oxygen metabolism states. In a microarray-based study we have begun to characterize the response signature linked with different receptor states. Study of 100 microarrays revealed 244 genes differentially affected by low-level teratogen exposure (10% average risk for malformation). Many changes correlated with p53 activating exposures and intervention with PK11195, a Bzrp antagonist. Preliminary analysis implies strong cross-regulation between p53-dependent and growth factor-mediated survival pathways (PTEN, v-akt/PKB), regulation of ribosomal protein expression, and chromatin remodeling (p300/CBP, HMG-A1, SMARC-A4) systems. An emergent property is inverse coupling between the Bzrp state and cellular plasticity; e.g., stimulating the receptor (as in respiratory distress) drives p53 to invoke checkpoints for cell growth, differentiation and death whereas antagonizing the receptor (pharmacologically) suppresses signals upstream to p53 induction and drives genes for chromatin remodeling and anaerobic (glycolytic) metabolism, pushing the system to a more primitive state. (Supported by grants AA13205 and ES09120 from the NIH, T32 ES07282 from the NIH/NIEHS, and grant R 827445 from the EPA but does not reflect agency policy).

1986 PROTEOMIC IDENTIFICATION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-6 INDUCED BY SUBLETHAL H_2O_2 STRESS FROM HUMAN DIPLOID FIBROBLASTS.

L. Xie and Q. M. Chen. *Pharmacology, University of Arizona, Tucson, AZ.*

Fibroblasts are the most ubiquitous cell type within various organs of our body. They produce various factors such as proteases, cytokines, growth factors and extracellular proteins to maintain the structure and texture of a particular organ or tissue. To identify the secreted protein factors and the alteration of these protein factors upon oxidative stress, we exposed confluent human diploid fibroblasts (HDFs) in culture to sublethal oxidative stress induced by 600 μM H_2O_2 for 2 hours. Such treatment caused the cells to develop a phenotype of senescence prematurely after 3 days. The control and premature senescent cells were kept in a medium deprived of serum for analyzing secreted proteins after collecting the conditioned medium. Concentrated conditioned media were used for Electrospray Ionization Liquid Chromatography Tandem Mass Spectrometry (ESI-LC-MS/MS) analyses and Sequest database search. Insulin-like growth factor binding protein-6 (IGFBP-6) was reproducibly detected in the H_2O_2 stressed group but not in the control group. Other proteins such as fibronectin, lumican, matrix metalloproteinase-2 (MMP-2) and collagen alpha1(VI) chain were consistently detected in both control and H_2O_2 stressed groups. Western blot analysis was used to verify the data obtained from ESI-LC-MS/MS approaches. The conditioned media from H_2O_2 treated HDFs showed a significant elevation of IGFBP-6, whereas no difference for fibronectin and MMP-2 was detected between the control and stressed groups. We conclude that H_2O_2 causes HDFs to secrete IGFBP-6, and that proteomics is a powerful technique for identifying proteins secreted by cells.

1987 NEW OPPORTUNITIES TO EXPLOIT THE HAZARDOUS SUBSTANCES DATA BANK USING TEXT MINING.

A. Porter[2], M. Szczur[1], H. F. Chang[1], J. Goshorn[1] and D. Schoeneck[3]. [1]*National Library of Medicine, National Institutes of Health, Bethesda, MD,* [2]*Technology Policy and Assessment Center, Georgia Institute of Technology, Atlanta, GA and* [3]*Search Technology Inc., Norcross, GA.* Sponsor: V. Hudson.

Biomedical research findings and toxicological information are compiled in rich databases, including the Hazardous Substances Data Bank (HSDB), MEDLINE, and TOXLINE. This project explores ways that text mining can help extract embedded information to aid toxicology researchers. We imported 73 selected fields of 4586 HSDB records into VantagePoint software. The full record file and the citation record file were created to facilitate knowledge discovery. The capability to easily compile cross-record information generates "bird's eye" perspectives. It also facilitates "drilling down" to identify agent information salient to particular research questions. Five essential steps in exploiting the body of knowledge on a selected topic are search, retrieval, data cleaning, analysis, and representation of findings. We illustrate these for one agent (phenol) and one effect (asthma). Most exciting is the capability to deliver quick "research profiles" focused on particular user needs. Tailored scripts can automate the five steps to generate otherwise inaccessible topical profiles. We present a sample profile based on asthma-associated information from thousands of HSDB, MEDLINE, and TOXLINE records. This "one-pager" targeted for a novice researcher spotlights: a) research activity trends b) leading sources c) "top, " "hot, " and "new" topics identification d) a topical cluster map We explore ways that this new method to access information can enrich toxicology. "Research profiles" can alert researchers to the advent of new approaches, tools, and issues. We see special promise in improving cross-disciplinary access to scientific findings. Desktop database mining can help researchers uncover agent-effect-organ interactions, including second-order linkages. Such research domain profiling, performed at the onset of new projects, could enhance research efficacy by increasing awareness of related findings.

1988 USING A CDNA MICROARRAY FOR GENE EXPRESSION PROFILING OF WHOLE BLOOD IN LIPOPOLYSACCHARIDE-TREATED RATS.

R. D. Fannin, J. T. Auman, M. E. Bruno, S. O. Sieber, S. M. Ward, C. J. Tucker, B. A. Merrick and R. S. Paules. *National Ctr Toxicogenomics, NIEHS, Research Triange Pk, NC.*

cDNA microarrays have been used to evaluate the expression of thousands of genes in diseased tissues. However, very few studies have investigated the change in gene expression profiles in one of the most easily accessible tissues, whole blood. We utilized an acute inflammation model to investigate the possibility of using a cDNA microarray to measure the gene expression profile in whole blood. Whole blood was collected in male F344 rats at 2 and 6 hr after treatment with 5 mg/kg i.p. lipopolysaccharide (LPS). Hematology showed marked neutrophilia accompanied by lymphopenia at both time points. TNFα and IL6 levels were elevated at 2 hr indicating acute inflammation, but by 6 hr the levels had declined. Total RNA was isolated from whole blood and hybridized to a rat 6, 700 cDNA microarray chip. Results showed that 216 and 180 genes were differentially expressed after 2 and 6 hr of LPS treatment, respectively. Many of the differentially expressed genes are known to play a role in the inflammation process, but differential expression was also noted in genes involved in cytoskeleton/cell adhesion, oxidative respiration, and transcription. Genes prominently upregulated at both time points include those involved in acute phase response, such as lipocalin 2 and IL1β. Genes commonly downregulated by LPS at both time points include those for antigen/processing, ribosomal proteins and protein kinase C related genes. In addition, a number of genes were differentially expressed at only one time point, such as TIMP2 that was upregulated at only 2 hr, while IL6 receptor was downregulated at only 6 hr after LPS treatment. Real-time PCR confirmed the differential regulation of 9

genes discovered by cDNA microarray. Principle component analysis could discriminate between acute inflammation at 2 hr and recovery at 6 hr. These studies indicate that inflammatory changes in gene expression profiles can be detected in whole blood despite the adaptive shifts in leukocyte populations that accompany inflammatory processes.

1989 APPLICATION OF CDNA MICROARRAYS FOR SCREENING CARCINOGENICITY OF CHEMICALS IN THE 28-DAY REPEAT-DOSE TOXICITY STUDY.

M. Otsuka[1], H. Matsumoto[1], Y. Yakabe[1], M. Takeyoshi[1], K. Saito[2], K. Sumida[2], M. Sekijima[3], K. Nakayama[3], Y. Kawano[3], M. Tsuchiya[3], Y. Shinohara[4] and T. Shirai[5]. *[1]Chemical Evaluation and Research Institute, Japan, Tokyo, Japan, [2]Sumitomo Chemical Co., Ltd.,, Osaka, Japan, [3]Mitsubishi Chemical Safety Institute Ltd.,, Ibaraki, Japan, [4]Hokkaido University, Sapporo, Japan and [5]Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan.*

In the present study, male Fischer 344 rats aged 5-week old were treated by daily oral gavages with known carcinogenic and non-carcinogenic compounds, such as peroxisome proliferators, enzyme inducers or hepatotoxicants, at two dose levels for 28 days. The livers were sampled at the following 5 timepoints: Day 1, 3, 7, 14 and 28. Physical signs, organ/body weight ratios, plasma enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin, and histological changes were measured. For monitoring the gene expression levels, the in-house microarray containing approximately 8, 000 genes and ESTs from the liver, kidney, spleen and colon of rats was prepared and GeneChip (Affymetrix) was also applied for comparison. Clofibrate, for example, produced hepatocellular granular eosinophilic cytoplasm and swelling of hepatocytes without any changes in plasma enzymes. In the liver tissue, gene expression such as acyl-CoA oxidase, acyl-CoA dehydrogenase, mitochondrial acyl-CoA thioesterase, 3-hydroxy-3-methylglutaryl-CoA synthetase, cytosolic epoxide hydrolase, malic enzyme, CYP4A1, and aldehyde dehydrogenase were upregulated, whereas apolipoprotein AIV or cyclin D1 were downregulated. These gene expression profiles obtained were consistent with previous observations reported elsewhere. We are going to build database of gene- and protein-expression profiles and develop data processing algorithms using reference compounds being carcinogenic and non-carcinogenic to establish a test system for screening of the carcinogenicity of chemicals. Acknowledgement: This work was sponsored by NEDO (New Energy and Industrial Technology Development Organization)

1990 QUANTITATIVE PROTEOMIC ANALYSIS OF HEPATIC RESPONSES TO 28-DAY REPEATED DOSE.

H. Yamanaka[1], M. Otsuka[1], Y. Yakabe[1], M. Takeyoshi[1], K. Saito[2], K. Sumida[2], M. Sekijima[3], K. Nakayama[3], Y. Kawano[3], M. Tsuchiya[2], Y. Shinohara[4] and T. Shirai[5]. *[1]Chemical Assessment Center, Chemical Evaluation and Research Institute, Japan, Saitama, Japan, [2]Sumitomo Chemical Co., Ltd.,, Osaka, Japan, [3]Mitsubishi Chemical Safety Institute Ltd.,, Ibaraki, Japan, [4]Hokkaido University, Sapporo, Japan and [5]Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan.*

We ran quantitative proteomic analysis on liver homogenates of rats by thirty chemicals with known carcinogenicity using the quantitative fluorescent 2D-DIGE (2D-Differential Gel Electrophoresis) method (Amersham Biosciences, UK Limited). Thirty chemicals tested includes 2 genotoxic carcinogen, 11 non-genotoxic carcinogen and 17 non-carcinogen. F344 rats were repeatedly dosed for 28 days with two levels of chemicals together with vehicle control. Individual liver sample of low and high dosed rats was labeled with Cy3 and Cy5, respectively. Each gel image of them was matched to one master gel, which generated with a pooled one of all samples (livers of low and high dosed and control rats) labeled with Cy2, with Decyder software to identify and quantify the spot of protein expressed in rat liver. These protein expression patterns potentially represent signatures of organ responses to specific chemicals or offer insights into tumorigenesis processes. Significant change in expression level compared to control was observed in response to administration of chemical depending on the test chemical and dose level. For example, 2D-DIGE of Clofibrate treated liver (250mg/kg/day, 28days) indicated significant ($p<0.001$) quantitative changes in 108 liver proteins compared to controls. The cluster analysis was applied with all protein expression data to find the protein expression profile specific to carcinogenicity of chemicals. Acknowledgement: This work was sponsored by NEDO (New Energy and Industrial Technology Development Organization) .

1991 BIOMARKERS OF ASPHALT FUME EXPOSURE.

C. Keshava[1], N. Keshava[1], B. Law[2], D. Lewis[2] and A. Weston[1]. *[1]TMBB, National Institute for Ocupational Safety and Health, Morgantown, WV and [2]Analytical Services Branch, NIOSH, Morgantown, WV.*

It has been estimated that at least 24, 000 occupationally related cancers are diagnosed in the US each year. The introduction of occupational cancer biomarkers in cross-sectional epidemiologic research may be a useful approach in the development of strategies for intervention. Here we have used "gene chip" technology (high density DNA-oligonucleotide arrays) to identify potential biomarkers of asphalt fume condensate (AFC) exposure. A rat lung epithelial cell line (RLE-6TN) was exposed to AFC (270μg/ml) *in vitro* for 12h and 24h. At the end of the treatment period, RNA was extracted from the cells, subjected to reverse transcription, *in vitro* transcription, fluorescence labeling and hybridization to Affymetrix™ rat genome microarrays (U34A). Transcription of 348 genes on the array was observed to be altered by a factor of at least 2 fold. A battery of genes induced ($p<0.01$) by this exposure included Phase I and II metabolism genes (*CYP1A1, CYP1B1, GST-A1* and *NQO1*), oxidative stress and inflammatory genes (*ALDH, CAT* and *COX-2*), DNA damage response genes (*GADD45, GADD153*), and transcription factors and oncogenes (*EIF-2* and *FRA-1*). These genes were selected for further investigation by reverse transcription and polymerase chain reaction (RT-PCR). The RT-PCR confirmed the induction of this battery of genes and RT-PCR was reasonably well correlated with the oligonucleotide array data ($r^2 = 0.6$, $t = 3.3$, $p<0.01$, $df = 20$). Genes that are turned-on in response to such exposures are potential candidate biomarkers that should be investigated further. This study identified several candidate biomarkers of asphalt fume exposure. Future studies will investigate the effects of chemopreventive agents on these candidate biomarkers and associated end-points like DNA damage in human epithelial cells.

1992 USING CORRELATION ANALYSIS AND HIERARCHICAL CLUSTERING TO DISCRIMINATE GENES ASSOCIATED WITH HEPATIC VASCULITIS FROM SUBSEQUENT HEPATIC INFLAMMATION.

B. lu[1], L. Nelms[1], G. Floyd[2] and M. Lawton[1]. *[1]Molecular and investigative toxicology, Pfizer, Groton, CT and [2]Pathology, pfizer, Groton, CT.*

During a pre-clinical study, compound X was found to induce hepatic vasculitis in rats. In an experiment designed to understand the molecular details of this lesion, groups of male Sprague-Dawley rats were given daily oral doses of vehicle, 10 mg/kg or 1000 mg/kg of compound X and animals were euthanized 24, 48, 144 and 240 hours after the first dose. Liver samples were harvested for gene expression profiling with RG_U34A Genechip and serum from blood was used for proteomics analyses. Clinical chemistry, hematology and histopathology data were also collected from the study. At 24 hours, histological vascular changes induced by high-dose compound X were consistent with endothelial activation, and frank vascular inflammation that sometimes extended secondarily into the hepatic parenchyma was evident at 48 hours. Clinical chemistry and hematology data both reflected the presence of inflammation at 48 hours. We then focused on genes that were differentially regulated at 24 or 48 hours to look for potential biomarkers for liver vasculitis. Changes in gene expression could be due to the endothelial proinflammatory response, vascular inflammation, inflammation of the perivascular hepatic parenchyma or activation of the hepatocellular acute phase response. To differentiate genes in these tandemly linked but different processes, we used statistical methods to calculate and identify genes that are highly correlated with severity of the vascular and perivascular inflammation. In addition, we have compared the hepatic gene expression profile induced by compound X to other compounds known to induce a hepatic acute phase response secondary to inflammation at sites other than the liver. Using data from Gene Logic's ToxExpress database, we also subtracted genes activated by hepatic inflammation from the compound X response and helped to in silico select candidate genes that are involved in the hepatic vascular inflammatory response rather than in the hepatic acute phase response that occurs secondary to inflammation.

1993 DBZACH: A COMPREHENSIVE TOXICOGENOMIC INFORMATION MANAGEMENT AND KNOWLEDGE DISCOVERY SYSTEM.

E. Dere, L. D. Burgoon, P. C. Boutros, S. Doran, S. S. Pai, R. Aiyar, J. Vakharia, R. Rotman, A. Adams, B. Lau and T. R. Zacharewski. *Pharmacology & Toxicology, Biochemistry & Molecular Biology, National Food Safety & Toxicology Center, Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.*

dbZach (http://dbzach.fst.msu.edu), a Minimum Information About a Microarray Experiment -Toxicology (MIAME/Toxicology) supportive toxicogenomic relational database, uses Java-based data mining and visualization tools to facilitate knowledge discovery. The system consists of the dbZach database back-end, and the dbZach software suite of data mining and knowledge discovery tools. The architecture for the dbZach database is modular and flexible, consisting of core subsystems (i.e. Clones, Genes, Protocols, Sample Annotation) which are required, in silico subsystems (Gene Regulatory, Pathway) which facilitate data interpretation, and 3) experimental subsystems (Microarray, Affymetrix, Real-Time PCR, Toxicology) that store raw as well as processed data . The modular architecture allows portions of the database to be cordoned off to facilitate development, fixes, fu-

ture expansion of the project, and incorporation into other existing database systems. The software suite allows the system to go beyond information management by providing query and knowledge discovery tools that support 1) data mining, 2) quality control and inspection of microarray data, 3) up-to-date gene annotation, 4) orthologous gene identification, 5) protocol cataloguing and inspection, 6) integration of disparate biological and toxicological data, and 7) data analysis and interpretation. Future developments for dbZach include management of biological pathway information, the development of a pipeline from dbZach to the ArrayExpress genomic data repository, and other data visualization tools. The dbZach System will be made source-available for local implementation as an independently-operated laboratory information management and knowledge discovery system. Supported by NIH grants *T32 ES07255 ES 04911-12, ES 011271 and ES 011777.

1994 COMPARISON OF THE MODIFIED LOOP AND INDEPENDENT REFERENCE DESIGNS FOR MICROARRAY STUDIES.

L. D. Burgoon[1], D. R. Boverhof[1], J. E. Eckel[2], C. Gennings[2] and T. R. Zacharewski[1]. *[1]Pharmacology & Toxicology, Biochemistry & Molecular Biology, National Food Safety & Toxicology Center, Institute for Environmental Toxicology, Michigan State University, East Lansing, MI and [2]Biostatistics, Virginia Commonwealth University, Richmond, VA.*

It remains unclear what impact the choice of experimental design may have on the identification of changes in gene expression following drug or chemical exposure. To examine this further, a hepatic time-course experiment combining the modified loop (MLD) and independent reference (IRD) designs was used to identify changes in genes expression following treatment by gavage with ethynyl estradiol (EE), an orally active estrogen. Immature, ovariectomized female mice were gavaged once or 3x24hr with 0.1mg/kg EE or vehicle (sesame oil) and hepatic tissue was harvested at 0, 2, 4, 8, 12, 24 or 72 hrs. Murine cDNA microarrays with 3, 068 clones, spotted in duplicate, representing 2, 259 distinct genes, were used to examine changes in gene expression elicited by EE. Each experimental design was replicated three times with independent biological samples. The data were normalized using a General Linear Mixed Model (GLMM), and gene effects were estimated using a gene-specific GLMM. The designs were compared using percentile range lists constructed using t-values from a model-based t-test. For comparison purposes, the 4hr time-point was chosen, as it was representative of the other time points. A 40% overlap exists between the designs at the highest 10th percentile, where the most active genes reside. 50% and 90% overlaps were evidenced at the 30th and 90th percentiles, respectively. The difference in gene lists is due to the number of measures taken per gene in the MLD, evidenced as a greater amount of variance per gene-treatment-time unit compared to the IRD. Cost-comparisons between the designs illustrate a significant cost-savings in performing the IRD. These results suggest the use of the IRD with a greater number of biological replicates, bringing the cost in-line with the MLD, to increase statistical power. Supported by NIH grants: *T32 ES07255 and ES011271.

1995 ASSESSING TOXICOGENOMIC DATA: ANALYSIS OF MICROARRAY QUALITY CONTROL MEASURES.

M. Ramer, L. D. Burgoon, D. R. Boverhof, B. Jeremy, C. Fong and T. Zacharewski. *Pharmacology & Toxicology, Biochemistry & Molecular Biology, National Food Safety & Toxicology Center, Institute for Environmental Toxicology, Michigan State University, East Lansing, MI.*

Adoption of the microarray technology brings with it many benefits, such as the ability to measure the expression of thousands of genes simultaneously. However, in addition to substantial costs, it also harbors significant technical difficulties. Although the technology continues to mature, quality assurance and quality control measures for assay performance and data quality are lacking. Average signal intensity, background intensity and dynamic range were compared across five independent microarray data sets to assess their predictive value in determining data quality using spread vs. level (SVL), principal components biplots, shewhart, feature not detected (FND), boxplots, and saturated features (SF) plots. The anomalies identified included 1) high global background, 2) low feature signal intensity, and 3) compressed dynamic range which skewed or abbre*via*ted normalized distributions, resulting in disrupted comparisons and confounding the results of statistical tests as well as pattern recognition algorithms (i.e., clustering). Average signal intensity, background intensity and dynamic range proved to be valuable measures to monitor assay and operator performance within and between studies. Moreover, these analyses suggest background intensity values should be sacrificed in favor of a higher PMT gain setting in order to maximize dynamic range. This work was supported by NIH grants: *T32 ES07255 and ES011271.

1996 PREDICTION OF TOXICITY FROM *LISTERIA MONOCYTOGENES* INFECTION IN MICE USING GENOMIC DATA.

H. H. Ng[1], C. E. Frantz[1], L. Rausch[1], J. Shimon[1], E. Riccio[1], S. Phillips[1], S. Smith[1], C. Litterst[2] and J. C. Mirsalis[1]. *[1]BioSciences, SRI International, Menlo Park, CA and [2]National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.*

The infection of mice with *Listeria monocytogenes* (LM) is a widely used model to study host response to intracellular bacterial pathogens and to examine potential immunotoxic effects of therapeutics. We have previously demonstrated increases in the liver enzymes AST and ALT and hepatic necrosis in mice 24 hr after infection with 5.3 x 10^4 CFU of LM, confirming that the liver is a target organ for LM infection. The purpose of this study was to characterize the short term changes in gene expression in mice after infection with LM, and to evaluate the predictive value of gene expression in liver and blood. Female BALB/c mice (14/group) were iv administered medium broth (controls) or 2 x 10^3 CFU (non-lethal dose) or 7 x 10^4 CFU (lethal dose) of LM and sacrificed 6 hr later. Gene expression was determined using Affymetrix Murine U74Av2 GeneChips. Multiple genes were found to be differentially expressed including genes associated with immune function, and liver function or toxicity, such as T-cell specific GTPase, cytochrome P450 and serum amyloid A protein. In addition, gene expression profiles in selected samples (designated as Training Set) were used in a K-nearest neighbor algorithm to establish a prediction rule to classify samples designated as unknowns (Test Set). Small sets of genes were identified that could predict control, non-lethal, and lethal doses of LM 6 hr after infection. An 8-gene set in liver samples was ≥90% accurate at predicting survival, and a 10-gene set in blood was ~80% accurate; however, different genes were predictive in each tissue. Our results suggest that class predictor genes should be able to distinguish samples in treatment groups with varying severity of infection, and serve as an early biomarker for subsequent conventional toxic endpoints. This approach will also be useful for the assessment of gene expression changes following administration of therapeutics for treatment of infection. Supported by NIAID Contract N01-AI-05417.

1997 TISSUE SPECIFIC OXIDATIVE STRESS AND GLUTATHIONE METABOLISM IN STREPTOZOTOCIN-INDUCED DIABETIC RATS.

H. Raza, A. John and L. A. Kumar. *Biochemistry, Faculty of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates.*

Increased oxidative stress is an important factor in the etiology and complications of diabetes. In this study we have investigated hyperglycemia-associated changes in cellular oxidative stress and glutathione level in different tissues of rats treated with streptozotocin (STZ). In addition, we also studied the level of oxidative stress in neonates of diabetic rats. Our results showed that ROS production and oxidative stress differentialy affect GSH metabolism in different tissues of rats and neonates of diabetic mothers. The pancreas and kidney appeared to be the target organs and were affected more severely than the liver. Based on catalytic activities, immunohistochemistry and Western blot analysis, we demonstrated an increased expression of cytochrome P450 2E1, specific gluathione S-transferase (GST) isoenzyme and other stress related proteins in diabetes. Increased membrane lipid peroxidation and accumulation of 4-hydroxynonenal (HNE), accompanied by a decreased enzymatic regeneration of glutathione appeared to be the main factors for tissue specific degeneration in diabetes. HNE induced oxidative stress and alterations in GSH metabolism were also confirmed by using cell culture model under *in vitro* conditions. These results suggest the possible role of oxidative stress and GSH metabolism in the etiology and pathology of diabetes.

1998 ASSESSMENT OF S-NITROSOTHIOLS IN BIOLOGICAL FLUIDS: CONTENT OF S-NITROSOTHIOLS IN PLASMA.

V. A. Tyurin[1], Q. Zhao[1], J. Jiang[1], G. G. Borisenko[1], R. E. Gandley[1, 4], Y. Y. Tyurina[1], H. Bair[2], A. A. Kapralov[1], A. A. Shvedova[6], A. M. Komarov[7], C. A. Hubel[4], R. N. Taylor[5], D. A. Stoyanovsky[3] and V. E. Kagan[1]. *[1]EOH, University of Pittsburgh, Pittsburgh, PA, [2]CCM, University of Pittsburgh, Pittsburgh, PA, [3]Surgery, University of Pittsburgh, Pittsburgh, PA, [4]OBGJN, University of Pittsburgh, Pittsburgh, PA, [5]OBGJN&RS, UCSF, San Francisco, CA, [6]PPRB, NIOSH, Morgantown, WV and [7]Physiology, George Washington University, Washington, DC.*

In plasma, nitrosylated thiols, mainly S-nitrosoalbumin, (Alb-S-NO), are the major reservoirs of releasable NO. Estimates of plasma concentrations of RSNO have been reported to range from 5 nM up to 15 μM. Both underestimates and overestimates in the assessment of RSNO are implicated as potential sources of high variability. We utilized four independent protocols to assess Alb-S-NO in plasma. The samples were pretreated with NEM and separated by affinity-chromatography to

obtain an albumin-enriched fraction. Decomposition of RSNO results in the stoichiometric production of NO● and a reduced thiol as the two primary relatively stable products. We employed two assays to detect released NO● (an EPR-based trapping using an NO● chelator, MGD2-Fe, as well as a fluorogenic scavenger of NO●, DAF2) as well as two protocols to evaluate Alb-SH (biotin-switch assay using NitroGlo™ kit, and ThioGlo-1™-based assay with a maleimide fluorogenic SH-reagent, and subsequent fluorescence assay of Alb-SH after reduction of Alb-S-NO by high concentrations of ascorbate). Procedures utilizing sulfanilamide gave false negative results due to decomposition of RSNO by the reagent. This may explain very low concentrations of RSNO reported by workers who utilized sulfanilamide treatments. All the protocols used give reasonably concordant results: low micromolar concentrations (20-100 pmol/mg protein). These levels are physiologically justified as they provides for its physiologically-relevant vasodilatory functions as assessed in our experiments with isolated mouse mesenteric arteries. Supported by NIH HL64145.

1999 4-HYDROXYNONENAL AND MALONDIALDEHYDE SUPRESS CONSTITUTIVE ACTIVITY OF NFKB IN PRIMARY RAT HEPATOCYTE CULTURES BY MODULATING MAPKS UP-STREAM OF IKK.

B. P. Sampey, M. S. Taylor and D. R. Petersen. *Pharmaceutical Sciences, University of Colorado HSC, Denver, CO.*

Lipid peroxidation (LPO) and the accumulation of proteins covalently modified by the resulting lipid-aldehydes are early events that persists through the initiation and progression of alcoholic liver disease (ALD). Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are products of LPO involved in the pathogenesis of alcohol-induced liver injury, likely through their ability to form protein-adducts. 4-HNE has been implicated in the modulation of NFκB activity *in vitro* and in various cells of differing pathologic states. However, a complete understanding of the cell-specific effects 4-HNE and MDA impose on this pathway remains to be defined. The purpose of this study was to use control, primary rat hepatocytes to identify the kinases associated with constitutive NFκB activity that are affected by the lipid-aldehydes 4-hydroxynonenal and malondialdehyde. Primary hepatocytes were isolated from naive, shelf-fed male Sprague-Dawley rats using a collagenase-perfusion method. Isolated cells were seeded onto 100mm tissue culture plates coated with extracellular matrix (ECM) at a density of 3.5X10E6 in 5ml of RPMI-1640 with serum and Penicillin/Streptomycin. After a 12 hour incubation under normal culture conditions, the media was removed along with non-adherent cells and replaced with serum-free media containing 0-100μM 4-HNE or 0-100μM MDA. After a 4 hour treatment period, the cells were washed with PBS (3X), the ECM removed, and total cellular protein isolated from cells of each treatment group for SDS-PAGE gel seperation. Utilizinging antibodies raised toward the activated kinases of the NFκB pathway, immunoblot analysis of the gel-separated lysates demonstrates, when compared to control, both 4-HNE and MDA decrease the activated forms of SAPK & JNK over the physiologically relevant doses used here, and that these reductions correlate well with decreases observed in the activated form of p65/NFκB. (supported by NIH/AA09300: D R P)

2000 INAPPROPRIATE CELL CYCLE CHECK POINT CONTROL DURING ROS- INDUCED ONCOTIC CELL DEATH.

S. Ramachandiran, S. S. Lau and T. J. Monks. *Pharmacology/Toxicology, University of Arizona Health Sciences Center, Tucson, AZ.*

2, 3, 5-*tris*-(Glutathion-S-yl)hydroquinone (TGHQ) is a potent ROS generating metabolite of the nephrotoxicant, hydroquinone. TGHQ catalyzes the formation of single strand breaks in DNA, followed by growth arrest and oncotic/necrotic death in renal proximal tubule epithelial cells (LLC-PK1). Cyclin dependent kinase1 (p34 cdc2) is the key protein kinase regulating mitotic entry in eukaryotic cells. Treatment of LLC-PK1 cells with TGHQ (200 μM) causes an initial (0- 2h) phosphorylation and activation of the DNA damage inducible protein kinases Chk1 and Chk 2, followed by increases in inhibitory tyrosine15 phosphorylation of p34 cdc2, indicative of cell cycle arrest. However subsequent (> 2h) cell death is associated with a clear decrease in Chk1 and Chk2 phosphorylation, followed by decreases in cdc2 Y15 phosphorylation, indicating inappropriate activation of cdc2 during the growth arrest and DNA damage repair response. TGHQ induced ERK and p38 MAPK also contribute to LLC-PK1 cell death, probably *via* histone H3 phosphorylation, which occurs concurrently with premature chromatin condensation. The activation of cdc2 in growth arrested LLC-PK1 cells suggests that premature mitosis might induce mitotic catastrophe, which may be the mechanism of cell death in this model. A similar phenomenon also occurs during H2O2 induced oncotic/necrotic death of LLC-PK1 cells. In contrast, cisplatin, an alkylating agent that induces apoptotic LLC-PK1 cell death, does not activate cdc2. Indeed, cisplatin treatment results in prolonged inhibitory Y15 phosphorylation of cdc2, even after 12h of treatment. The data suggests that inappropriate activation of cdc2 may contribute to oncotic /necrotic death of otherwise growth arrested LLC-PK1 cells. (Supported by NIH grant DK 59491)

2001 USING CRYOPRESERVED NEURONAL CELLS FOR ASSESSMENT OF CHEMICALLY INDUCED OXIDATIVE CELL STRESS IN PRIMARY CELL CULTURE.

A. Krantis[1, 2], W. Staines[1, 2], S. VandenHoek[2, 1] and T. Durst[1, 2]. [1]*Cellular& Molecular Medicine, University Ottawa, Ottawa,, ON, Canada and* [2]*CRBB, University Ottawa, Ottawa, ON, Canada.* Sponsor: M. Aschner.

Cryopreserved primary neuronal cells from the rat brain represent an ideal and sensitive cell culture assay for screening drug neuroactivity. When thawed and grown in cell culture, these dissociated neuronal cells display normal morphology, neurochemistry and physiological responsiveness within 10 days. We have obtained similar results with neuronal cells dissociated from the mouse brain. However, the susceptibility of cryopreserved rodent brain neuronal cells to neurotoxic stress in primary culture is unknown. Such information would be useful in determining their suitability for employment in assay screens for chemically induced neurotoxicity. On this basis, we examined cryopreserved neuronal cells prepared from mouse and rat cortex for the effects of exposure to a classic oxidative stress, hydrogen peroxide (H2O2), and to a series of novel compounds for their putative oxidative vs protective properties. Neuronal cells dissociated from brain cortex and cryopreserved by QBM Cell Science, were thawed and cultured in 96 well plates (100, 000 cells/well) for 10days. At this stage they displayed typical neuronal neurite networks and morphology. Wells were then treated for 24hrs with H2O2 (50-100μM), or with novel compounds over a range of concentrations (0.1-50μg/ml). The wells were then assayed using the OxyDNA (Biotrin) fluorescence-based assay for detection of DNA damage. Typical cell damage and disruption of neurite networks were evident with H2O2 50μM as monitored by phase contrast microscopy. A concentration dependent increase in fluorescence associated with 8-oxoguanine fragments, typical of DNA damage due to free radical action was also evident. The novel compounds could be classified as either protective or deleterious. These results show the sensitivity of cryopreserved rodent brain neuronal cells for plate based cell culture assay of oxidation induced neurotoxicity.

2002 OXIDATIVE INACTIVATION OF THE THIOREDOXIN SYSTEM.

W. H. Watson[1] and D. P. Jones[2]. [1]*Environmental Health Sciences, Johns Hopkins School of Public Health, Baltimore, MD and* [2]*Medicine, Emory University School of Medicine, Atlanta, GA.*

The thioredoxin system, composed of thioredoxin 1 (Trx1), thioredoxin reductase, NADPH, and the peroxiredoxins, protects against the toxicity of peroxides and other oxidants by supporting both detoxification and repair mechanisms. The active site of Trx1 becomes oxidized to a disulfide as part of its catalytic mechanism and must be restored to the dithiol form by thioredoxin reductase and NADPH. We recently described the formation of a non-active site disulfide (Cys62-Cys69) in Trx1 under oxidizing conditions within the cell. In the present study, the effect of the non-active site disulfide of Trx1 on the reduction of the active site was examined in an *in vitro* assay system. Fully oxidized Trx1 (containing both disulfides) was initially reduced very slowly by thioredoxin reductase. After this initial lag phase, Trx1 was rapidly reduced to the two dithiol form. The early lag phase was dependent on the Cys62-Cys69 disulfide because a mutant of Trx1 bearing Ser at positions 62 and 69 exhibited no lag phase. An active site mutant of Trx1, bearing only the Cys62-Cys69 disulfide, was not a substrate for thioredoxin reductase. However, addition of a catalytic amount of wild type Trx1 resulted in the complete reduction of this non-active site disulfide. These findings suggest a mechanism by which Trx1 can be temporarily inactivated under oxidizing conditions, facilitating oxidant-mediated signal transduction and the induction of oxidative stress response machinery.

2003 THE ROLE OF CYSTEINE REGULATION IN ADIPOGENESIS.

M. Cimafranca[2], P. R. Hanlon[2] and C. R. Jefcoate[1, 2]. [1]*Pharmacology, University of Wisconsin, Madison, WI and* [2]*Molecular & Environmental Toxicology Center, University of Wisconsin, Madison, WI.*

2, 3, 7, 8 - tetrachlorodibenzo-p-dioxin (TCDD) inhibits adipogenesis in the mouse embryo fibroblast cell line C3H10T1/2 (10T1/2). cDNA microarray analysis of 10T1/2 after 24 hours of exposure to the adipocyte differentiation stimulus was performed in the presence and absence of TCDD. An 8-fold increase in both cysteine dioxygenase (CDO) and γ-glutamyl cysteine ligase (GCL), together with their partial suppression by TCDD under adipogenic conditions, suggest that cys-

teine is an important factor for early regulation in adipogenesis. In the presence of TCDD, our preliminary data shows the addition of N-acetylcysteine (NAC) or the GCL inhibitor buthionine sulfoximine (BSO) substantially inhibits adipogenesis in a dose-dependent manner; these agents each potentially increase intracellular levels of cysteine but have opposing effects on glutathione. This report further addresses the effects of these agents on PPARγ1 expression, a key mediator of adipogenesis that is suppressed by TCDD. A more detailed analysis of the time course of CDO and GCL expression in relation to PPARγ1 will be presented along with the effects of manipulating intracellular cysteine levels *via* NAC, BSO, and DL-propargylglycine (PPG), an inhibitor of cystathionine γ-lyase which blocks endogenous production of cysteine. We will also examine whether a putative AhR response element found in the CDO flanking region is indeed responsive to TCDD.

2004 FREE RADICAL DETERMINANTS OF AMPHETAMINE NEURODEGENERATION: PROSTAGLANDIN H SYNTHASE (PHS)-CATALYZED FREE RADICAL FORMATION AND REACTIVE OXYGEN SPECIES (ROS)-MEDIATED OXIDATIVE DNA DAMAGE IN NEURONAL DEGENERATION AND FUNCTIONAL DEFICITS.

W. Jeng[1] and P. G. Wells[1, 2]. *[1]Pharmacy, University of Toronto, Toronto, ON, Canada and [2]Pharmacology, University of Toronto, Toronto, ON, Canada.*

When incubated *in vitro* with purified PHS-1, the amphetamines 3, 4-methylenedioxymethamphetamine (MDMA, Ecstasy), 3, 4-methylenedioxyamphetamine (MDA) and methamphetamine (METH) were bioactivated to free radical intermediates, characterized by electron paramagnetic resonance spectrometry, which initiated the formation of ROS that caused oxidative DNA damage. These PHS-dependent processes were time- and amphetamine concentration-dependent, stereoselective, and blocked by the PHS inhibitor eicosatetraynoic acid. Similarly *in vivo*, these amphetamines stereoselectively caused oxidative DNA damage, dopaminergic neurodegeneration and permanent motor coordination deficits, which all were blocked by a single pretreatment with the PHS inhibitor aspirin (with MDA), or reduced in a gene dose-dependent manner in PHS-1 knockout mice (with MDMA). Regional amphetamine-enhanced DNA oxidation correlated positively with the level of PHS-1, which varied significantly among brain regions. These results implicate PHS-catalyzed bioactivation and ROS-mediated oxidative damage in amphetamine-initiated neurodegeneration, providing a novel hypothesis potentially relevant to the mechanisms and risk factors for xenobiotic-enhanced neurodegeneration. (Support: Canadian Institutes of Health Research; SOT Covance Fellowship)

2005 INCREASED MITOCHONDRIAL THIOREDOXIN INHIBITS OXIDANT-INDUCED APOPTOSIS BY A GSH-INDEPENDENT MECHANISM IN SH-SY5Y NEUROBLASTOMA CELLS.

J. Cai, Y. Chen and D. P. Jones. *Department of Medicine, Emory University, Atlanta, GA.*

Aging and age-related degenerative diseases often involve increased production of reactive oxygen species (ROS) and oxidative damage in neuronal tissues. A major proportion of ROS are produced from mitochondria which contain multiple defensive mechanisms to protect against oxidative injury. Mitochondrial thioredoxin (mtTrx; Trx2) is a newly identified thioredoxin family protein that is localized in mitochondria and functions as an antioxidant protein. In the present study, we have measured whether an overexpression of mtTrx in SH-SY5Y human neuroblastoma cells could protect against apoptosis induced by t-butylhydroperoxide (tBH). Results showed that treatment with 50 microM tBH caused over 75% of SH-SY5Y cells to undergo apoptosis that was inhibited by overexpressing mtTrx. The level of protection correlated with the level of mtTrx overexpression. MtTrx protected loss of mitochondrial membrane potential induced by tBH but not by calcium ionophore (Br-A23187) treatment. Although no significant changes were observed in total cellular GSH, mitochondrial glutathione pool was oxidized by tBH and that was not inhibited by increased mtTrx. Mutations of Cys90 and Cys93 to serine rendered mtTrx ineffective in protection against tBH toxicity. These data indicate that mtTrx controls mitochondrial redox status independently of GSH and is a major component of the protective mechanism against oxidative injury in neuronal cells. (Supported by NIH grant ES09047 and a pilot grant from Emory Parkinsons Disease Center).

2006 FETAL HEMATOPOIETIC STEM CELLS ARE SENSITIVE TARGETS OF 4-HYDROXYNONENAL.

C. G. Moneypenny, C. M. Huisden and E. P. Gallagher. *Department of Physiological Sciences, University of Florida, Gainesville, FL.*

During fetal development, the liver serves as the primary hematopoietic organ in which hematopoietic stem cells (HSC) comprise a large component of hepatic cell populations. Because, HSC are pluripotent and are capable of initiating long-term hematopoiesis, injury to these cells may have toxicological ramifications with regards to the etiology of blood-borne diseases. In the current study, we examined the effects of 4-hydroxynonenal (4-HNE), a mutagenic α, β-unsaturated aldehyde that can be produced *in utero*, on the extent of HSC proliferation, differentiation, *via*bility and 4-HNE-protein adduct formation. Exposure of HSC to 5 μM 4-HNE resulted in a decrease in the rate of HSC proliferation and a loss of *via*bility. These effects were not observed at 1 μM 4-HNE. HSC exposed to either 5 μM or 50 μM 4-HNE resulted in the formation of multiple 4-HNE-protein adducts that were resolved by two-dimensional gel electrophoresis. MALDI-TOF and HPLC-mass spectral analysis revealed that several cell structure proteins and their precursors, as well as heat shock and tumor rejection antigen proteins were targets of 4-HNE. Collectively our data indicate that cellular levels of 4-HNE consistent with oxidative injury cause a loss of proliferation and *via*bility in human fetal HSC. Furthermore, these data are supportive of 4-HNE-protein adduct formation as a mechanism of 4-HNE toxicity in HSC. Supported by NIH ES09427 and USEPA STAR R827441.

2007 MOUSE GLUTAMATE-CYSTEINE LIGASE CATALYTIC AND MODIFIER SUBUNITS COMPLEX IN-VITRO, TO FORM HOLOENZYME EXHIBITING OPTIMIZED CATALYTIC EFFICIENCY.

Y. Chen, S. N. Schneider, H. G. Shertzer, D. W. Nebert and T. P. Dalton. *Environmental Health and Center for Environmental Genetics, University of Cincinnati, Cincinnati, OH.*

Glutamate-cysteine ligase (GCL) is the rate-limiting enzyme in glutathione (GSH) biosynthesis. The holoenzyme is a heterodimer formed by the catalytic subunit (GCLC) and the modifier subunit (GCLM). GCLC alone can catalyze the formation of L-γ -glutamyl-L-cysteine (γ-GC); however, GCLM can increase its catalytic efficiency by lowering the Km for glutamate and decreasing sensitivity to GSH inhibition in experiments with rat and human purified proteins. To determine the subunit interaction and catalytic characteristics of mouse GCL, we expressed and purified mouse GCLC and GCLM. Interaction of GCLC with GCLM to form holoenzyme could be monitored because GCLC, GCLM and GCL have distinct mobilities in nondenaturing PAGE. Western immunoblot analysis demonstrated that all detectable GCLC and GCLM were interaction-competent. The apparent Km of glutamate for GCLC was 2.6-fold higher than that for GCL (1.8 mM vs 0.51 mM), and GCLC was more sensitive to inhibition by GSH. Titration of GCLC with GCLM gave a linear increase in GCL activity, and GCL was ~4-fold more active than mGCLC alone (185 vs 800 nmol/min/mg protein). A similar increase in GCL activity was observed when complementing tissue cytosol from *Gclm(-/-)* mice with recombinant GCLM. Thus, *in vivo* GCLC stands poised to accept GCLM. Interestingly, addition of GCLM to wild-type tissue cytosol resulted in increased GCL activity and the relative increase was reproducibly dependent on the tissue type. Thus, in many tissue cytosols, GCLC is more abundant than GCLM, and perhaps an increase in GCLM alone in the context of many cell types would lead to an increase in γ-GC production. ——**Supported, in part, by NIH grants P30 ES06096 and R01 ES10416.**

2008 GENERATION OF REACTIVE OXYGEN BY HALOGENATED AROMATIC HYDROCARBONS IN MOUSE LIVER MICROSOMES.

T. P. Dalton, M. Genter, C. D. Clay, M. C. Chames, S. N. Schneider, G. G. Oakley, D. W. Nebert and H. G. Shertzer. *Department of Environmental Health and Center for Environmental Genetics, University of Cincinnati Medical Center, Cincinnati, OH.*

Using liver microsomes from 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD)-induced or vehicle-treated (non-induced) mice, we evaluated the *in vitro* effects of added chemicals on the production of reactive oxygen due to substrate/P450-mediated uncoupling. Catalase-inhibited NADPH-dependent H2O2 production was lower in induced than non-induced microsomes. Various chemicals, added to microsomes at 2.5 μM, were evaluated for their effects on H2O2 production. There were three groups of compounds that elicited distinct effects. Group 1 were highly-halogenated and coplanar compounds that increased H2O2 production, as well as the H2O2/O2 and H2O2/NADPH ratios in induced, but not in non-induced, microsomes. Group 2 were non-coplanar halogenated biphenyls that did not affect H2O2 production nor H2O2/O2 or H2O2/NADPH ratios. Group 3 were minimally-halogenated biphenyls and benzo[a]pyrene. Group 3 compounds decreased H2O2 production, and also H2O2/O2 and H2O2/NADPH ratios. Microsomal lipid peroxidation was proportional to H2O2 production. Although TCDD induction decreased microsomal production of H2O2, addition of Group 1 compounds to TCDD-induced microsomes *in vitro* stimulated the second-electron reduction of CYP1A1 and CYP2E1 and subsequent release of H2O2 production. This pathway is likely to contribute to the oxidative stress response, and associated toxicity, produced by many of these biologically-persistent environmental chemicals. (Supported by grants R01 ES10133, R01 ES06321, R01 ES08147, R01 ES08799, T32 ES07250, and P30 ES06096)

2009 GLUTATHIONE DEFICIENCY IN PANCREATIC BETA CELLS PREDISPOSES MALE MICE TO THE DEVELOPMENT OF DIABETES.

S. N. Schneider, Y. Chen, Y. Yang, H. G. Shertzer, D. W. Nebert and T. P. Dalton. *Environmental Health and Center for Environmental Genetics, University of Cincinnati, Cincinnati, OH.*

Diabetes is a complex disease caused by the combination of genetic and environmental factors. Many studies implicate oxidative stress as an important factor in the development of diabetes; the results of such studies suggest that the insulin-producing pancreatic beta cells are sensitive to oxidative stress because they possess relatively low levels of antioxidant enzymes. To develop an animal model in which beta cells have increased susceptibility to oxidative stress, we have made a glutamate-cysteine ligase catalytic subunit (*Gclc*) conditional knockout mouse line in which *Gclc* gene disruption occurs specifically only in beta cells. GCLC is essential for production of the antioxidant glutathione (GSH). Beta cell-specific knockout of *Gclc* occurs when a floxed allele [*Gclc(f)*] allele is present in a mouse that carries the rat insulin2 gene promoter (RIP)-Cre transgene. *Gclc(f)* behaves like the wild-type *Gclc* allele but, in the presence of RIP-Cre, beta cell *Gclc(f)* becomes the *Gclc(β)* null allele. We monitored the development of diabetes in a colony of male and female *Gclc(β/β)* and littermate *Gclc(f/f)* controls by measuring fasting blood glucose (FBG) at 5, 20, 40 and 60 weeks of age. Male and female *Gclc(f/f)* mice and female *Gclc(β/β)* had normal fasting blood glucose levels throughout the course of the study. In contrast, male *Gclc(β/β)* mice displayed a trend for elevated FBG as early as 5 weeks of age; FBG in this group by 20 weeks was significantly elevated to diabetic levels (greater than 126 mg/dL), compared with all other groups and continued to rise through 60 weeks. We conclude that male mice, lacking the capacity to synthesize GSH in pancreatic beta-cells, develop diabetes as characterized by significantly increased blood glucose levels associated with age. These mice will be useful in understanding the processes underlying oxidant stress-induced diabetes and in developing therapies for its prevention. ———***Supported, in part, by NIH grants P30 ES06096 and R01 ES012463.***

2010 BRAIN UPTAKE, METABOLITE DISTRIBUTION AND METABOLIC SHIFT FOLLOWING [^{14}C]-1, 3-DINITROBENZENE ADMINISTRAION IN THE SPRAGUE-DAWLEY RAT.

J. Tobias, R. T. Miller and P. Venkatakrishnan. *Toxicology, University of Kentucky, Lexington, KY.*

1, 3-Dinitrobenzene (1, 3-DNB) is a CNS toxicant. Studies regarding 1, 3-DNB toxicity in multiple systems have been done and while some data is available regarding 1, 3-DNB toxicity in the brain, uptake studies and possible shifts in metabolism following multiple doses have not yet been conducted. This study attempts to address these questions. Male Sprague-Dawley rats were dosed ip with 20 mg/kg of [^{14}C]-1, 3-DNB in corn oil every 12 hr x 3. Following the last dose, brain levels of 1, 3-DNB were measured as a function of time. Results showed 1, 3-DNB was rapidly taken up by brain with levels decreasing steadily over time. In separate experiments, rats were dosed ip with 20 mg/kg of [^{14}C]-1, 3-DNB in c. o. either singly or every 12 h x 3. At 45 and 90 min thereafter, brains were removed and dissected into forebrain, brainstem and cerebellar regions. In both groups, the major metabolites were 1, 3-DNB and 3-nitroaniline (3-NA). We identified a reactive intermediate, N-nitroso-3-nitrobenzene (NNB) in cerebellum and brainstem in each group. Major cerebellar metabolites at 45 min were 1, 3-DNB and 3-NA (58 and 24% of total radioactivity after single dose and 52 and 39% after 3 doses.) NNB accounted for 18% after a single dose and 9% after 3 doses. At 90 min, 1, 3-DNB and 3-NA represented 38 and 33% after a single dose, 56 and 31% after 3 doses. NNB accounted for 28% after a single dose and 13% after 3 doses at 90 min. In brainstem at 45 min, 1, 3-DNB and 3-NA accounted for 45% each after a single dose and 39 and 52% after 3 doses. NNB represented 10% after a single dose and 9% after 3 doses. At 90 min both 3-NA and NNB accounted for 27% after a single dose and 1, 3-DNB for 44%. In multiple dose animals, NNB was 71% and 1, 3-DNB and 3-NA 24 and 5%. Data are expressed as percent of total radioactivity in the brain at 45 or 90 minutes after single or third dose. Collectively, these data demonstrate both that 1, 3-DNB is taken up by the brain and that there are differences in its metabolic profile between regions in the brain.

2011 PROTECTIVE ROLE OF URIC ACID AGAINST NITRIC OXIDE-MEDIATED OXIDATIVE INJURY.

R. M. Uppu and B. Kandlakunta. *Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA.* Sponsor: J. Ward.

Uric acid (UA) was viewed as a toxic metabolite till recently. Excessive production of UA can induce gout, a disease that affects joints and the kidneys. A landmark hypothesis by Ames that UA constitutes an important defense mechanism against oxidative damage, which occurs in aging and other degenerative diseases, has contributed to a change in the perception of UA as a toxic metabolite. Uric acid exists as urate monoanion at physiological pH, and demonstrates a potent antioxidant activity as seen in several *in vitro* assays. It exhibits strong therapeutic effects in animals suffering from experimental allergic encephalitis in which peroxynitrite (PN) is suspected to be the causative agent. The PN-mediated oxidation of UA was examined in the present work as PN is a potent oxidant formed during the down regulation of nitric oxide. Our results show that PN reacts with urate both directly and through a carbon dioxide-dependent pathway, producing identical product profiles. Interestingly, allantoin, which is often considered to be the major product, is formed in yields less than 25% of the overall oxidation of UA. The product profile in the PN/carbon dioxide/UA resembles that of the UA/hydrogen peroxide/peroxidase reaction, which proceeds *via* the formation of the urate radical. In view of this, we hypothesize that PN/carbon dioxide oxidizes urate to a metastable nitroderivative; this reaction occurs *via* a free radical mechanism involving nitrogen dioxide and the carbonate radical. The nitroderivative thus formed loses nitrite to become dehydrourate, which, in turn, hydrolyzes to allantoin, parabanic acid, and other products. The current efforts are directed towards examining pathways for the decay of dehydrourate (hydrolysis-driven) that can result in electrophilic intermediates with potential for binding DNA or cause depletion of cellular antioxidants. (Funding support from NIEHS grant ES10018 is acknowledged. Corresponding author: Rao M. Uppu. E-mail: rao_uppu@cxs.subr.edu. Fax: 25/771-5350.)

2012 SYNTHESIS OF PEROXYNITRITE USING ISOAMYL NITRITE AND HYDROGEN PEROXIDE IN A HOMOGENOUS SOLVENT SYSTEM.

R. R. Kancharla, B. Kandlakunta and R. M. Uppu. *Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA.* Sponsor: J. Ward.

The peroxy isomer of nitrate, peroxynitrite is a versatile oxidant that can be formed in biological systems from the reaction of nitric oxide with superoxide and oxygenated heme proteins, in particular, oxyhemoglobin. Several different methods of synthesis of peroxynitrite have been reported (for a review, see R.M. Uppu *et al.* (1996) *Methods Enzymol.* 269, 285-295). These include synthesis in a two phase system wherein alkaline-H_2O_2 in water (aqueous phase) was allowed to react with a water-insoluble alkyl nitrite, isoamyl nitrite (organic phase) (R.M. Uppu and W.A. Pryor (1996) *Anal. Biochem.* 236, 242-249). In the present studies, we modified the method of Uppu and Pryor by way of introducing solvents of intermediate polarity that abolish phase separation of the reactant pair hydroperoxide anion and isoamyl nitrite. This simple modification allows a rapid and efficient synthesis of peroxynitrite with much less nitrite, nitrate, and carbonate contamination. The modification does not necessitate any additional post-processing other than the one reported earlier for reactions performed in the two-phase system. In view of shortening of reaction times, the modification eliminates the need for metal ion chelators such as diethylenetriaminepentaacetic acid. The present method is amenable for large scale synthesis and, therefore, promotes industrial applications of peroxynitrite as an oxidizing agent. For example, we find that peroxynitrite and its secondary reaction products with CO_2 afford oxidation of indigo and indigo carmine, the two major organic pollutants in waste waters from textile industries. Studies are underway to examine other industrial applications of peroxynitrite; including its use as bleach additive (US Pat. 5, 914, 305). (Funding support from NIEHS grant ES10018 is acknowledged. Corresponding author: Rao M. Uppu. E-mail: rao_uppu@cxs.subr.edu. Fax: 225/771-5350.)

2013 PROTECTIVE EFFECTS OF ENHANCED GLUTATHIONE SYNTHESIS ON TNFA-INDUCED HEPATOTOXICITY IN GLUTAMATE-CYSTEINE LIGASE TRANSGENIC MICE.

S. Shi, D. Botta, C. C. White, P. A. Vliet, S. Chatterton-Kirchmeier and T. J. Kavanagh. *Environmental & Occupational Health Sciences, University of Washington, Seattle, WA.*

Oxidative stress plays a critical role in tumor necrosis factor-a (TNFa) cytotoxicity, which involves both necrotic and apoptotic processes. Glutathione (GSH) is a major free radical scavenger and an important factor in detoxification of reactive oxygen species and xenobiotics. The rate-limiting enzyme in the synthesis of GSH is glutamate-cysteine ligase (GCL) which consists of two subunits: GCLC and GCLM. We have generated transgenic mice that conditionally express GCLC and GCLM in the liver using a mifepristone (RU486)-responsive transactivator. To test the hypothesis that enhanced GSH synthesis would prevent TNFa-induced hepatotoxicity, GCL transgenic mice and their non-transgenic littermate controls were treated with TNFa after sensitization by actinomycin D (ActD). GCLM protein levels and GCL activities were significantly increased in GCL transgenic mice with RU486 induction as revealed by Western Blotting and biochemical assay. TNFa /ActD treatment caused liver injury as indicated by serum alanine aminotransferase (ALT) activity. However, ALT levels in GCL transgenic mice induced with RU486

were lower than those in transgenic mice not induced and also lower than control mice receiving TNFa /ActD. Phospho-Erk levels were greatly increased 15 minutes after treatment with TNFa, but this response was attenuated in GCL transgenic mice induced with RU486. These results support the role of oxidative stress in TNFa induced liver injury, and the protective role of GSH synthesis in this process. Supported by NIH grants 1P42ES04696, P30ES07033 and 1R01ES10849-02.

2014 HYPEROXIA-INDUCED MAP KINASE ACTIVATION IN LUNG CELLS.

M. Wu[1, 2], L. Volk[2, 1] and W. J. Martin[2, 1]. [1]*Biochemistry & Mol Biol, University of North Dakota, Grand Forks, ND and* [2]*College of Medicine, University of Cincinnati, Cincinnati, OH.* Sponsor: D. Sens.

Oxygen toxicity is a well established model for studying adult respiratory distress syndrome. Mitogen-activated protein (MAP) kinases are a group of enzymes that have been well identified to be activated by exposure to various stresses. However, the role of hyperoxia in regulating MAP kinase in lung cells is not clear. We have studied the signaling transduction of MAPK in lung epithelial cell line A549 under hyperoxia. The main method was the MAP kinase activity assay, using Western blotting with specific antibodies against p38 and p44/42 following immunoprecipitation of phosphorylated p38 and p44/42 molecules. The results demonstrate that phosphorylation of p44/42 in A549 cells was increased by exposure to 95% O2 compared with the control (in room air). It has also been shown that p38 activity in A549 cells was enhanced by exposure to 95% O2 compared with the control. Furthermore, we have transduced A549 cells with human enzyme 8-oxoguanine DNA glycosylase (hOgg1) using retroviral vector pSF91 and tested the regulatory function of hOgg1 on MAP kinase. Our results demonstrate that over-expression of hOgg1 did not alter the p38 activation in the presence of 95% O2 but decreased the expression of p44/42. This is consistent with that MAP kinases are related to the cell cycle arrest. When oxygen damages DNA, cells are halted at G1 or S phase. With the repair of damaged DNA, cells may resume their cycle and progress into M phase. Our results indicate that base excision repair proteins may be useful for repairing oxygen damage in lung cells.

2015 DEP-INDUCED *FRA-1* EXPRESSION CORRELATES WITH A DISTINCT ACTIVATION OF AP1-DEPENDENT GENE TRANSCRIPTION IN ALVEOLAR EPITHELIAL CELLS.

Q. Zhang[1], S. R. Kleeberger[2] and S. P. Reddy[1]. [1]*Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD and* [2]*Laboratory of Pulmonary Pathobiology, NIEHS, Research Triangle Park, NC.*

Recent studies indicate a potential role for Fra-1, a heterodimeric partner of AP1, in toxicant-induced epithelial injury, repair, and cellular transformation. Here, we have investigated the effects of diesel exhaust particles (DEP) on *fra-1* expression in C10 cells, a nonmalignant murine alveolar type II-like epithelial cell line. DEP caused a marked up-regulation of *fra-1*, but not *fra-2*, expression. The increase in *fra-1* mRNA expression correlated well with its protein and DNA binding activity. DNA binding assays with antibodies specific to each of the other AP1 family members revealed a predominant presence of Jun-B and Jun-D in the AP1 complex. Interestingly, DEP did not alter Jun-B and Jun-D protein levels. Transcriptional analysis revealed that *fra-1* induction is regulated in part at the transcriptional level. The -379 to +32 bp 5′-flanking region mediated this induction. Furthermore, inhibitors of ERK1/2, JNK1 and p38 mitogen-activated protein kinases (MAPKs) significantly suppressed DEP-stimulated *fra-1* promoter activity and expression, suggesting their involvement in the induction process. Consistent with this finding, DEP stimulated phosphorylation of ERK1/2, JNK1 and p38 MAPKs with a distinct activation pattern. Overexpression of Fra-1 down-regulated both basal and c-Jun-enhanced AP1 transcriptional activity. It had a similar effect on basal and Nrf2-enhanced ARE-mediated reporter gene expression. In contrast, Fra-1 had the opposite effect on MMP-9 promoter activity. In particular, it bound to the functional TRE sequence of the MMP-9 promoter after DEP stimulation. Consistent with this result, DEP also markedly up-regulated MMP-9 promoter activity. Collectively, these findings suggest that *fra-1* induction by DEP may play a role in selectively regulating gene expression involved in alveolar epithelial cell injury and repair. Funded by: ES09606, ES11863, and EPA-R82672401

2016 HUMAN MITOCHONDRIAL THIOREDOXIN (MTTRX) IS MORE SENSITIVE TO PEROXIDE-DEPENDENT OXIDATIVE STRESS THAN CYTOPLASMIC THIOREDOXIN (TRX1).

Y. Chen, J. Cai and D. P. Jones. *Department of Medicine, Emory University, Atlanta, GA.*

Thioredoxins (Trx) are a family of small proteins containing a conserved -Cys-X-X-Cys- motif at their redox active center. In the presence of thioredoxin reductase and NADPH, Trx undergoes reversible oxidation/reduction in the active center. Trx has been implicated in the regulation of fundamental cellular functions such as proliferation, transcriptional regulation and apoptosis. The majority of these biological functions, if not all, rely on the reversible oxidation/reduction in the active center. A mitochondria-localized Trx family protein, mitochondrial Trx (mtTrx, also known as Trx2), has been described in different species including human. MtTrx has been shown essential to cell survival and has been linked to regulation in apoptosis. Trx system is a crucial antioxidant system which not only scavenges reactive oxygen species (ROS) but also functions to prevent protein damage, and furthermore, repair protein damage if happened. The existence of two complete and separate Trx system in different compartments, the cytosol and mitochondria, raises an interesting question, i.e, whether different Trx systems have different responses to oxidant insult. In this study, we used a Redox Western technique which detects endogenous redox status of proteins of interest following thiol alkylation and SDS-PAGE. We investigated the redox status change of both Trx1 and mtTrx in response to three different chemical oxidant treatments. Results showed that mtTrx was more sensitive than Trx 1 to t-butylhydroperoxide (tBH) and hydrogen peroxide-induced oxidation, while the responses to diamide treatment were similar. In cells treated with 300 μM of tBH, recovery of the reduced form or mtTrx was slower than Trx 1. At 60 min, when the majority of Trx 1 returned to reduced state, nearly 60% of mtTrx was still oxidized. These data shows that the redox change of mtTrx is different from that of the cytosolic Trx1 in response to oxidants. MtTrx may be a sensitive biomarker for measurement of mitochondrial oxidative stress. (Supported by NIH grant ES09047).

2017 INJURY DYNAMICS FOLLOWING SUBLETHAL BLAST OVERPRESSURE EXPOSURES.

N. M. Elsayed[1, 2], N. V. Gorbunov[3], S. J. McFaul[3] and J. L. Atkins[3]. [1]*Hurley Consulting Associates, Chatham, NJ,* [2]*SUNY Downstate Medical Center, Brooklyn, NY and* [3]*Walter Reed Army Institute of Research, Silver Spring, MD.*

Detonation of explosives produces blast overpressure (BOP) waves with magnitudes proportional to the exploding charges. Primary blast injury (PBI) from direct exposure to BOP includes primarily hearing loss and pulmonary contusion. Both military personnel during combat or training and civilians exposed to terrorist bombings sustain PBI. While physical protection has a greater military priority, treatment is more critical for injured civilians. We have shown previously that exposure to BOP waves induce lung injury characterized by rupture of capillaries and alveolar walls leading to hemorrhage, transient hypoxia, inflammation and oxidative stress. In a previous study, we found that rats exposed to multiple low-level BOP (~60 kPa) sustained lung injury after the first exposure, and that the effect of subsequent exposures was not additive. A progressive increase of red blood cells infiltration into the alveoli was observed microscopically, but no gross hemorrhage was visible. However, iron-transferrin [Fe3+]TRF complexes did not increase in lung tissue after BOP exposures. In the present study, we exposed rats to a moderate, sublethal level of BOP (~120 kPa) which produced greater lung contusion and assessed TRF and polymorphonuclear leuckocytes (PMNs) in lung and blood after 1, and 3 h. At 1 h, steady state TRF concentration remained unchanged. At 3 h, TRF was markedly decreased (38%, $P<0.05$). In contrast, PMNs increased in blood during the same period: 2.7-fold at 1 h and 5-fold at 3 h. Increased PMN count could reflect disruption of PMN adhesion to the vascular endothelium leading to their trans-endothelial migration. Resident macrophages in lung tissue were also increased suggesting activation of phagoctytosis. These observations combined with other findings suggest that a dynamic and complex process continues to develop after initial PBI and stresses the importance of initiating medical triage within the first hour, even in the absence of visible signs of injury.

2018 GENERATION AND CHARACTERIZATION OF A GLUTAMATE-CYSTEINE LIGASE MODIFIER SUBUNIT NULL MOUSE.

L. McConnachie[1], F. N. Hudson[2], C. B. Ware[3], C. Fernandez[1], P. A. Vliet[1], C. C. White[1] and T. J. Kavanagh[1]. [1]*Environmental and Occupational Health Sciences, University of Washington., Seattle, WA,* [2]*Pathology, University of Washington, Seattle, WA and* [3]*Comparative Medicine, University of Washington, Seattle, WA.*

The rate-limiting step in glutathione (GSH) biosynthesis is catalyzed by glutamate cysteine ligase (GCL). This enzyme is a heterodimer composed of a catalytic and a modifying subunit. The modifying subunit, GCLM, functions to modify the activity of GCL by increasing its affinity for glutamate and attenuating the feedback inhibition of GCL by GSH. To more thoroughly characterize the role of GCLM in GSH biosynthesis, we have created a GCLM-null mouse model. We replaced exon 1 of the GCLM gene with a beta-galactosidase/neomycin phosphotransferase fusion protein, allowing in situ detection of GCLM promoter activity. Western blots performed on tissues, including liver and kidney, indicate the -/- (null) mice do not express immunodetectable levels of GCLM, while the +/- mice express GCLM at 50% of wild-type (+/+) controls. Furthermore, hepatic GCL activities in -/- and +/-

mice are 23% and 71% of controls while GSH levels were 22% and 96% of controls, respectively. Conversely, beta-galactosidase activity was increased in the -/- and +/- mice when compared to the controls. DNA microarray analysis of hepatic gene expression confirmed that the -/- mice express no GCLM, whereas expression in the +/- mice is 50% of wild-type. Furthermore, this analysis revealed compensatory alterations in gene expression exclusively in the null mice, relative to the controls. Up-regulated genes include those involved in antioxidant defense and amino acid transport. Down-regulated genes include those involved in signal transduction and protein translation. In sum, these data indicate that the targeted disruption of GCLM results in mice with severely compromised GSH biosynthesis. These mice will be very useful in further characterizing the function of GCLM. (Supported by NIH Grants 1P42ES04696, 1R01ES10849, 1T32ES07032, 1P30ES07033, and 2T32AG000057).

2019 THE ROLE OF ANTIOXIDANTS IN URAEMIC PATIENTS. Z A FADHEL, UNIVERSITY OF PETRA, AMMAN, JORDAN.

Z. A. Fadhel. *Division of Neurotoxicology, HFT-132, National Center for Toxicological Research/FDA, Jefferson, AR.*

Many studies have implicated oxygen radicals and other oxygen-derived species as important causative agents of many human diseases. High levels of reactive oxygen species (ROS) can overwhelm the antioxidant defenses and cause pathological conditions. Dietary factors and nutrition are considered to be of major importance in the etiology of diseases. The present study was undertaken to evaluate the extent of serum and RBCs lipid peroxidation, as well as the role of various free radicals in uraemic patients. Methods: Twenty uraemic and 12 healthy males, aged 50 ± 10 years were studied. Serum and RBC membranes were evaluated for their ability to form thiobarbituric acid reactive substances (TBARS) as an index of oxidative stress. Various free radical scavengers such as mannitol, ethanol, sodium benzoate, histidine, aminopyrine, β-carotene were added to the RBC incubation mixtures from control and uraemic patients. Results: The results indicate that patients with high levels of urea showed 20-30% increases in the serum and RBC content of TBARS. Furthermore, addition of free radical scavengers to the incubation mixtures decreased TBARS formation with RBC from uraemic patients to 5.2 –7.1%, while they decreased TBARS formation with control to 22-43% of the values contained in the absence of free radical scavengers. Conclusions: The results suggest the existence of low levels of endogenous antioxidants and indicate that dietary factors could be used to provide some protection in uraemic patients.

2020 DETERMINATION OF PARTITION COEFFICIENTS FOR SELECTED N-ALKANES.

A. Q. Smith, J. L. Campbell and J. Fisher. *Environmental Health Science, University of Georgia, Athens, GA.*

JP-8 is the primary a*via*tion fuel source used by US and NATO a*via*tion assets. JP-8 is comprised of a complex mixture of hydrocarbons. Exposure occurs primarily *via* inhalation and dermal contact. A complex mixture PBPK model for JP-8 is in development to aid in understanding target tissue dosimetry. The n-alkanes, particularly n-octane through n-dodecane (C8-C12) comprise a large majority of neat JP-8 (by weight). As part of the model development efforts, partition coefficients for C8-C12 n-alkanes were determined using Sprague-Dawley rat tissues. Rat tissue:air partition coefficients for liver, muscle, brain, fat, and whole blood were determined using a modified version of the *via*l equilibration method used by Sato and Nakajima (1979), and later by Gargas et al. (1989). A tissue smear technique was used to place the solid tissue on the side of the *via*l. The mean blood:air and fat:air partition coefficients for C8, C9, C10, C11, and C12 were 3.13, 5.80, 8.13, 20.41, 24.57, and 771.91, 1588.16, 3037.59, 10797.14, 18777.12, respectively. Tissue:air partition coefficient values increase with carbon length for C8-C12 hydrocarbons, with few noted exceptions. Additional chemicals found in JP-8 with similar volatility are currently being studied. Funded by AFOSR [grant # F49620-03-1-0157].

2021 COMPARATIVE METABOLISM OF HYDROQUINONE IN RAT AND HUMAN HEPATOCYTES.

T. S. Poet[1], H. Wu[1], J. C. English[2] and R. A. Corley[1]. *[1]Cntr for Biological Monitoring and Modeling, PNNL, Richland, WA and [2]Eastman Kodak Company, Rochester, NY.*

Hydroquinone (HQ) is an important industrial chemical that also occurs naturally in several plant species and food products. Exposure of laboratory animals to HQ may result in a species, sex, and strain-specific nephrotoxicity. The sensitivity appears to have a pharmacokinetic component related to differences in the rates of formation and further metabolism of key nephrotoxic metabolites formed *via* a minor glutathione conjugation pathway. To improve our understanding of the role of pharmacokinetics in renal sensitivity, an iterative, nested approach was used to determine metabolic rate constants for each step in the metabolism of HQ in hepatocytes isolated from male F344 rats and human donors. HQ and each subsequent metabolites along the mono-glutathione conjugation pathway were each used separately as substrates with analysis by reversed phase HPLC/UV detection. A pharmacokinetic model was used to analyze each experiment by simultaneously fitting the disappearance of each substrate along with the appearance of subsequent metabolites over a range of substrate concentrations using Michaelis-Menten equations to define metabolism. The pharmacokinetic model was constrained by the requirement that rate constants determined during analysis of individual steps in the metabolism of HQ must also satisfy the complete, integrated metabolism scheme including competitive metabolic pathways such as the formation of di-glutathione conjugates and the major pathways of glucuronide and sulphate conjugation. The results from this study indicated that the overall capacity for metabolism of HQ and its mono-glutathione conjugate is greater in hepatocytes from humans than those isolated from rats. Rats were found to have a greater capacity for forming di-glutathione conjugates than humans, which could favor the production of more toxic metabolites. These data will form the basis for modifications of an existing physiologically based pharmacokinetic model to improve current estimates of target tissue doses.(Sponsored by the HQ Panel of the American Chemistry Council)

2022 A COMPREHENSIVE APPROACH FOR PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELS USING THE EXPOSURE RELATED DOSE ESTIMATING MODEL (ERDEM) SYSTEM.

A. Ruiz[1], A. M. Tsang[1], J. Licitra[1], F. Power[2], J. Blancato[2] and C. Dary[2]. *[1]Las Vegas Technical Center, Anteon Corporation, Las Vegas, NV and [2]Human Exposure & Atmospheric Sciences Division, USEPA, Las Vegas, NV.*

The implementation of a comprehensive PBPK modeling approach resulted in ERDEM, a complex PBPK modeling system. ERDEM provides a scalable and user-friendly environment that enables researchers to focus on data input values rather than writing program code. ERDEM efficiently manages these inputs and provides reporting and model output assessment capabilities, for toxicologists, risk assessors, exposure scientists and managers. As PBPK models grow in complexity, the implementation of models, the preparation and maintenance of inputs, and the assessment of model output results has become more tedious and error prone. Combining this experience with intensive research produced a comprehensive modeling approach for designing ERDEM and other PBPK modeling projects. The goal of this modeling approach is to improve the modeling process from planning to application for multimedia exposure assessments. The core activities for this approach include identification and implementation of the model design and performance criteria, the model hardware/software specifications, and the model testing and evaluation. As a comprehensive modeling system, ERDEM contains a PBPK model engine component that can create scenario-based simulations and target dose estimates in multiple compartments for exposure of a species to multiple chemicals and metabolites. The input management component uses a Windows based graphical user interface and relational database, enabling the user to enter, edit, report, and export data sets of user assigned physiological information. The stochastic component evaluates the uncertainty associated with input parameters and output model results. ERDEM replaces the traditional program-oriented modeling approach, which is error prone, with a sophisticated and user-friendly model simulation and data management system. DISCLAIMER This work has been funded by the USEPA. It has been subjected to review and approved for publication.

2023 APPLICATION OF THE EXPOSURE DOSE ESTIMATING MODEL (ERDEM) TO ASSESSMENT OF DERMAL EXPOSURE IN THE RAT TO MALATHION.

M. V. Evans[1], F. W. Power[2], C. C. Dary[2], R. Tornero-Velez[2] and J. N. Blancato[2]. *[1]PKB, USEPA, Research Triangle Park, NC and [2]National Exposure Research Lab., USEPA, LV, NY.*

Renewed interest in the study of dermal exposure to malathion has emerged as a consequence of the prescriptive use of this organophosphorus (OP) insecticide as a pediculicide. The present PBPK/PD model in ERDEM was expanded to include metabolic descriptions needed to link exposure scenarios involving use patterns of malathion with potential health effects. This model allows for the estimation of malathion and malaoxon concentrations in the following organs: skin, fat, kidneys, brain, liver, rapidly perfused, and slowly perfused organs. Physiological values were obtained from the literature. Partition coefficients for parent and metabolites were calculated using tissue-composition based equations. An oral gavage study combining time course metabolite data at different doses was used to calibrate the complex

metabolic scheme described in the model. An acceptable fit was obtained for all the metabolite data by adjusting the metabolic parameters in the model. Computer predicted values for malaoxon concentration were then related to acetylcholinesterase (AChE) levels. A preliminary estimate for the maximum velocity (Vmax) of malaoxon activation was 3x10-5 mg/hr-kg, with an affinity constant (Km) of 8x10-3 mg/liter. The AChE equations described aging, as well as regeneration of AChE. The rat PBPK model was used to extrapolate to humans to predict AChE inhibition in children of different ages exposed dermally to malathion when treating head lice. (This work has been funded by the United States Environmental Protection Agency. It has been subjected to Agency review and approved for publication).

2024 KINETIC MODELING OF ORAL UPTAKE AND ELIMINATION OF ^{54}MN FOLLOWING ORAL AND COMBINED ORAL/INHALATION EXPOSURE.

H. J. Clewell[1], J. G. Teeguarden[2] and M. E. Andersen[3]. *[1]ENVIRON Health Sciences Institute, Ruston, LA, [2]ENVIRON Health Sciences Institute, Collegeville, PA and [3]CIIT Centers For Health Research, Research Triangle Park, NC.*

Simple two-compartment pharmacokinetic (PK) models have been the standard for describing the bulk kinetics of radiolabeled tracer studies with metals such as manganese (Mn). The resulting characterizations, especially the elimination rates, are of limited usefulness as predictive kinetic tools for describing exposure:tissue dose relationships; nonetheless, these descriptions can serve as building blocks of more comprehensive PK models. Here we have attempted to link two simple (two compartment) PK models developed by us previously to describe (1) the influence of oral and inhalation Mn exposure rates on tracer (^{54}Mn) elimination kinetics and (2) uptake and elimination of ingested Mn. The unified model failed to describe both tracer kinetics and uptake and elimination for three tested oral Mn exposures and four combined inhalation/oral Mn exposures. Measured liver Mn concentrations could not be maintained while fitting elimination kinetics, and changes in the flux of Mn between a central (plasma) compartment and the deep tissue and liver compartments with dose did not follow an identifiable pattern. Failure of the simple combined PK model structure indicated the existence of kinetically distinguishable pools of Mn, one in the GI-tract derived portal blood and the other in the venous/arterial blood. This conclusion is consistent with our knowledge about the speciation of Mn in the body. Revised to account for route differences in availability, the model provided much improved descriptions of oral and inhalation route kinetics. Route dependent differences in handling of absorbed Mn are a necessary component of physiologically based PK models for Mn.

2025 DOSE METRIC SENSITIVITY TO CHANGES IN PBPK MODEL INPUT FUNCTIONS USED TO SIMULATE DAILY ORAL OR INHALATION EXPOSURE.

R. S. DeWoskin[1] and H. A. Barton[2]. *[1]ORD/NCEA/HPAG, USEPA, Research Triangle Park, NC and [2]ORD/NHEERL/ETD, USEPA, Research Triangle Park, NC.*

Some dose metrics are more sensitive than others to variations in how an oral or inhalation daily exposure is modeled. Continuous exposure *via* drinking water or inhalation can be simulated in a variety of ways in a physiologically based pharmacokinetic (PBPK) model. The total dose amount may be modeled to enter the lung or GI compartment: 1) as a uniform concentration that is continuously inhaled or ingested over 24 hours; 2) as a specified number of equal or unequal bolus doses (ingestion) or peak concentrations (inhalation) delivered at certain times and intervals throughout the day; or 3) as a single daily bolus or peak concentration. In a simulation of the kinetics of a parent compound using a perfusion-limited PBPK model without metabolism, peak concentration (PC) or time above some critical concentration (TCC) are more sensitive dose metrics to the amount of dose delivered per unit time than total area under the curve (AUC). When nonlinear metabolism terms are added, dose metric sensitivity for both parent and metabolite is further influenced by the parameter values for Vmax, and Km. Sample PBPK models for bromodichloromethane or 1, 1, 2-trichloroethane were used to evaluate the sensitivity of different dose metrics to variations in model input functions for a continuous daily ingestion or inhalation exposure. In the standard oral exposure animal bioassay, test chemical is administered either as a single bolus gavage dose or as a continuous exposure in drinking water or food. For inhalation studies, exposure to the test chemical in air is generally for 6 hours per day, 5 days per week. The PBPK models were parameterized to fit standard bioassay data, and the concentration-time curves for various dose metrics were then compared based upon the use of different model input functions for a daily exposure. The simulation results demonstrate the importance of the choice of dose metric and model input function when extrapolating from bioassay data to a continuous daily exposure. [This abstract does not necessarily reflect EPA policy.]

2026 PHYSIOLOGICAL PARAMETERS OF RATS FOR PHARMACOKINETIC MODELS OF PRENATAL EXPOSURE.

T. Leavens, B. Elswick and D. Dorman. *Center for Developmental Dosimetry, CIIT Centers for Health Research, Research Triangle Park, NC.*

Physiologically based pharmacokinetic (PBPK) models that predict the fate of environmental chemicals in developing animals during pregnancy provide a tool for reducing uncertainty in animal to human extrapolation. Currently there is a lack of information concerning representative values for physiological parameters in rats during pregnancy and how these values compare with simulations from physiological models. The objectives of our research were to compile values for physiological parameters during the entire period of gestation and to compare the literature values with simulations derived from a model that has been used extensively to simulate physiological changes in rodents during pregnancy (O'Flaherty, et al., 1992, Toxicol Appl Pharmacol 112: 245-56). Values for physiological parameters including maternal and fetal body weight, maternal and fetal organ volumes, maternal cardiac output, and maternal organ blood flows were obtained from peer-reviewed, published research and unpublished data generated from reproductive and developmental studies. In general the gestational blood flow data was not as robust as data on body weights and tissue volumes, partly because of the scarcity of measurements made in nonanesthetized rats. The majority of blood flow data was measured at gestation day (GD) 0 and 18-21 and reported as normalized tissue flow. Model simulations of blood flow fell within the range of data over the period of gestation for most tissues. The model-simulated change in cardiac output, which peaked at GD 11 before decreasing and then increasing in later pregnancy, could only be supported by the original dataset used to generate the equations. The model under-predicted weight gain in the dam, particularly during the period from GD 0 to 16. The compilation of data will serve as the basis for updating a physiological model for rat pregnancy. The ranges of data will be useful for conducting sensitivity analyses to determine the impact of uncertainty in model parameters on blood concentrations of environmental chemicals in rats exposed during pregnancy.

2027 PHARMACOKINETIC MODEL FOR TESTOSTERONE AND ITS METABOLITES, DIHYDROTESTOSTERONE AND ESTRADIOL, IN THE PERINATAL RAT.

R. Clewell and M. E. Andersen. *CIIT Centers for Health Research, Research Triangle Park, NC.*

Male sexual development is dependent upon both adequate testosterone (T) levels and specificity of timed surges in T production and release. In order to evaluate time-dependent changes in T levels, a physiologically-based pharmacokinetic model has been developed, which describes T production, distribution and conversion to its active metabolites, dihydrotestosterone (DHT) and estradiol (E2), in the perinatal male rat. Physiological and hormone-specific kinetic parameters, such as T production rates and affinity constants for metabolizing enzymes, were available from literature. The T, DHT and E2 models include diffusion-limited compartments for testes, brain and body. The three models are linked *via* conversion of T to DHT and E2 described with Michaelis-Menten metabolism in the blood. Thus, mass is transferred from the T model to the appropriate metabolite model. Fitted parameters included tissue permeability, first order metabolism of E2 and DHT and maximum capacity for DHT and E2 production. The current model successfully reproduces age-dependent changes in fetal and neonatal testes T and serum T, DHT, and E2 levels from gestation day (GD) 15 to post-natal day 3. Two peaks in serum T occur in development, the first on GD 18 and another at 1 to 3 hours after birth. Changing T production appears to be responsible for the prenatal T surge and the model adequately reproduces measured T using production rates from published *in vitro* data. The post-natal T surge, however, is much shorter-lived than that in the fetus, and the increase in serum levels is much greater (3-fold increase in serum T). The mechanism of this observed hormone surge is unknown, although T stored in the testes may be released at birth. This testes-release hypothesis is supported by the ability of the model to fit measured data based on this mode of action. This model will be used to develop a predictive tool in the assessment of perinatal risk resulting from decreased T production induced by perinatal exposure to endocrine active compounds, such as di-n-butylphthalate.

2028 PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING OF DI-N-BUTYL PHTHALATE (DBP) IN PREGNANT RATS.

J. Kremer, R. A. Clewell and S. J. Borghoff. *CIIT Centers for Health Research, Research Triangle Park, NC.*

DBP is a phthalic acid ester widely used as a plasticizer and solvent and a known reproductive toxicant. PBPK models incorporate physiological and chemical specific parameters to make quantitative predictions of tissue concentrations over time. The

objective of this study was to develop a PBPK model to predict fetal exposure to monobutylphthalate (MBP), the proposed toxic metabolite of DBP, during the critical window of reproductive development in rats (gestation days GD12-20). A flow-limited PBPK model for a pregnant rat was developed based on an existing model (O'Flaherty et al., 1992, Toxicol. Appl. Pharmacology 112: 245-256). Physiological parameters were obtained from peer-reviewed published literature reports. Chemical specific parameters were obtained from or calculated using data available from literature. Specifically, DBP to MBP metabolism in the gastrointestinal (GI) tract was modeled using Michaelis-Menten kinetics, and MBP uptake from GI fluid was calculated using a first-order rate constant. MBP metabolism was assumed to follow Michaelis-Menten kinetics in the liver. We evaluated the ability of this PBPK model to predict MBP concentration by comparing model output with data on maternal plasma, placenta, and fetal plasma following oral administration of DBP (0.5 and 1.5 g/kg) in pregnant rats on GD14 (Saillenfait et al., 1998, Toxicol. Sciences. 45: 212-224). Model simulations predicted the time course of MBP uptake, distribution, and elimination from maternal plasma, placenta, and fetal plasma. Experimental data were underpredicted but model outputs were consistent with the rapid clearance of DBP and MBP, greater than 90% excreted within 48 hours, seen experimentally. Sensitivity analysis indicated that model predictions depend strongly on the rate-function used for MBP absorption from the GI tract following oral administration of DBP. Targeted experimental data on pathways of oral absorption and metabolism parameters during pregnancy will be required to improve predictions of MBP concentrations in maternal and fetal tissues.

2029 PBPK MODELING OF PHARMACOKINETIC INTERACTIONS FOR THE LACTATIONAL TRANSFER OF METHYLMERCURY AND PCB CONGENERS: IMPLICATIONS OF TRANSPORT PROTEINS.

S. Lee, M. B. Reddy, M. Lohitnavy and R. Yang. *Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO.*

A PBPK model was developed to simulate pharmacokinetic interactions for the lactational transfer of methyl mercury (MeHg) and PCB congeners (PCB 153, PCB 126) in mice. Model structure and parameters were based on previously developed PBPK models describing the lactational transfer of MeHg or PCB congeners. However, mechanistic involvement of transport proteins were incorporated into the PBPK model. Earlier studies suggested: (1) MeHg and PCB congeners could affect the plasma levels of albumins and lipoproteins; and (2) MeHg binds to albumins in plasma, and a major portion (~60%) of PCBs could binds to lipoproteins in plasma. Thus, we investigate the effects of lipoproteins and albumins on the lactational transfer of MeHg and/or PCBs using PBPK modeling. The following hypotheses regarding the roles of lipoproteins and albumins were tested using PBPK modeling: (1) Only unbound MeHg or PCB congeners could be transferred to the pups; (2) Only bound MeHg or PCB congeners could be transferred to the pups; and (3) The rate of association with transfer proteins could determine the transfer levels of MeHg or PCBs to the pups. Simulation results showed that the levels of lipoproteins and albumins were important factors determining the amounts of lactational transfer of MeHg and PCB congeners. These model simulations are validated iteratively with perinatal pharmacokinetic studies using lactating mice and their pups. Our strategy in using PBPK modeling with incorporation of mechanistic information may improve risk assessment for the mixture of MeHg and PCBs in the developing organisms (Supported by NIEHS R03 ES 10116 and ATSDR Cooperative Agreement U61/ATU 881475).

2030 SIMULATING THE DOSE-DEPENDENT LAG IN BILIARY EXCRETION OF GENISTEIN USING A HEPATIC DISPERSION MODEL.

M. G. Zager[1, 2], H. T. Tran[2] and P. M. Schlosser[1]. *[1]CIIT Centers for Health Research, Research Triangle Park, NC and [2]Center for Research in Scientific Computation, NCSU, Raleigh, NC.*

Genistein is an endocrine-active compound found naturally in soy products and linked to both beneficial and adverse health effects. Data suggest that genistein is extensively conjugated in the liver and these conjugates undergo enterohepatic recirculation. Sfakianos, et al. (J Nutr, 127:1260-1268, 1997) infused four doses of genistein into the hepatic vein of rats and observed a dose-dependent delay in the biliary excretion of genistein conjugates. Typical physiologically based pharmacokinetic (PBPK) models fail to accurately describe these data. We developed a model to simulate the distribution, metabolism and biliary excretion of genistein and conjugates in the liver that accounts for spatial variations in compound concentrations (dispersion) and in physiological parameters. Our model treats the liver as two parallel tubes, representing the blood vessel space and bile duct space, respectively, with hepatocytes in between. Numerical code was developed for model simulations and to calibrate the unknown model parameters using the data of Sfakianos, et al. Parameters that determine the extent of dispersion within the tubes were adjusted to obtain biologically relevant concentration distributions for genistein and conjugates. The model was first calibrated to each of the four doses separately, but this led to a distinct parameter set for each dose. Analysis of the parameter variation between doses suggests that several parameters in the model are concentration-dependent. When a single parameter set was fit to the data for all four doses the model over-predicted some data and under-predicted others in a manner that supports this conclusion. Possible explanations for this observed dose-dependence are that excretion into the bile duct is saturable or that genistein causes a dose-dependent change in the rate of biliary excretion. Ultimately, the dispersion model can serve as a template for other chemicals that undergo enterohepatic recirculation. (This research was supported by the American Chemistry Council.)

2031 MATHEMATICAL MODELING OF FAST AND SLOW GENISTEIN PHARMACOKINETICS (PK) IN RATS.

P. M. Schlosser[1], S. J. Borghoff[1], N. G. Coldham[2], H. T. Tran[3] and M. G. Zager[1, 3]. *[1]CIIT Centers for Health Research, Research Triangle Park, NC, [2]Department of Bacterial Diseases, eterinary Laboratories Agency, Surrey, United Kingdom and [3]Center for Research in Scientific Computation, NCSU, Raleigh, NC.*

Phytoestrogens such as genistein are endocrine-active compounds produced naturally by plants. High concentrations of genistein occur in soy products such as tofu and soy infant formula. Genistein is associated with a range of effects from chemoprevention of breast and prostate cancer and cardiovascular disease to observed reductions in weight and anogenital distances of rodents at birth. To estimate circulating levels of genistein during health-effects studies, physiologically based pharmacokinetic (PBPK) models for genistein in rats are being developed. Tissue concentration data were obtained in rats dosed with 4 mg/kg of ^{14}C-genistein (IV or oral; 30-min to 48-hr time-points). These data show a terminal half-life of ~ 1 hr for elimination from the blood after IV dosing and rapid appearance in the blood after oral dosing (peak by 30 min). After oral dosing, total activity in gastro-intestinal (GI) tissue was 1-2 orders of magnitude greater than any other tissue and the elimination half-life from GI tissue was greater than 5 hr. An initial PBPK model with a single GI tissue compartment and perfusion- or diffusion-limited transport between the GI compartment and blood, could not describe these data. Because the oral doses were administered in corn oil, we considered uptake into GI-associate lymph tissues, which were assumed to be included in measures of total GI activity, and this allowed us to successfully describe the fast and slow PK after IV vs. oral dosing. We also observed biphasic elimination of genistein from serum after IV administration and found that it could be described if binding of genistein to serum proteins was included. Experimental evidence already exists for binding to serum proteins but the suggestion that transport is occurring *via* GI-associated lymph ducts should be confirmed experimentally. (This research was supported in part by the American Chemistry Council and the Food Standards Agency, UK.)

2032 A PRAGMATIC METHOD FOR SIMULATING THE PHARMACOKINETICS OF INTERACTING CHEMICALS IN MIXTURES: A CASE STUDY WITH TOLUENE IN MIXTURES.

K. Price and K. Krishnan. *Occupational and Environmental Health, Universite de Montreal, Montreal, QC, Canada.*

The change in tissue dose of components of mixtures is often a consequence of metabolic interactions. When metabolic interactions are well characterized, their impact on tissue dose can be simulated with PBPK models. It is not always possible to characterize all metabolic interactions, especially with increasing complexity of mixtures. The aim of this study was therefore to develop a pragmatic method that does not require interaction data for simulating change in kinetics of chemicals competing for hepatic metabolism during mixed exposures. A case study was undertaken to predict the inhalation pharmacokinetics of toluene in rats exposed to this chemical (50 or 100 ppm, 4 hr) in the presence of 50 or 100 ppm of increasing number of interacting chemicals (m-xylene, p-xylene, o-xylene, ethylbenzene, dichloromethane, benzene, perchloroethylene, trichloroethylene, styrene). The methodology involved the use of the individual chemical PBPK model for toluene along with the hepatic extraction ratio (E) specific for mixed exposures, calculated on the basis of total dose of mixture components expressed as toluene-equivalents. The results indicate that the E value for toluene changed from 0.71 for an individual exposure at 50 ppm to 0.08, 0.03 or 0.02 in mixtures of 4, 8 or 10 components, respectively. The E also decreased from 0.3 for a single exposure at 100 ppm to 0.07 or 0.03 during mixed exposures to 3 or 5 chemicals. With the use of the modified value of E in the toluene PBPK model, its blood concentrations during mixed exposures were simulated. The toluene blood concentrations at the end of 4-hr inhalation exposures, predicted by this method, for different mixture complexity (n = 2, 3, 4, 5, 8, 10), chemical combinations and exposure concentrations, varied by a factor of 0.9 to 1.2 of experimental values previously obtained in the rat. These results suggest that the kinetics of chemicals in mixtures can be predicted using individual chemical PBPK models, when competitive inhibition is the underlying interaction mechanism.

2033 A GENERAL PHYSIOLOGICAL AND TOXICOKINETIC (GPAT) MODEL FOR SIMULATION OF COMPLEX TOLUENE EXPOSURE SCENARIOS IN HUMANS.

E. M. Kenyon[1], T. Colemen[2], C. Eklund[1] and V. Benignus[3]. *[1]ORD/NHEERL, USEPA, Research Triangle Park, NC, [2]Biological Simulators, Inc., Jackson, MS and [3]HSD, USEPA, Chapel Hill, NC.*

Many environmental exposure scenarios require dynamic methods in which exposures and human activities vary continuously as a function of time. Simulation of physiological scenarios with commonly available software packages and simulation languages is often highly programming intensive and complex. To simplify this process, a "whole-body" human physiological simulation model, Quantitative Circulatory Physiology, Research Version 4.0, (QCP4), was coupled with a physiologically-based toxicokinetic (PBTK) model for toluene. Chemical-specific parameters and initial organ volumes and blood flow rates were obtained from the literature. Compartments in the model included lung, slowly and rapidly perfused tissue groups, fat, liver, gut and brain. QCP4 updated changing physiological parameters required by the PBTK model appropriate to varying activity level of the human subject as the model was iteratively executed over time. The GPAT model adequately predicted toluene blood concentrations under varying exercise levels and exposure scenarios from 4 different studies reported in the peer reviewed literature. In comparison to a similar model executed in Advanced Continuous Simulation Language (ACSL), the GPAT model required less coding and had greater flexibility to vary activity levels. The coupled GPAT modeling framework can also simulate physiological alterations in response to changes in altitude, temperature, diet, and illness. When linked with a toxicokinetic model this provides a powerful tool for both risk assessment (e.g., effects on sensitive subpopulations) and experimental design applications. (This abstract does not necessarily reflect EPA policy.)

2034 IMPACT OF PEAK EXPOSURE AND BIOLOGICAL VARIABILITY ON THE KINETICS OF TOLUENE IN MAN - A PBTK ANALYSIS.

J. Bessems, J. Lammers, G. Schaafsma, T. Bouwman, L. Ravensberg and A. Freidig. *Toxicological Risk Assessment, TNO Chemistry, Zeist, Netherlands.* Sponsor: V. Feron.

Occupational exposure limits generally reflect to time-weighted average exposures and an average healthy worker. However, peak exposures and interindividual variation in physiological and biochemical parameters may influence dosimetry. Using a PBTK (physiologically based toxicokinetic) modeling approach and toluene as model compound, we investigated the impact of these variables on internal exposure. Male volunteers were exposed by inhalation to a constant toluene concentration of 40 ppm for 4 h or to three peaks of 110 ppm for 0.5 h (within 4 h) such as to make total dose similar in both exposure scenarios. Blood toluene concentration (BTC) and urinary o-cresol excretion were measured. In the constant exposure scenario, BTCs increased constantly. For some individuals, it appeared that BTC was almost reaching equilibrium after 4 h. For this scenario, the largest interindividual differences in BTCs during exposure were about a factor of two. BTCs were very similar 2 h after the exposure had stopped. In the peak scenario, external peaks were reflected by fluctuating BTCs, with the highest BTC measured at the end of the third exposure peak. At this time-point, the lowest and highest BTC differed about a factor of two again. Our PBTK simulation provided a fairly good prediction of the means of the BTCs in both exposure scenarios. According to model calculations, BTC appeared not to be impacted by interindividual variation in body weight and body fat content nor to variation in the blood:air partition coefficient. In contrast, the model was sensitive to changes in the metabolic clearance parameters Vmax and Km as well as to changes in the blood flow to the fat compartment and the liver compartment. It is concluded that the impact of peak exposure and variability in biological parameters on BTC can be modeled reasonably well using the PBTK approach. PBTK modeling may provide an additional instrument in the setting of short-term exposure limits (STEL), thereby taking into account human variability in physiological and biochemical parameters.

2035 PRELIMINARY DEVELOPMENT OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC/ PHARMACODYNAMIC(PBPK/PD) MODEL FOR LOW LEVEL EXPOSURE TO CHEMICAL WARFARE AGENT (CWA) IN MINIPIG.

K. O. Yu[1], R. J. Mioduszewski[2], E. M. Jakubowski[2], S. Hulet[2], S. M. Thompson[2] and J. M. Gearhart[1]. *[1]Air Force Research Laboratory, US Air Force, Wright-Patterson AFB, OH and [2]Edgewood Chemical and Biological Center, US Army, Aberdeen Proving Ground, MD.*

Chemical warfare organophosphorous agents inhibit acethylcholine esterase (AChE) that hydrolyzes a neurotransmitter, acethylcholine. A PBPK/PD model for the CWA sarin was developed in a miniature swine (Gottingen strains) to better estimate human exposure compared to rodent models. The vapor exposure data and model have different routes including inhalation and absorption through dermal and ocular contact. The majority of organ volumes and blood flows of the minipig were obtained from the literature, while some parameter values were scaled from other species using standard allometric relationships. Since one of the observed effects of low-level Sarin exposure is constriction of the ocular pupil (miosis), the ocular compartment for eyes was added to address this pharmacodynamic effect. In this model sarin is absorbed through the eyes where it internally binds with AChE. Pupil constriction from external, systemic or combined delivery of sarin could be predicted based on AChE inhibition in the ocular muscles. The PBPK/PD model was used to simulate AChE inhibition after different routes and different doses of exposure and to predict potential pharmacodynamic effects at the eye and other target tissues. Sensitivity analysis will be performed to find which model parameters have the greatest influence on AChE inhibition. This model will provide quantitative dose-response relationships to assess the physiologically significant consequences of low-level exposures of sarin in the minipig.

2036 A QUANTITATIVE DESCRIPTION OF SUICIDE INHIBITION OF DICHLOROACETIC ACID IN RATS AND MICE.

D. A. Keys[1], I. R. Schultz[2], R. D. Stenner[3], D. A. Mahle[4] and J. W. Fisher[1]. *[1]Environmental Health Science, University of Georgia, Athens, GA, [2]Pacific Northwest Division, Battelle, Sequim, WA, [3]Pacific Northwest Division, Battelle, Richland, WA and [4]Mantech Environmental Technology Inc., Dayton, OH.*

Dichloroacetic acid (DCA) is a minor metabolite of trichloroethylene that remains an important risk assessment issue because of its carcinogenic potency and its distribution in water supplies as a production of chlorination. DCA has been shown to inhibit its own metabolism by irreversibly inactivating glutathione transferase zeta (GSTZ). To better predict internal dosimetry of DCA, a physiologically based pharmacokinetic model was developed which included a description of DCA suicide inhibition. Suicide inhibition was described dynamically by having the rate of maximal metabolism of DCA (Vmaxt) vary with time. Resynthesis (zero-order) and degradation (first-order) of metabolic activity were also described. Published iv pharmacokinetic studies in rats following exposure to 1, 5, or 20 mg/kg DCA were used to estimate initial VmaxC and Km values. Degradation and resynthesis rates were set at values estimated previously from rat immunoreactive GSTZ protein time courses. The first-order inhibition rate, kd, was estimated to these same GSTZ protein time courses. Residual linear metabolism (Kfc) was estimated from DCA time courses following exposure to DCA in drinking water. With all parameters set to estimated values, the PBPK model predictions were validated by comparing predicted DCA concentrations to measured DCA time courses in published studies of rats pre-treated with DCA following iv exposure to 0.05, 0.25, 1, 5 or 20 mg/kg DCA. The same model structure was used to simulate DCA time-courses following 5, 20 and 100 mg/kg iv exposures in naive and pre-treated mice and exposure of mice to DCA in drinking water. Comparisons of PBPK model predicted values to measured was favorable lending support for the further development of this model for application to DCA or TCE human health risk assessment. (Supported by DOE Coop. Agreement DE-FC09-02CH11109)

2037 PBTK-TD MODEL FOR ACUTE POISONING BY HYDROGEN CYANIDE.

G. Johanson. *Work Environment Toxicology, IMM, Karolinska Institutet, Stockholm, Sweden.*

Several studies suggest that fire fatalities are primarily caused by fire smoke. Hydrogen cyanide (HCN), formed in the combustion of polyurethane and other N-containing materials, may contribute significantly. The low awareness of this risk is in part due to lack of knowledge on the absorption, distribution, metabolism, and excretion (ADME) of HCN. Further, it is difficult to estimate HCN exposure in fire victims since HCN is eliminated from blood also after death. Yet a number of studies show markedly elevated blood HCN levels in fire victims and even higher levels in deceased victims. The aim of this study was to develop a PBTK-TD model for HCN, as no such models have hitherto been published. The model has 5 compartments: lungs, plasma/liver, muscle tissues, other tissues, sulphur pool. Chemical independent model parameters were taken from the literature. Chemical dependent parameters (partition coefficients, sulphur-dependent saturable detoxification to thiocyanate, and saturable methemoglobin binding) were estimated from published studies on HCN. The effect of exercise on ADME was also incorporated. The TD model describes the degree of cytochrome oxidase inhibition as a sigmoid function of plasma HCN. The cut-off level for acute toxicity (lethality) was set to 70% inhibition. All simulations were performed with Berkeley Madonna. The model accurately reflected reported fatal HCN air and blood levels. Simulated human exposures agreed well with experimental animal data. Thus, the model predicted a value of 1.8 compared to 1.9 in monkeys for the exponent n in the equation C^n x T=k, where C is the exposure concentration and T the time to lethality.

The strong non-linearity in kinetics and dynamics is further illustrated by T values of 13 h and 1 h for exposures to 50 ppm and 200 ppm HCN, respectively. The influence of work was also dramatic. At light exercise (50W) the corresponding T values dropped to 1.5 h and 0.15 h. The PBTK-TD model for HCN may be used to study different exposure scenarios, to improve the basis for diagnosis and treatment, and for Acute Exposure Guideline Levels (AEGLs).

2038 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR DECANE, A CONSTITUENT OF JET PROPELLENT-8.

R. Perleberg[1], D. A. Keys[1], J. L. Campbell[1], W. Everett[1], A. Q. Smith[1], T. Almekinder[1], K. Frank[1], M. G. Bartlett[2] and J. Fisher[1]. *[1]Environmental Health Science, University of Georgia, Athens, GA and [2]Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA.*

Decane, a 10 carbon alkane, is one of the highest vapor phase constituents of jet propellent-8 (JP-8). Decane was selected as a representative compound of the semi-volatile fraction of JP-8. Male Fisher 344 rats were exposed to decane vapors in a 31 L leach chamber for 4 hours to time weighted average (TWA) concentrations of either 1200, 781, or 273 ppm. Time course samples for the 1200 ppm decane exposure were collected from blood, brain, liver, fat, bone marrow, lung, skin, and spleen tissues, while end of exposure samples were collected with these tissues for 781 and 273 ppm vapor exposures. A physiologically based pharmacokinetic (PBPK) model for decane was developed using flow-limited (liver and lung) and diffusion-limited (brain, bone marrow, fat, skin, and spleen) equations to describe the uptake and the clearance of decane in the blood and tissues. *In vivo* partition coefficient values for blood/air and tissue/blood were estimated by fitting end of exposure blood and tissue concentrations. Decane pharmacokinetic behavior is unlike short chain halogenated hydrocarbons, which are described primarily by flow-limited conditions using in-vitro derived partition coefficients.

2039 DEVELOPMENT OF A PBPK-CHEMICAL LUMPING MODEL FOR GASOLINE VOLATILES.

J. E. Dennison[1], M. E. Andersen[2], H. J. Clewell[3], M. M. Mumtaz[4] and R. S. Yang[1]. *[1]ERHS, Colorado State, Fort Collins, CO, [2]CIIT Centers for Health Research, Research Triangle Park, NC, [3]Environ Corp, Ruston, LA and [4]ATSDR, Atlanta, GA.*

A PBPK model was previously developed for several representative GASOLINES using a 6-component mixture model structure. We extended the model to volatile components of gasoline, reflecting common human exposures. The mixture PBPK model includes benzene, toluene, ethylbenzene, o-xylene, and n-hexane and a lumped component that represents all remaining components. Competitive inhibition of saturable metabolism between each component was included. We prepared samples of gasoline that were enriched in the more volatile components by heating the liquid and collecting the vapors. Samples represented (1) the first third of the neat liquid to evaporate and (2) the first two thirds to evaporate. Closed chamber experiments were performed with male F344 rats using an autosampling gas uptake system with GC/FID. At higher exposure concentrations, the data indicated that ventilation rates (QP) declined, possibly due to irritation and/or CNS depression. The best fits were obtained with QPs of 12.5, 13.5 and 14.9 L/hr/kg^0.74 for high, medium, and lower exposures. The different fractions required modification of the blood:air partition coefficient (PB) for the lumped chemical. The optimal PBs were 1.6, 2.3, and 3.0 for the 1/3 cut, 2/3 cut, and whole gasoline, respectively. Other parameters were similar to those used in the previous model. Except for o-xylene, which may be affected by absorption to the animals fur, an excellent correspondence between the model and the chamber uptake data was obtained for all chemicals, including the lumped chemical. We conclude that complex mixture PKs can be described using this lumping approach and that different fractions of a mixture can be incorporated into the general method with appropriate adjustments in physicochemical parameters.

2040 PHYSIOLOGICAL MODELING OF DECAMETHYLCYCLOPENTASILOXANE (D5) INHALATION KINETICS IN RATS AND HUMANS.

M. Reddy[1], J. M. Tobin[2], D. A. McNett[2], M. L. Jovanovic[2], M. J. Utell[3], P. E. Morrow[3], K. P. Plotzke[2] and M. E. Andersen[4]. *[1]Quantitative Toxicology Group, Center for Environmental Toxicology and Technology, Colorado State University, Fort Collins, CO, [2]Toxicology, Health and Environmental Sciences, Dow Corning Corporation, Midland, MI, [3]Departments of Medicine and Environmental Medicine, University of Rochester Medical Center, Rochester, NY and [4]Division of Biomathematics and Physical Sciences, CIIT Centers for Health Research, Research Triangle Park, NC.*

D5 is a silicone fluid used in a variety of consumer applications including personal care. An extensive dataset of D5 distribution in the rat following inhalation exposure was used to develop a physiologically based pharmacokinetic (PBPK) model. The D5 model followed a similar model structure that successfully described the distributional characteristics of another cyclic siloxane, octamethylcyclotetrasiloxane (D4). The rat model structure for D5 incorporated deep compartments in the lung and liver and had two fat compartments as well as an unusual combination of low blood:air and high fat:blood partitioning. For the disposition of D5 in humans, a PBPK model structure was developed based on the rat model but was simplified due to the less extensive human dataset. However, a similar model structure successfully described D5 pharmacokinetics in both rats and humans. A key component of both the rat and human model was a sequestered pool of D5 that was presumed to be in lipoproteins. This bound D5 was released from the liver, distributed by the blood and cleared into the fat. Although D5 metabolism is essentially flow-limited, due to its low blood:air partition coefficient (0.2 in rats and 0.5 in humans *in vivo*), the primary mechanism of D5 elimination was exhalation. Despite high fat:blood partitioning, D5 is not expected to accumulate due to rapid clearance by exhalation and metabolism. Supported in part by the Silicones Environmental, Health and Safety Council of North America.

2041 INCORPORATION OF THE GENETIC CONTROL OF ALCOHOL DEHYDROGENASE INTO A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR ETHANOL IN HUMANS.

G. M. Pastino[2], L. G. Sultatos[1], C. A. Rosenfeld[1] and E. J. Flynn[1]. *[1]Pharmacology and Physiology, UMD- New Jersey Medical School, Newark, NJ and [2]Schering-Plough Research Institute, Lafayette, NJ.*

The assessment of the variability of human responses to foreign chemicals is an important step in characterizing the public health risks posed by non-therapeutic hazardous chemicals, as well as the risk of encountering adverse reactions with drugs. Of the many sources of interindividual variability in chemical response so far identified, hereditary factors are some of the least understood. Physiologically based pharmacokinetic modeling linked with Monte Carlo sampling has been shown by various investigators to be a useful tool for quantification of interindividual variability in chemical disposition and/or response when applied to biological processes that displayed single genetic polymorphisms. The current project has extended this approach by modeling the complex hereditary control of alcohol dehydrogenase, which includes polygenic control, as well as polymorphisms at two allelic sites. The physiologically based pharmacokinetic model for ethanol indicated that peak blood ethanol levels and time to peak blood ethanol levels were marginally affected by alcohol dehydrogenase genotypes, with simulated subjects possessing the B2 subunit having slightly lower peak blood ethanol levels and shorter times to beak blood levels compared to subjects without the B2 subunit. In contrast, the area under the curve (AUC) of the ethanol blood decay curve was very sensitive to alcohol dehydrogenase genotype, with AUCs from any genotype including the *ADH1B2* allele considerably smaller than AUCs from any genotype without the *ADH1B2* allele. Furthermore, the AUCs in the *ADH1C1/C1* genotype were moderately lower than the AUCs from the corresponding *ADH1C2/C2* genotype. Moreover, these simulations demonstrated that interindividual variability of ethanol disposition is affected by alcohol dehydrogenase, and that the degree of this variability was a function of the ethanol dose. (Supported by a grant from the American Chemistry Council).

2042 TOWARDS A GENERIC PBPK MODEL OF PYRETHROID PESTICIDES: MODELING DELTAMETHRIN AND PERMETHRIN IN THE RAT.

R. Tornero-Velez[1], H. Nichols[4], M. V. Evans[2], M. J. DeVito[2], C. C. Dary[1], M. Dellarco[3] and J. N. Blancato[4]. *[1]National Exposure Research Laboratory, USEPA, Las Vegas, NV, [2]National Health and Environmental Effects Research Laboratory, USEPA, Research Triangle Park, NC, [3]National Center for Environmental Assessment, USEPA, Washington DC, DC and [4]National Exposure Research Laboratory, USEPA, Research Triangle Park, NC.*

Pyrethroids have emerged as a major class of insecticide due to their selective potency in insects and their relatively low potency in mammalian studies. Pyrethroids exert toxicity by binding to voltage-gated sodium channels, thereby eliciting excitatory neurotoxicity. The Food Quality Protection Act (FQPA) of 1996 requires the USEPA to consider the cumulative effects (multiple chemicals by aggregate routes of exposure) of pesticides having a common mechanism of toxicity. In such evaluations it is necessary to accurately estimate the dose available to critical tissues. Towards this end, studies examining the pharmacokinetics of permethrin and deltamethrin in rodents were used to derive a common PBPK model structure. The model included skin, fat, liver, brain, and lumped tissue compartments. Physiological values were obtained from the literature. Tissue-blood partition coefficients were based on Log P and tissue composition. Diffusion-limited tissue uptake in all tissues provided a better visual fit to the data than flow-limited kinetics. Apparent coefficients of permeability were held constant for both pyrethroids at 5% of tissue flow for fat and muscle tissues, 2% for brain tissue, and 0.2% for richly

perfused tissue. While the terminal half life of deltamethrin in blood was 2.5 times longer than permethrin, the kinetics of these two pyrethroids were adequately described by the same model structure. This initial modeling exercise suggests that the kinetics of other pyrethroids may be described by a common model structure. This may allow the development of a single unified PBPK model for use in the cumulative risk assessment of pyrethroid pesticides. *(This work has been funded by the United States Environmental Protection Agency. It has been subjected to Agency review and approved for publication).*

2043 PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF CHLOROETHANE DISPOSITION AND GLUTATHIONE DEPLETION.

L. M. Sweeney[1], J. W. Holder[2] and M. L. Gargas[1]. [1]*The Sapphire Group, Dayton, OH and* [2]*USEPA, Washington, DC.*

A validated physiologically based pharmacokinetic (PBPK) model of chloroethane (CE) for rats, mice, and humans would useful for assessing internal dose-response relationships and performing high-to-low dose and interspecies extrapolations. Such extrapolation is useful because of species differences in incidence of uterine tumors in CE-exposed animals in a single, high-concentration bioassay. An existing PBPK model for chloroethane in rats (Gargas et al., 1990) was evaluated, expanded, and refined. Considerations in the expansion and refinement were the available pharmacokinetic data pertinent to evaluation of chloroethane disposition and potential mode(s) of action for uterine cancer observed in the bioassay. Physiologic compartments were included for complete mathematical description as related to potential mode(s) of action in target organs, storage depots, major site of oxidative metabolism and glutathione (GSH) conjugation, secondary sites of GSH metabolism with available GSH depletion data, and for tissue mass/blood flow balance. Rat model parameters were taken from the literature (physiology, partitioning, and oxidative metabolism by cytochrome P450) or determined through best fit methods for the most complete data set for CE-induced GSH depletion (parameters for GSH conjugation and synthesis). Limited validation was achieved by comparison to other GSH depletion data sets and studies in which 14C-CE exhalation was monitored in 14C-CE-exposed rats. Model predictions were used to elucidate the behavior, as inhaled concentration increases (1-15000 ppm), of the flux through two elimination pathways, and blood and tissue concentrations. Nonlinear behaviors were observed due to saturation of oxidative metabolism and GSH conjugation with increasing inhaled concentration. Similar high-dose nonlinear kinetic behaviors are anticipated for mice, a species susceptible to CE-induced uterine tumors; such behavior may have implications for identification of potential mode of action and risk assessment of chloroethane in humans.

2044 PARTITIONING OF BISPHENOL A (BPA) IN RAT TISSUE FOR PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING.

I. A. Ross[1], H. M. Luu[2], P. P. Sapienza[1], W. Johnson[1], J. C. Hutter[2] and C. S. Kim[1, 2]. [1]*Toxicology, US Food and Drug Administration, Laurel, MD and* [2]*Radiological Health, USFDA, Rockville, MD.*

The rat tissue partitioning of BPA in artificial cerebrospinal fluid (CSF) for brain and Krebs phosphate buffer (Krebs) for other organs was measured *in vitro* by using the BPA concentration ratio of tissue to medium at steady state. Tissue slices were prepared and incubated in artificial CSF or Krebs, containing 14C-BPA (140, 000 dpm/ml medium, sp. act. 55 mCi/mmol) plus cold BPA 0.1 mg/ml medium. The tissues were incubated under 95% oxygen/5% carbon dioxide at 37oC with constant agitation until steady-state uptake was reached (90 min). At the end of the incubation period, tissue slices were processed for radioactivity measurement. The BPA tissue/blood or tissue/CSF partition coefficients were estimated by dividing tissue concentration (mg/g) by incubation medium concentration (mg/ml) in Krebs or CSF. The following results were obtained from the study: heart, 7.5; liver, 6.1; kidney, 6.4; fat, 3.6; muscle, 2.6; breast, 3.6; ovaries, 9.1; uterus, 5.9; stomach, 5.1; small intestine, 6.7; pituitary gland, 12.8; brain stem, 6.1; cerebellum, 6.4; hippocampus, 7.1; hypothalamus, 6.1; frontal cortex, 4.9 and caudate nucleus, 6.8. This information is a requisite for constructing the PBPK model of BPA for its quantitative risk assessment.

2045 HALOACETIC ACID PHARMACOKINETICS IN RHESUS MONKEYS AND HUMANS: CLASSICAL AND PBPK MODELING APPROACHES.

I. Schultz[1], R. E. Shangraw[2], R. D. Stenner[3], D. A. Keys[4] and J. W. Fisher[4]. [1]*Battelle PND, Sequim, WA,* [2]*OHSU, Portland, OR,* [3]*Battelle PND, Richland, WA and* [4]*University of Georgia, Athens, GA.*

Halogenated acetic acids (HAs) are a major class of drinking water disinfection by-product. Several of the chlorine and bromine substituted forms are established rodent carcinogens and reproductive/developmental toxins. Epidemiological studies have not ruled out a link between disinfection by-product exposure and adverse reproductive outcomes. Because of potential health risks, current EPA standards limit the combined total of five common HAs in drinking water to 60 ug/L with an eventual goal of eliminating certain HAs from drinking water. Thus, improving the understanding of HA pharmacokinetics and oral bioavailability is critical to accurately assessing public health. However, measurement of HA pharmacokinetics in humans at actual drinking water exposure rates is problematic because of analytical detection limitations and the influence of prior exposures. In our studies, we measured dichloroacetate (DCA; used as a model di-HA) bioavailability in human volunteers and tri-HA bioavailability in rhesus monkeys (used as a surrogate for humans) using a semi-simultaneous experimental design. Volunteers were given an oral dose of 12C-labeled DCA followed 10 min later by a iv dose of 13C-labeled DCA, which serves as a reference for elimination. Monkeys were given a mixture of four 12C / 13C labeled tri-HAs. To reduce the influence of prior exposure, volunteers or monkeys were provided purified drinking water for 2 weeks prior to studies. Estimation of bioavailability using classical PK modeling approaches indicated large inter-volunteer variation ranging from 5 - 65 %. A previous PBPK model for DCA based on rodent and human *in vitro* data proved inadequate for describing the observed data due to underestimation of peak plasma levels and observed elimination rates. A modified PBPK model was developed that more accurately describes oral absorption and incorporates key aspects of tri-HA disposition. (Supported by EPA STAR grants R825954, R825954).

2046 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL FOR INTRAVENOUS AND INHALATION-ROUTE PHARMACOKINETICS OF BUTYL ACETATE (BA) AND METABOLITES N-BUTANOL (BOH) AND N-BUTYRIC ACID (BOOH).

J. G. Teeguarden[1], P. J. Deisinger[2], T. S. Poet[3], C. English[2], R. A. Corley[3], H. A. Barton[4], H. J. Clewell[1] and W. D. Faber[5]. [1]*ENVIRON Health Sciences Institute, Collegeville, PA,* [2]*Eastman Kodak Co., Rochester, NY,* [3]*PNNL, Richland, WA,* [4]*NHEERL, USEPA, Research Triangle Park, NC and* [5]*WFTC, LLC, Victor, NY.*

Risk assessment for n-butyl acetate and metabolites n-butanol and n-butyric acid (the butyl series) can be accomplished with limited toxicity data and pharmacokinetic data for each compound through application of the "family approach" (Barton et al., 2000). The necessary quantitative and interpretive tool is a PBPK model describing the inhalation-route blood kinetics of the series members. A series of revisions of the initial model were carried through to validation of the inhalation route (BA and BOOH) for male SD rats. Rats were implanted with dual indwelling cannulae and administered BA, BOH or BOOH by IV bolus dose, IV infusion or by inhalation in a recirculating closed chamber. Hepatic, vascular and extravascular metabolic constants for metabolism were estimated by fitting the model to the blood time course data. The respiratory bioavailability of BA (100%) and BOH (~50%) was estimated from a novel closed chamber inhalation study design (Poet, 2002) involving simultaneous measurement of ventilation rates, chamber loss (uptake) and blood kinetics. The resulting PBPK model successfully reproduces the blood time course of these compounds following inhalation exposure to BA and BOH, verifying the description of the blood kinetics based on i.v. studies. This effort highlights the value of new, cost effective dosimetry based approaches to risk assessment for metabolically related compounds. (This abstract does not represent EPA policy).

2047 DEVELOPMENT OF A PRIMATE PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR DI-2ETHYLHEXYL PHTHALATE AND ITS METABOLITE MONO-2ETHYLHEXYL PHTHALATE.

E. D. McLanahan[1], R. Conolly[2] and D. Keys[1]. [1]*Environmental Health Science, University of Georgia, Athens, GA and* [2]*Center for Computational Biology & Extrapolation Modeling, CIIT Centers for Health Research, Research Triangle Park, NC.*

Di-2ethylhexyl phthalate (DEHP) is a commercially important plasticizer and environmental contaminant. Many studies have established that oral dosing of DEHP in rats and mice results in testicular toxicity and teratogenic effects at high doses. In contrast, non-human primates appear relatively insensitive to the testicular effects of DEHP. DEHP is rapidly metabolized in the gut to mono-2ethylhexyl phthalate (MEHP), which is the active metabolite for testicular toxicity. A physiologically based pharmacokinetic (PBPK) model for DEHP and MEHP has been developed in adult male rats previously. The objective of this work was to test the hypothesis that the same PBPK model structure developed for the rat could adequately predict primate pharmacokinetic data. Blood concentrations of DEHP and MEHP were available in marmosets following single and repeated oral exposures to 30 and 500 mg/kg DEHP. Physiological parameters were set to marmoset-specific values. Simple allometric scaling of metabolic parameters was inadequate for predicting

blood concentrations. Metabolic and absorption parameters were fit to the marmoset pharmacokinetic data. The maximum rate of marmoset metabolism of DEHP to MEHP in the gut, VmaxgC (mg/hr/kg$^{0.75}$) was lower than previously estimated for the rat, indicating a species difference in DEHP gut metabolism. Alternatively, the fecal loss rate, loss (1/hr) was higher in the primate model than the rat model, suggesting less complete absorption. The MEHP concentration time courses following repeated exposure were well predicted with parameters estimated from acute exposure time courses. Once validated, a primate PBPK model for DEHP will be able to predict dose metrics for use in human health risk assessment. (Supported by a gift from the American Chemistry Council)

2048 USING *IN VIVO* GAS UPTAKE STUDIES TO ESTIMATE METABOLIC RATE CONSTANTS FOR CCL CHEMICALS: 1, 1-DICHLOROPROPENE AND 2, 2-DICHLOROPROPANE.

C. T. Mitchell, M. V. Evans and E. M. Kenyon. *ORD/NHEERL, USEPA, Research Triangle Park, NC.*

The Safe Drinking Water Act Amendments of 1996 required the USEPA to develop Candidate Contaminant Lists (CCL), to aid in the setting of priorities for the Agency's drinking water research program. 1, 1-Dichloropropene (1, 1-DCPe) and 2, 2-dichloropane (2, 2-DCP) are two of the high priority chemicals identified from the first CCL. Since both are volatile organic compounds, the gas uptake technique was used to estimate rates of metabolism. The gas uptake system is a closed inhalation chamber system in which an initial bolus injection of chemical is made into the chamber, and allowed to decline to obtain a set of decay curves. Individual male F344 rats (200-250g) were exposed to initial chamber concentrations of 50 ppm, 200 ppm, 500 ppm, or 1200 ppm of either 2, 2-DCP or 1, 1-DCP for up to six hours (n=4 rats/exposure level/chemical). Each rat was exposed only once to a single concentration of each chemical. Partition coefficients were estimated using a published quantitative structure-property relationship methodology (Beliveau et al., 2003). These partition coefficients were used with the PBPK model to generate estimates of VmaxC (mg/hr/kg) and KM (mg/L). Optimized VmaxC and KM were 4.9 and 3.17, respectively, for 2, 2-DCP. Optimized VmaxC and KM were 5.76 and 0.31, respectively, for 1, 1-DCPe. The shape of the gas uptake curves for 2, 2-DCP compared to 1, 1-DCPe and the V/K ratios suggest that 2, 2-DCP is more slowly metabolized. These metabolic rate estimates are critical parameters for PBPK models that can ultimately be used for animal to human extrapolation. They are also a vital part of a developing data base comparing *in vivo* to *in vitro* methods for determination of metabolic rate parameters. (This abstract does not reflect EPA policy).

2049 HUMAN MITOCHONDRIAL DNA AMPLIFICATION AND SEQUENCING STANDARD REFERENCE MATERIALS 2392 AND 2392-I.

B. C. Levin[1], D. K. Hancock[1], H. Cheng[1], K. Holland Deckman[2] and K. L. Richie[1]. *[1]Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD and [2]Chemistry Department, Gettysburg College, Gettysburg, PA.*

The National Institute of Standards and Technology (NIST) supplies industry, academia, and government with over 1300 Standard Reference Materials (SRMs) of the highest quality. These SRMs provide laboratories the quality control and assurance that their results with unknown samples are accurate and compatible with other measurements. NIST has revalidated the human mitochondrial DNA (mtDNA) SRM 2392 and developed the new SRM 2392-I to provide quality control when performing the polymerase chain reaction (PCR) and sequencing of mtDNA for forensic identification, medical diagnosis, or mutation detection. They may also serve as controls when performing PCR and sequencing any DNA. SRM 2392 is certified for the sequences of the entire human mtDNA (16, 569 base pairs) from two lymphoblastoid cell lines (CHR and 9947A) from apparently normal individuals and the cloned HV1 region of CHR containing a C-stretch, following which it is difficult to sequence. SRM 2392-I is certified for the entire mtDNA sequence from HL-60, a promyelocytic cell line from the blood leukocytes from an individual with acute promyelocytic leukemia. The mtDNA sequences (but not the DNA) from two other cell lines (GM03798 and GM10742A) that were amplified and sequenced in their entirety at NIST are provided for information and comparison purposes. The sequences of fifty-eight unique primer sets that allow any area or the entire mtDNA to be amplified and sequenced under the same conditions are also given. Many of the single nucleotide polymorphisms (SNPs) found in these five mtDNA templates did result in amino acid changes when compared with the Cambridge Reference Sequence. Two interlaboratory evaluations for the amplification, sequencing, and data analysis of SRM 2392 and SRM 2392-I were each conducted by three different laboratories and NIST. Corroboration of the results in these SRMs will provide quality assurance that any unknown mtDNA is also being amplified and sequenced correctly.

2050 POLYMORPHISMS IN CYTOCHROME P4503A5 (CYP3A5) MAY BE ASSOCIATED WITH TUMOR SIZE IN BREAST CANCER PATIENTS.

A. N. Deigert, L. Lewis, K. Tkaczuk and J. Flaws. *Epidemiology and Preventive Medicine, University of Maryland, Baltimore, MD.*

CYP3A5 is one of the most important members of the cytochrome P450 superfamily. Although this enzyme has been shown to have two polymorphisms (CYP3A5*3 and CYP3A5*6), little is known about the prevalence of these polymorphisms, and whether they are associated with biologic outcomes. Since polymorphisms in other enzymes differ by race, and have been associated with tumor characteristics, the purpose of this work was to test the hypothesis that there are racial/ethnic differences in the odds of having a CYP3A5 polymorphism and that polymorphic status in CYP3A5 affects tumor status in women with breast cancer. To test this hypothesis, 107 women with breast cancer were recruited from a single clinic at the University of Maryland. Each patient completed a survey that obtained information on race and potential confounding factors (age, date at diagnosis, body mass index) and provided a blood sample. Information on tumor characteristics (size, receptor status, nodal involvement) was collected by abstraction of medical records. Blood samples from each patient were subjected to genomic DNA extraction followed by PCR for CYP3A5*3 and CYP3A5*6. The associations between race, polymorphic status, and tumor characteristics were assessed using t-tests and logistic regression models. The data indicate that 40.7% of the women had the CYP3A5*3 polymorphism, and 9.1% had the CYP3A5*6 polymorphism. In addition, white women were 26 times more likely to carry the CYP3A5*3 polymorphism than black women, whereas black women were 9 times more likely to carry the CYP3A5*6 polymorphism than white women. Further, there was significant difference in mean tumor size in women with the CYP3A5*6 polymorphism (3.6cm ±0.98) compared to those without the polymorphism (2.0cm ±0.18) ($p<0.02$). These findings suggest that there are racial/ethnic differences in the prevalence of CYP3A5 polymorphisms, and that polymorphic status may be associated with the size of breast tumors. Supported by DOD grant DAMD 17-00-1-0321 and the University of Maryland, Maryland Statewide Health Network.

2051 GSTP1 A1578G (ILE105VAL) POLYMORPHISM IN BENZIDINE-EXPOSED WORKERS: AN ASSOCIATION WITH CYTOLOGICAL GRADING OF EXFOLIATED UROTHELIAL CELLS.

Q. Ma[1], G. Lin[1], Y. Qin[2], D. Lu[3], K. Golka[4], F. Geller[5], C. Jigang[2] and J. Shen[1]. *[1]Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China, [2]Disease Prevention and Control, Municipal Center, Shanghai, China, [3]State Key Laboratory of Genetics Engineering, Fudan University, Shanghai, China, [4]Institute for Occupational Physiology, University of Dortmund, Dortmund, Germany and [5]Institute of Medical Biometry and Epidemiology, Philipps-University Marburg, Marburg, Germany.*

A polymorphism at codon 105 (Ile/Val) in the GSTP1 gene has been associated with a higher risk for different cancer types. To assess the role of GSTP1 polymorphisms in the development of benzidine-related bladder cancer, GSTP1 AA, AG and GG alleles were determined in occupationally benzidine-exposed Chinese workers without known disease and benzidine-exposed bladder cancer patients from the same cohort of the Shanghai area. An increased but not significant frequency of GSTP1 AG or GG carriers was observed in the occupationally exposed bladder cancer patient group (OR=1.95, 95% CI 0.70-5.46). The odds ratios for the most important non-genetically determined risk factors for bladder cancer in males were as follows: Age (increase per year): OR 1.05, 95% CI 0.99-1.11, ever smoker: OR 1.31, 95% CI 0.47-3.69, duration of exposure (increase per year): OR 1.19, 95% CI 1.10-1.29, and high exposure: OR 4.50, 95% CI 0.70-5.46. Significant differences were found between all benzidine-exposed workers without known disease with modified exfoliated urothelial cells (grade II and higher) and all workers without known disease with at most minor changes (less than grade II) according to Papanicolaou (OR 1.90, 95% CI 1.13-3.20). These findings show for the first time an association between the GSTP1 AG or GG genotype and higher cytological gradings of exfoliated urothelial cells from formerly benzidine-exposed workers.

2052 GENETIC VARRIATION IN TGF-BETA1 BUT NOT ANTIOXIDANT GENES IS ASSOCIATED WITH PROGRESSIVE MASSIVE FIBROSIS IN COAL WORKERS.

B. Yucesoy, V. J. Johnson, M. L. Kashon, K. Fluharty, P. Willard, V. Vallyathan and M. I. Luster. *Health Effects Laboratory Division, NIOSH/CDC, Morgantown, WV.*

Progressive massive fibrosis (PMF) is the severe form of coal worker's pneumoconiosis. Although the severity of PMF is mostly dependent on the total amount and duration of dust exposure, genetic factors also play an important role in the devel-

opment of disease and modify the individual susceptibility. Studies have implicated reactive oxygen species (ROS) in the pathogenesis of fibrosis and other lung diseases. Many chemicals and physical agents including mineral dusts are potent generators of ROS. Lung tissue is protected against ROS by a variety of antioxidant mechanisms such as superoxide dismutases (SOD) and glutathione s-transferases (GSTs). Transforming growth factor beta (TGFβ) is a key profibrotic growth factor implicated in fibrosis, including the deposition of extracellular matrix proteins. Antioxidant enzyme and cytokine genes are subject to polymorphisms in their regulatory regions which affect their expression level and thus contribution to disease process. This study was undertaken to examine the association between the functional polymorphisms of TGFβ and antioxidant enzyme genes and progression of PMF as well as possible gene-gene and gene-environment interactions. We genotyped DNA collected from lung autopsy tissues from 270 miners diagnosed with PMF as well as in 270 control miners with no disease using a case-control study design. Polymorphisms in GSTP1, GSTM1, GSTT1, MnSOD and TGFβ were anylised by Taqman® assay to determine each genotype. Our results showed an association of PMF prevalence with TGFβ1 (-509) TT genotype (OR 1.77; 95% CI 1.03-3.09), whereas no statistically significant differences in the allele frequencies of antioxidant genes were observed between the cases and controls. The results suggest that interactions of genetic background with environmental exposure may affect the pathogenesis of PMF.

2053 GENETIC INFLUENCES ON HUMAN ENDOTHELIAL CELL FUNCTION AND SURVIVAL: INFLUENCE OF THE NOS3 EXON7 (GLU298ASP) AND ACE (I/D) POLYMORPHISMS ON GLUCOSE AND FREE 3-NITROTYROSINE INDUCED CELL TOXICITY *IN VITRO*.

M. S. Joshi[2, 1] and J. A. Bauer[1, 2]. [1]*Center for Cardiothoracic Medicine, Columbus Children's Research Institute, Columbus, OH and* [2]*Division of Pharmacology, The Ohio State University, Columbus, OH.* Sponsor: C. Smith.

Genetic polymorphisms of NOS3 and ACE have been implicated in diabetes related cardiovascular disease risk in humans. The functional relevance of NOS3 and ACE genetic variations to endothelial cell function is largely unstudied. The objective of this study was to test the functional relevance of NOS3 (Glu298Asp) polymorphism and ACE (I/D) polymorphism to the endothelial cells in-vitro. The central hypothesis was that the presence of these genetic polymorphisms alters endothelial cell sensitivity to glucose and 3-nitrotyrosine, two known diabetes-related vascular toxicants. Primary cell preparations (HUVECs) were initially screened for NOS3 and ACE genotypes. Unstimulated growth characteristics were in initially investigated and cells were incubated with various concentrations of glucose (10, 20, and 30mM), free 3-nitrotyrosine (10nM-5mM), or a combination of these two toxicants. No significant differences in unstimulated growth rates were observed among genotype groups (n=3-6 per group), but significant differences in glucose induced cell death (at 20mM Glucose: 9.27%: 30.35%: 28.69%; Glu/Glu: Glu/Asp: Asp/Asp. $p<0.05$) and free 3-nitrotyrosine induced cell death (LC50 values: 12.94±1.38 μM: 1.55±1.48 μM: 10.78±1.20 μM; Glu/Glu: Glu/Asp: Asp/Asp. $p<0.05$) were observed among the NOS3 genotypes. Combined exposures of glucose/3NT caused increased toxicity among the NOS3 genotypes. In contrast, no differences were observed among the ACE genotypes in terms of their responses to these concentrations of the toxic agents. These data demonstrate that the exon 7 NOS3 genotype may an important predictor of or mechanistically involved in endothelial vulnerability to these two diabetes related toxicants, whereas the ACE I/D genotype is apparently less important. Thus the NOS3 (Glu298Asp) genetic variation may play a specific role in vulnerability to diabetes related vascular complications

2054 PARAOXONASE STATUS IN A MEXICAN POPULATION AND ITS RELATIONSHIP TO THE SUSCEPTIBILITY TO DNA DAMAGE ON CULTURED HUMAN LYMPHOCYTES TREATED WITH METHYL-PARATHION AND PARAOXON.

E. Rojas-Garcia, L. Vega L, M. J. Solis-Heredia and B. Quintanilla-Vega. *Toxicology Section, CINVESTAV-IPN, Mexico City, D.F., Mexico.*

Human paraoxonase (PON) is an enzyme that participates in the detoxification of organophosphorus pesticides (OP). PON polymorphisms responsible for different catalytic activities and expression levels have demonstrated the relevance of PON in OP toxicity (mainly acethylcholinesterase inhibition), and the importance of evaluating PON status (genotype/phenotype). No information is available about the role of PON in the susceptibility to DNA damage. The aim of this study was to evaluate the role of PON status in the susceptibility to cytogenetic effects on cultured human lymphocytes treated with methyl-parathion or paraoxon. Serum PON activities were assayed using phenylacetate and paraoxon as substrates. Three PON variants (−108, 55 and 192) were evaluated by PCR-RFLP. Induction of sister-chromatid exchange and mitotic index were determined in cultured lymphocytes added with homologous serum from two individuals with the haplotypes PON $C_{-108}L_{55}R_{192}$ ("resistant") or two individuals with the haplotypes PON $T_{-108}M_{55}Q_{192}$ ("susceptible"). Participants (n=74, 28.6 years old) were individuals of both sexes. PON activity with paraoxon was 632.9 IU/L (range 123.4-1645.6), and with phenylacetate was 199.2 IU/ml (range 85.2-422). Genotype frequencies for PON_{108} were 0.14CC, 0.5CT and 0.36TT; for PON_{55} were 0.09MM, 0.27ML and 0.64LL, and for PON_{192} were 0.24RR, 0.33QR and 0.43QQ. Enzymatic activities were different ($p<0.005$) according to genotype. Paraoxon and methyl-parathion showed cytogenetic effects in cultured lymphocytes but a difference in response according to PON genotype was observed only with paraoxon: lymphocytes from "resistant" individuals had lower cytogenetic effect than the "susceptible" individuals. The difference between paraoxon and parathion effect could be explained by the fact that PON hydrolyzes oxons and not the parent compound. Our results suggest that PON could have an important role in the cytogenetic effects caused by OP exposure.

2055 THE ARYL HYDROCARBON RECEPTOR 1 (AHR1) LOCUS IS HIGHLY POLYMORPHIC IN ATLANTIC KILLIFISH (FUNDULUS HETEROCLITUS): RELATIONSHIP TO DIOXIN RESISTANCE.

M. E. Hahn[1], B. R. Evans[1, 2], S. I. Karchner[1] and D. G. Franks[1]. [1]*Biology, Woods Hole Oceanographic Institution, Woods Hole, MA and* [2]*Biology, Boston University, Boston, MA.*

AHR agonists such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and polynuclear aromatic hydrocarbons (PAHs) are highly toxic to most vertebrate animals. To understand the role of the AHR pathway in differential sensitivity to TCDD and PAHs, we are studying populations of the Atlantic killifish (Fundulus heteroclitus) that differ in their sensitivity to the toxic and biochemical effects of these compounds. Killifish inhabiting New Bedford Harbor (MA, USA), a federal Superfund site, have developed heritable resistance to AHR agonists. To investigate the mechanism of resistance and the possible role of AHR genetic variability, we cloned killifish AHR1, AHR2, ARNT2, and AHR repressor. AHR1, but not AHR2, ARNT, or AHRR, is differentially expressed between dioxin-sensitive and -resistant fish. Sequencing of AHR1 cDNAs from multiple individuals from several sites in Massachusetts, New York, and New Jersey revealed extensive polymorphism at the AHR1 locus. We identified 39 single nucleotide polymorphisms, 14 of which were non-synonymous. Alleles were assigned to three groups: AHR1*1, AHR1*2, and AHR1*3. AHR1*1 alleles were under-represented in a New Bedford Harbor fish (dioxin resistant) as compared to fish from Scorton Creek, MA (dioxin-sensitive). Initial functional analysis of the two most divergent variants (AHR1*1A and AHR1*3A) expressed by *in vitro* transcription and translation indicated similar [3H]TCDD-binding affinities. In transient transfection assays using mammalian cells, AHR1*1A and AHR1*3A exhibited similar transactivation potentials in the presence of TCDD. Thus, the killifish AHR1 locus is highly polymorphic and allele distributions differ between some dioxin-sensitive and dioxin-resistant populations. The role of AHR1 polymorphisms in the resistant phenotype remains uncertain. (Superfund Basic Research Program (5P42 ES07381) and the Hudson River Foundation.)

2056 EFFECT OF ALDH2 GENE POLYMORPHISMS ON THE METABOLISM AND TOXICITY OF 2-ETHOXYETHANOL IN THE EXPOSED WORKERS.

R. WANG[1], M. Suda[1], X. Gao[2], B. Wang[2] and T. Honma[1]. [1]*Department of Health Effects Research, National Institute of Industrial Health, Kawasaki, Kanagawa, Japan and* [2]*Beijing Institute of Industrial Hygiene and Occupational Diseases, Beijing, China.* Sponsor: M. Chiba.

2-Ethoxyethanol (ethylene glycol monoethyl ether) has been known to be toxic to testes and blood system. It is metabolized by alcohol dehydrogenase and CYP P450 2E1 to ethoxyacetic aldehyde, and then transformed by aldehyde dehydrogenase (ALDH) to ethoxyacetic acid, which is excreted in urine. It is not clear which metabolite, the aldehyde or the organic acid, plays the role in the occurrence of the injuries induced by the solvent. ALDH2 is the major enzyme in the metabolism of many short chain alcohols. The single nucleotide polymorphisms at nucleotide 1510 (G/A) of the gene result in a substitution of Glu to Lys at amino acid position 487. Approximately 30% of the Orientals possess ALDH2*2 allele encoding the enzyme without activity. In this study, we investigated the toxic effects of 2-ethoxyethanol exposure among workers, and analyzed whether ALDH2 polymorphisms exerts any effect on the toxicity of the compound. It was found that the quantity and quality of sperm were both decreased in the males exposed to high concentration of 2-ethoxyethanol; the organic solvent seemed not to affect the blood concentrations of the sex related hormones such as testosterone, LH, FSH at

the present exposure levels; the RBC count and Hg content were decreased by the exposure to the solvent; liver function was normal even in the exposed subjects; the genetic polymorphisms of ALDH2 affected the toxicity of 2-ethoxyethanol in both the spermatogenesis and the hematopoiesis, and the ALDH2 *1/*2 seemed to be a protective factor from the damage caused by the compound.

2057 COMPARATIVE ASSESSMENT OF THE INHIBITION OF HUMAN RECOMBINANT CYP19 (AROMATASE) BY AZOLES USED AS FUNGICIDES IN AGRICULTURE, AS ANTIMYCOTIC DRUGS, AND FOR TUMOR THERAPY.

W. K. Lutz[1], K. Scholz[1], E. R. Troesken[1], R. W. Lutz[2], W. Voelkel[1] and J. A. Zarn[3]. *[1]Toxicology, University of Wuerzburg, Wuerzburg, Germany, [2]Statistics, Swiss Federal Institute of Technology, Zuerich, Switzerland and [3]Food Science, Swiss Federal Office of Public Health, Zuerich, Switzerland.*

Azole compounds (imidazoles, triazoles) are widely used as antifungal agents in agriculture and as antimycotic drugs in medicine. They are also used for the treatment of estrogen-dependent conditions, e.g. in tumor therapy. The antifungal/antimycotic activity is due to their inhibitory activity against CYP51 (lanosterol-14 alpha demethylase), the interference with the sex hormone balance is based on their inhibition of CYP19 (aromatase). For antifungal use, inhibition of CYP19 is a side effect that may affect the endocrine system. The inhibitory potency may therefore be a useful quantitative indicator of toxicity. Human recombinant CYP19 from Gentest was used in a fluorimetric assay for enzyme inhibition, with dibenzylfluorescein as substrate. Dose responses for 20 azoles were established in duplicate and the data were analyzed statistically with a nonlinear mixed-effects model using a probit curve with a logarithmic dose scale and attributing a random effect to the start value of the fluorescence. IC50 values of 13 fungicides ranged from 0.012 to 37 μM, the range of five antimycotics was from 0.014 to >88 μ M. Imidazoles tended to be more potent than triazoles. The two tumor therapeutic agents fadrozol and letrozol showed IC50 values of 0.0055 and 0.011 μM, respectively. The data indicate that azoles used as antifungal agents or antimycotic drugs can be as potent inhibitors of aromatase as the drugs used in tumor therapy. For the development of new antifungal agents, this activity should be taken into consideration. It may also be of interest to investigate the steroid hormone balance in humans treated with azole antimycotics and to monitor azoles used for crop protection in farmers and exposed wildlife.

2058 HUMAN EXPOSURE TO IMIDACLOPRID FROM DOGS TREATED WITH ADVANTAGE®.

M. Craig[2], R. C. Gupta[1], T. D. Canerdy[2] and D. A. Britton[1]. *[1]Toxicology, Murray State University, Hopkinsville, KY and [2]Agriculture, Murry State University, Murray, KY.*

The objective of this investigation was two-fold: (1) to determine the transferable residue of imidacloprid in gloves worn while petting experimental household dogs after the application of Advantage®and (2) to determine the imidacloprid residue in the dog's blood. Advantage® contains 9.1% imidacloprid, which controls fleas on dogs for up to 30 days. Imidacloprid produces toxicity by interacting with nicotinic receptors. Advantage® (240 mg imidacloprid/dog) was applied topically to six household dogs. The glove and blood samples were collected at 24hr, 72hr, and then on a weekly basis for 5 weeks post-Advantage® application. The glove samples were collected by petting each dog for five minutes while wearing a different glove per dog. The blood samples (5 ml from each dog) were collected into EDTA tubes. The imidacloprid residue was determined in the blood extracts and glove samples using RP-HPLC. The highest levels of imidacloprid residues were detected at the 24 hr interval in both the glove (254.163.00 ppb) samples. The blood imidacloprid residue was reduced by 1/3 at the 72 hr interval (18.73±2.00 ppb) and was not detected after 1 week. Imidacloprid residue in the glove samples decreased approximately 1/3 between each collection interval. The residue of imidacloprid in the glove extract by the fourth week was very low (0.08±0.02 ppm) and not detected by the fifth week. The findings suggested that following topical application of Advantage®, imidacloprid residue can be detected in the dog's blood for up to 72 hrs, and transferable residue on the dog's coat can be detected for up to 4 weeks. Repeated exposure to imidacloprid may pose possible health risks to veterinarians, veterinary technologists, and dog handlers.

2059 HUMAN EXPOSURE TO SELAMECTIN FROM DOGS TREATED WITH REVOLUTION®.

T. M. Acosta, R. C. Gupta, M. B. Masthay, T. D. Canerdy, R. J. Provost, B. H. Atieh and R. J. Keller. *Toxicology, Murry State University, Hopkinsville, KY.*

This study was undertaken to determine selamectin residue in dog's blood and in gloves worn while petting dogs after Revolution®application. Revolution®contains the active ingredient selamectin (a semi-synthetic avermectin), which controls ecto-and endoparasites including adult fleas, flea eggs, ticks, heartworms, ear mites, and sarcoptic mange in dogs, for 30 days. Revolution® was applied topically on a group of six adult household dogs (240mg selamectin/dog). The glove worn for 5 minutes while petting the dog and a serial blood sample (5ml/dog) were collected *via* the jugular vein at 0 hr, 24 hr, and 1, 2, 3, 4, and 5 weeks post-Revolution® application for selamectin residue determination. At no time during the study did the dogs show any signs of toxicity, weight loss, or change in body temperature. Extracts of the blood and the gloves were analyzed for selamectin residue using RP-HPLC, coupled with a UV detector (246nm). Selamectin standard used for peak identification and quantitation was purified from Revolution®. Selamectin residue was detected in the blood (10.26±1.06 ng/ml) only at 72 hrs post-Revolution®application, probably due to its poor dermal absorption and rapid elimination from the circulation. In the glove extracts, the highest concentration of selamectin (518.90±66.80 ppm) was detected 24 hrs after Revolution®application. Transferable residue of selamectin in gloves from dog's coat was detected at a lesser magnitude one week after Revolution®application, and that followed by a further descending trend during the 2nd, 3rd, and 4th weeks. No selamectin residue was detected in the glove extracts after the 5th week. This appears to be due to rapid elimination of selamectin from the dog's coat. In spite of selamectin's binding to the sebaceous glands of the skin, gloves contained significant transferable residue. Thus, these findings suggest that repeated exposure to selamectin can pose potential health risks, especially to veterinarians, veterinary technologists, dog trainers/handlers, and pet owners.

2060 MODULATION OF HEPATIC CYTOCHROME P450S IN RATS AND MICE BY TRIAZOLE CONAZOLES.

G. Sun[1], A. K. Goetz[2], D. Tully[1], G. R. Lambert[1], D. J. Dix[1] and S. Nesnow[1]. *[1]NHEERL, Research Triangle Park, NC and [2]North Carolina State University, Raleigh, NC.*

Conazoles are azole antifungal agents used as both pesticides and drugs. Some of these compounds are hepatocarcinogenic in mice and some can induce thyroid tumors in rats. The modes of conazole-based toxicity and tumorigenicity are not known. Many N-substituted azoles can induce and/or inhibit mammalian hepatic cytochrome P450s that are responsible for the metabolism of endogenous as well as exogenous compounds including drugs and xenobiotic chemicals. In the present study, four triazole-containing conazoles: fluconazole, myclobutanil, propiconazole, and triadimefon, were studied for their effects on hepatic P450s in Sprague-Dawley rats and CD-1 mice. Alkoxyresorufin O-dealkylation (AROD) methods were used as measures of P450 enzyme activities. The selectivities of AROD were studied using recombinant rat liver P450s. Animals were administered the following compounds (mg/kg body weight) by gavage daily for 14 days: fluconazole (2, 25, or 50), myclobutanil (10, 75, or 150), propiconazole (10, 75, or 150), or triadimefon (5, 50, or 115). Animals were sacrificed 24 hrs after the last dosing and hepatic microsomes were prepared. Each conazole significantly induced pentoxyresorufin O-dealkylation (PROD) and benzyloxyresorufin O-dealkylation (BROD) at mid and high doses in liver microsomes of both rats and mice (high dose fold-inductions: rats/fluconazole: BROD-7.1, PROD-5.3; rats/myclobutanil: BROD-13, PROD-8.1; rats/propiconazole: BROD-5.7, PROD-7.3; rats/triadimefon: BROD-28, PROD-18; mice/fluconazole: BROD-7.2, PROD-8.6; mice/myclobutanil: BROD-2.7, PROD-2.3; mice/propiconazole: BROD-3.8, PROD-5.4; mice/triadimefon: BROD-2.4, PROD-2.6). The inductions of PROD and BROD were dose dependent. No induction or slight induction of methoxyresorufin O-dealkylation or ethoxyresorufin O-dealkylation was detected. Triadimefon and fluconazole were the most potent AROD inducers in rats and mice respectively. Our results indicate that these four conazoles induced cytochrome 2B and/or 3A families of isozymes. This abstract does not reflect EPA policy

2061 DEVELOPMENTAL EFFECTS OF CHLORPYRIFOS EXTEND BEYOND NEUROTOXICITY: CRITICAL PERIODS FOR IMMEDIATE AND DELAYED-ONSET EFFECTS ON CARDIAC AND HEPATIC CELL SIGNALING.

A. Meyer[1, 2], F. J. Seidler[1] and T. A. Slotkin[1]. *[1]Pharmacology & Cancer Biology, Duke University Med. Ctr, Durham, NC and [2]Escola Nacional de Saude Publica, Rio de Janeiro, Brazil.*

The fetal and neonatal neurotoxicity of chlorpyrifos (CPF) and related insecticides is a major concern. Developmental effects of CPF involve mechanisms over and above cholinesterase inhibition, notably events in cell signaling that are shared by non-neural targets. In the current study, we evaluated the immediate and long-term effects of CPF exposure of rats during different developmental windows (gestational days 9-12 or 17-20, postnatal days 1-4 or 11-14), on the adenylyl cyclase (AC) signaling cascade in the heart and liver. In addition to basal AC activity, we assessed the responses to direct AC stimulants (forskolin, Mn2+), to isoproterenol

and glucagon, which activate signaling through specific membrane receptors, and to NaF, which activates the G-proteins that couple the receptors to AC. Few immediate effects on AC were apparent when CPF doses remained below the threshold for systemic toxicity. Nevertheless, CPF exposures on GD9-12, GD17-20 or PN1-4 elicited sex-selective effects that emerged by adulthood (PN60), whereas later exposure (PN11-14) elicited smaller, nonsignificant effects, indicative of closure of the window of vulnerability. Most of the effects were heterologous, involving signaling elements downstream from the receptors, and thus were shared by multiple inputs; superimposed on this basic pattern, there were also selective alterations in receptor-mediated responses. These results suggest that the developmental toxicity of CPF extends beyond the nervous system, to include cell signaling cascades that are vital to cardiac and hepatic homeostasis. Future work needs to address the potential implications of these effects for cardiovascular and metabolic disorders that may emerge long after the end of CPF exposure. Support: USPHS ES10387 and ES10356.

2062 INHIBITION OF DIAZINON METABOLISM BY CHLORPYRIFOS IN RAT LIVER MICROSOMES.

H. Wu, C. Timchalk and T. Poet. *Cntr for Biological Monitoring and Modeling, Battelle, Pacific NW Division, Richland, WA.*

Chlorpyrifos (CPF) and diazinon (DZN) are major organophosphorus pesticides that are structurally and mechanistically similar, but have very different rates of metabolism. Their toxicity is mediated *via* CYP450 metabolism to oxons. Detoxification of the oxons is mediated by CYP450 and A-esterases, resulting in the formation of trichloropyridinol from CPF and 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) from DZN. Since exposures often involve more than one pesticide, this study was conducted to assess the effect of co-incubation with both CPF and DZN on the metabolism of DZN. Substrate concentrations of DZN ranging from 20 to 575 μM were incubated with 0, 100, 300, or 750 μM CPF for 15 min and bioactivation to DZN-oxon and deactivation to IMHP quantified. Data was analyzed using Lineweaver-Burke (1/v; 1/s) plots, by fitting the Michaelis-Menten equation to the data with non-linear regression using SlideWrite Plus®, and by fitting the data to a mathematical model written in Simusolv®, which employed Michaelis-Menten equations including inhibition constants to describe metabolism. The 1/v; 1/s plots were used to estimate the types of inhibition. Metabolism to IMHP appeared to be competitively inhibited whereas metabolism to the oxon was uncompetitive (or suicide). Fitting the data with non-linear regression, apparent Km concentrations vary for the production of IMHP, but not the apparent Vmax; whereas, the apparent Vmax rates change for the production of oxon, confirming the types of inhibition. Inhibitory rate constants (Ki) for oxon and IMHP metabolism were ~250 μM and ~50 μM, respectively. Using the different methods to predict Vmax and Km, apparent Vmax and Km with inhibition, and Ki resulted in similar estimations. The mathematical model has the advantage of providing a methodology by which all parameters are considered at once. The relatively high Ki values indicate the importance of metabolic interactions on kinetics of *in vivo* co-exposures to DZN and CPF may be limited to high acute dose exposures. (Sponsored by CDC/NIOSH Grant R01 OH03629-01A2 and EPA grant R828608).

2063 COMPARISON OF CHLORPYRIFOS-OXON AND PARAOXON ACETYLCHOLINESTERASE INHIBITION DYNAMICS: POTENTIAL ROLE OF A PERIPHERAL BINDING SITE.

A. Kousba[1], L. G. Sultatos[2], T. Poet[1] and C. Timchalk[1]. *[1]Center for Biological Monitoring and Modeling, Pacific NW Nat'l Lab., Richland, WA and [2]UMDNJ, Newark, NJ.*

The primary mechanism of action for organophosphorus (OP) insecticides involves the inhibition of acetylcholinesterase (AChE) by active oxon metabolites. This inhibition has been attributed to the phosphorylation of the serine hydroxyl group in the active site of AChE. Inhibition is described by the bimolecular rate constant (Ki), which is used to assess OP inhibitory capacity. It has been reported that the Ki for inhibition of AChE by certain oxons changes as a function of oxon concentration, and that this phenomenon could be explained by the presence of a peripheral binding site. When such a putative site would be occupied, the capacity of additional oxon molecules to phosphorylate the active site is reduced. Therefore, the objective of the current study was to evaluate the *in vitro* inhibition of AChE by a wide range of chlorpyrifos oxon (CPO) and paraoxon (PO) concentrations (0.5 pM -100 nM) using the Ellman assay combined with a dynamic model. The results indicated that the Ki is inversely changed in proportion to the oxon concentrations. The Ki determined using high oxon concentrations, were 238 and 22 μM-1h-1 and the spontaneous reactivation rates were 0.087 and 0.078 h-1 for CPO and PO, respectively. While the estimated Ki using high oxon concentrations were similar to those reported previously, the Ki estimates following 1 pM oxon, which have not been reported previously, were >1000-fold higher. Surprisingly, the Ki estimated using 1 pM CPO was similar to the Ki estimate using an equivalent PO concentration (0.18 and 0.25 pM-1h-1, respectively). This implies that both oxons exhibit similar inhibitory potency at the lower concentration, in contrast to the marked difference following higher concentrations, which was explained by considering a peripheral binding site. The potential of a peripheral site should be considered when modeling AChE kinetics particularly at low environmentally relevant concentrations. (Sponsored by CDC/NIOSH Grant R01 OH03629-01A2 and EPA grant R828608)

2064 PHARMACOKINETIC & PHARMACODYNAMIC INTERACTIONS OF A BINARY MIXTURE OF CHLORPYRIFOS AND DIAZINON IN THE RAT.

C. Timchalk, T. S. Poet, M. N. Hinman, A. L. Busby and A. Kousba. *Center for Biological Monitoring and Modeling, Pacific NW Division, Richland, WA.*

Chlorpyrifos (CPF) and diazinon (DZN) are organophosphosphorus (OP) insecticides, with the potential for concurrent exposures. Their oxon metabolites are potent inhibitors of cholinesterases (ChE). This study evaluated the impact of an acute binary OP exposure on dosimetry and ChE inhibition in rats. Groups (3-4/time point) of male S-D rats were orally administered CPF, DZN or a CPF/DZN mixture (0, 15, or 60 mg/kg) and blood and brain were collected at 0, 3, 6, 12 and 24 hr post-dosing. CPF, DZN and their respective metabolites trichloropyridinol (TCP) and 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) were quantified in blood and/or urine using gas chromatography. A modified Ellman method was used to measure ChE inhibition in blood and brain. Co-exposure at 15 mg/kg, did not appreciably alter the pharmacokinetics of CPF, DZN or TCP. The total amount of urinary TCP was decreased ~50% for the co-exposed group, but the amount of urinary IMHP was not altered. Co-exposure to 60 mg/kg delayed and increased the Cmax for both CPF (1.6x) and DZN (7.2x), and resulted in the respective blood AUCs increasing 145 and 422%. The AUC for TCP decreased to 82%, but was slightly increased (107%) for IMHP. For the co-exposed group the total amount of urinary TCP was decreased ~60%, but did not appreciably alter the amount of IMHP excreted relative to the single exposure. These results suggest that both CPF and DZN are capable of inhibiting each other's metabolism, but the effect is observed at higher doses (>15 mg/kg). A dose-dependent inhibition of ChE activity was also noted in plasma, RBC and brain homogenates for both the single and co-exposures to CPF and DZN. In all tissues, CPF exposure resulted in greater ChE inhibition than DZN, however the overall ChE response appeared to be additive for the co-exposures. These results characterize both the pharmacokinetic and pharmacodynamic interactions of CPF and DZN in the rat and will be used to further develop a binary kinetic/dynamic model for OPs. (Sponsored by CDC/NIOSH R01OH03629-01A2)

2065 CONTINUOUS SYSTEMS MODELING OF THE INTERACTIONS OF PARAOXON WITH HUMAN RECOMBINANT ACETYLCHOLINESTERASE.

C. A. Rosenfeld and L. G. Sultatos. *Pharmacology and Physiology, UMD- New Jersey Medical School, Newark, NJ.*

Previous studies from this laboratory have reported that the inhibitory constant k_i for the inhibition of acetylcholinesterase by the organophosphate paraoxon (*O, O*-diethyl *p*-nitrophenyl phosphate) changes as a function paraoxon concentration. The k_i values were calculated by the application of continuous systems modeling to experimentally determined residual acetylcholinesterase activity following the addition of various paraoxon concentrations. The current study used the fluorescent compound N-methylacridinium to ascertain an independent measure of active site concentration for human recombinant acetylcholinesterase, which was then used in the model for a precise determination of k_i over a range of oxon concentrations at both 24°C and 37°C. Incubations at 24°C used oxon concentrations ranging from 78 pM-750 nM with active sites concentrations ranging from 3.01-4.87 pM. The k_i for 750 nM paraoxon was 0.111 $nM^{-1}h^{-1}$ whereas the k_i for 78 pM paraoxon was 0.410 $nM^{-1}h^{-1}$. Incubations at 37°C used oxon concentrations ranging from 15 pM-100 nM with active sites concentrations ranging from 4.36-5.60 pM. The k_i for 100 nM paraoxon was 0.146 $nM^{-1}h^{-1}$ whereas the k_i for 78 pM paraoxon was 4.21 $nM^{-1}h^{-1}$. These data suggest that individual paraoxon molecules at the lower concentration have a greater capacity to inhibit acetylcholinesterase than individual paraoxon molecules at the higher concentration (consistent with previous data from our lab). At 37°C the difference from the highest k_i to the lowest was approximately 30 fold whereas at 24°C the difference from the highest k_i to the lowest was approximately 4 fold. These data indicate that the kinetic scheme utilized for the derivation of the inhibitory constant k_i is inadequate to explain the interaction of human recombinant acetylcholinesterase and paraoxon.

2066 PERMETHRIN AND NICOSULFURON MIXTURES ALTER TOXICITY AND GLUTATHIONE S-TRANSFERASE ACTIVITY IN CORN EARWORMS (HELICOVERPA ZEA).

O. Jacobs, P. S. Burns, R. Massa and S. L. Chao. *Natural Sciences, Fayetteville State University, Fayetteville, NC.* Sponsor: J. Casida.

Recent studies of synergism or potentiation related to pesticide mixtures and neurotoxicity have affirmed the need to explore mechanisms underlying such interactions. This study explores synergism with mixtures of a widely used insecticide, herbicide, and ammonium nitrate found in fertilizers. Corn earworm larvae (Helicoverpa zea) were exposed to the following chemicals alone and in mixture: permethrin, nicosulfuron, and ammonium nitrate. Toxicity of larvae was measured using a dip bioassay. Increased mortality was observed consistently with mixtures of nicosulfuron and permethrin; however nitrate and mixtures of nitrate did not consistently increase toxicity. Acetylcholinesterase (AChE) and glutathione s-transferase activities were measured in larvae exposed to above pesticide mixtures. The increased toxicity observed with pesticide mixtures did not appear to be associated with changes in AChE activity; however, glutathione s-transferase activity was altered with exposure to mixtures of above pesticides.

2067 ROLE OF CYP3A METABOLISM IN HEPG2 CYTOTOXICITY OF ALACHLOR.

S. R. Miranda and S. A. Meyer. *Toxicology, University of Louisiana at Monroe, Monroe, LA.*

Alachlor [2-chloro-2', 6'-diethyl-N-(methoxymethyl)acetanilide] is a chloroacetanilide herbicide used in agriculture and is a drinking water contaminant (MCL = 2ppb). Previous studies in our laboratory have shown that alachlor is acutely hepatotoxic to rats at doses > 400mg/kg. N-dealkylation of alachlor is believed to be rate limiting to the formation of a reactive metabolite, diethylbenzoquinoneimine (DEBQI) and Coleman et al (1999, Chem.Biol.Interactions, 122:27-39) have shown that CYP3A4 catalyzes alachlor N-dealkylation. We propose that CYP3A mediated N-dealkylation leading to the ultimate toxicant DEBQI is causal to the hepatotoxicity of alachlor. The objectives of this study were to determine the toxicity of 2, 6-diethylaniline (DEA), a precursor to DEBQI, and secondly to examine the role of CYP3A in alachlor hepatotoxicity. HepG2 human hepatoblastoma cells were chosen as a model system since these cells express constitutive and dexamethasone-inducible CYP3A7 and can be further induced by dexamethasone to express CYP3A4 (Krusekopf, et al. 2003. Eur. J. Pharmacology 466:7-12). Release of lactate dehydrogenase (LDH) into the culture medium was used as the endpoint for cytotoxicity. In the present studies, we observed that alachlor hepatotoxicity was time-dependent with an EC50 of 700µM and 400µM after treatment for 6 hr and 24 hr, respectively. DEA at concentrations up to 800µM was not toxic after 24 hr. Further, pretreatment with dexamethasone (25µM, 48 hr) resulted in a two-fold increase in alachlor toxicity. Collectively, these data suggest that CYP3A-mediated metabolic activation of alachlor to a reactive metabolite other than DEBQI may be causal to hepatotoxicity. Support: Johnston Science Foundation and Louisiana Board of Regents.

2068 DIAZINON ALTERS SPERM CHROMATIN STRUCTURE BY NUCLEAR PROTAMINE PHOSPHORYLATION.

B. Pina-Guzman[1], B. E. Reyes-Marquez[2], M. J. Solis-Heredia[1] and B. Quintanilla-Vega[1]. [1]*Toxicology Section, CINVESTAV-IPN, Mexico City, D.F, Mexico and* [2]*Department of Cell Biology, CINVESTAV-IPN, Mexico City, D.F, Mexico.*

Diazinon (DZN) is an organophosphorous pesticide (OP) widely used in agriculture and pest control in Mexico, which has been associated with male reproductive effects. As an OP compound, DZN might phosphorylate nuclear protamines that pack sperm DNA during the latest steps of spermatogenesis, contributing to its reproductive toxicity. Shortly after their synthesis, protamines are phosphorylated followed by a dephosphorylation process required for the correct binding to DNA. Any alteration in protamine-DNA binding alters the sperm chromatin structure. The aim of this study was to evaluate the effect of DZN on the sperm chromatin structure and its ability to phosphorylate nuclear protamines as the mechanism of action, after a unique dose (8.12 mg/kg, i.p.) in adult male mice. Animals (NMRI, 9-weeks old) were sacrificed 8 and 15 days after treatment and sperm quality, chromatin condensation, phosphorous content in protamines and sperm heads, and phosphorylation sites in protamines were evaluated in spermatozoa from cauda epidydimis and vas deferens. Chromatin condensation was measured by the sperm chromatin structure assay (SCSA) parameters: DFI (αt) and DFI% (COMP-αt). At 8 days post-treatment, DFI and DFI% increased 50% and 4.5 fold, respectively, indicating an alteration on sperm chromatin condensation as well as DNA damage; significant alterations were also shown in sperm *via*bility, motility, and morphology. Increases in the phosphorous content in protamines (50%) and sperm heads (140%) were also observed at this time, as well as in the immunodetected phosphoserine residues at 8 days (99%) and 15 days (33%) post-treatment. These data suggest that spermatozoa exposed during the latest steps of spermatic maturation (late spermatide) were the target cells of DZN exposure. Finally, DFI and DFI% strongly correlated (r=0.8413 and r=0.8431, respectively) with phosphorous content in protamines, suggesting that nuclear protein phosphorylation is involved in the DZN effects observed on sperm chromatin structure.

2069 THE EFFECT OF THIOFLAVIN-T AND PARAOXON ON THE GROWTH PROMOTING FUNCTION OF ACETYLCHOLINESTERASE IN NG108-15 CELLS.

H. M. Campanha and E. J. flynn. *UMD-New Jersey Medical School, Newark, NJ.*

Acetylcholinesterase (AChE) is widely studied for its role in the hydrolysis of Acetylcholine (ACh). This enzymatic activity is inhibited by a number of organophosphate insecticides. Allowable human exposure to insecticide levels are set by their ability to inhibit AChE enzymatic activity, which leads to cholinergic crisis. However, recent evidence has accumulated indicating AChE may also have a non-cholinergic function. For example, studies have shown AChE expression in the cells of the chick neural tube, prior to synaptogenesis. It has also been shown that over expression of AChE leads to process extensions and increased cell adhesion. Concern has been raised that, in humans, this non-cholinergic function of AChE may be altered from the exposure to environmental pesticides and lead to effects on neuronal growth and development. In order to investigate the non-cholinergic function of AChE in the nervous system, we used a cultured hybrid mouse neuroblastoma-rat glioma cell line, NG108-15, which expresses AChE in its undifferentiated and differentiated state. These cells were grown on tissue culture plates on a substratum containing varying amounts of AChE. Following incubation times for up to 96 hours, the degree of cell adherence was assessed by staining attached cells with crystal violet. There was an increase in cell adherence dose related to the amount of AChE added to the substratum. The AChE induced adherence was decreased when cells were grown in the presence of Paraoxon (PO) at concentrations of 0.5 mM and 1 mM. Similarly, there was a decrease in adherence when NG108-15 cells were grown on AChE coated plates in the presence of Thioflavin-T at concentrations ranging from 1nM to 100 uM, in a dose dependent manner. In addition, cells grown in the presence of PO on uncoated plates, showed a decrease in cell adherence. These observations suggest that PO and Thioflavin-T can interfere with the growth promoting function of AChE by decreasing its effect on cellular adherence.

2070 *IN VIVO* AND *IN VITRO* EFFECTS OF THE ORGANOPHOSPHATE INSECTICIDE TETRACHLORVINPHOS ON CHOLINESTERASE AND CARBOXYLESTERASE ACTIVITIES IN HORSES.

S. Karanth[1], L. Mason[1], T. Holbrook[2], C. MacAllister[2] and C. Pope[1]. [1]*Physiol Sciences, Oklahoma State University, Stillwater, OK and* [2]*Vet. Clin Sciences, Oklahoma State University, Stillwater, OK.*

Tetrachlorvinphos (TCVP) is used in "feed-through" larvicides for fly control around horse stables. Relatively little is known, however, on the effects of TCVP on target (acetylcholinesterase, AChE) or non-target (butyrylcholinesterase, BChE; carboxylesterase, CE) enzymes in the horse. While erythrocytes exclusively express AChE, plasma ChE activity was primarily BChE and muscle contained predominantly AChE. Plasma ChE activity was substantially more sensitive (>10000 fold) *in vitro* than erythrocyte ChE activity to TCVP (IC_{50}: plasma, 97 nM; erythrocyte, >1mM). Muscle ChE and plasma CE were also markedly less sensitive than plasma ChE (IC_{50}, 116 uM and 27 uM respectively). Adult, male horses (n=3/group, 364-590 kg) were given either sweet feed alone or sweet feed supplemented with a TCVP-containing larvicide (Equitrol®) daily for 21 consecutive days to provide approximately 700 mg TCVP per animal/day (according to label instructions). Clinical signs (vital signs, abdominal auscultation, ophthalmic exam, body temperature) were recorded on a daily basis. Heparinized blood samples were taken at days -1, 1, 3, 7, 21, 28 and 42 while muscle (semimembranous) biopsies were taken under aseptic conditions on days -1 and 21. No signs of toxicity were noted at any timepoint. Plasma ChE activity was significantly inhibited (33%) in larvicide-treated horses after 1 day of treatment and apparent peak inhibition (68 and 71%) was noted at days 7 and 21. Following cessation of dosing, plasma ChE activity recovered (46 and 83% of control on days 28 and 42). Neither erythrocyte ChE activity nor plasma CE activity was affected by larvicide treatment *in vivo*. Muscle ChE activity was surprisingly variable among individual horses (pre-treatment range: 0.50-4.92 nmole.min^{-1}.mg protein), but there was no evidence of treatment-related reduction in muscle ChE activity. The results suggest remarkable tissue- and enzyme-specific differential effects of TCVP on target and non-target enzymes in horses.

2071 *IN VITRO* METABOLISM OF PYRETHROIDS IN RAT LIVER MICROSOMES.

S. J. Godin[1], R. A. Harrison[2], M. F. Hughes[2] and M. J. DeVito[2]. *[1]Toxicology, University of North Carolina, Research Triangle Park, NC and [2]NHEERL, USEPA, Research Triangle Park, NC.*

Pyrethroids are neurotoxic pesticides that bind to sodium channels. These pesticides are used in a variety of agricultural and household applications. In order to determine *in vitro* kinetic parameters for pyrethroids, an LC/MS method was developed for the detection and quantification of deltamethrin, esfenvalerate and cyfluthrin. These chemicals were separated using a Zorbax Eclipse XDB - C18 column (4.6 x 50 mm, 3.5 micron) and an isocratic mobile phase of 90:10 methanol:water with 0.01% formic acid. Using a flow rate of 0.5 ml/minute all chemicals were separated and eluted within 5 minutes. In our LC/MS system (Agilent 1100 series ion trap LC/MS) all three chemicals formed sodium adducts. The adducted molecular ions used to quantify these chemicals were m/z 527.8, 442.1 and 456.0 for deltamethrin, esfenvalerate and cyfluthrin, respectively. Detection limits were as low as 10 ng/ml. Initial kinetic studies were performed with deltamethrin. Liver microsomes were prepared from 65 day old male Long Evans rats. The *in vitro* assay was performed in 1ml of 100 mM Tris (pH 7.5), 1 mg/ml NADPH and 1mg microsomal protein/ml. The reaction was carried out for 0, 5, 10, 20, and 30min, and was done in duplicate. The reaction was started with the addition of deltamethrin at concentrations ranging from 2 - 200nM. The reaction was stopped by the addition of 5 ml of cold n-pentane. Extraction efficiencies were greater than 80%. Nonlinear regression analysis was used to estimate Km and Vmax. Estimates of Km and Vmax for deltamethrin are 53.8 nM and 185 pmoles/min/mg protein, respectively. (This abstract does not represent USEPA policy. SJG supported by NHEERL-DESE, USEPA CT826513)

2072 TOXICITY OF SODIUM METAM IN THE RAINBOW TROUT.

T. Bunch[1], M. A. Haendel[2, 3] and G. S. Bailey[1, 2, 3]. *[1]Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, [2]Linus Pauling Institute, Oregon State University, Corvallis, OR and [3]MFBSC, Oregon State University, Corvallis, OR.*

Sodium metam (sodium methyldithiocarbamate (NaM) CAS# 137-42-8) was the third most used pesticide by volume in 1999. Primarily used as a soil fungicide, it is also a nematocide, herbicide and wood preservative. Applied up to 300 pounds per acre, it has the potential to contaminate groundwater. Therefore, there exists the possibility for human or aquatic organism exposure and the long-term effects of NaM have not been intensely investigated. We have selected the rainbow trout model to initiate studies of NaM toxicity in an aquatic species. Rainbow trout fry (5-months post-spawn) were exposed to varying concentrations between 0 and 100ppm of pure (99%) NaM for 48-hours with solution replacement after 24-hrs in aerated containers. The LC_{50} was determined to be 0.8ppm. Subsequently, a long-term experiment was conducted to examine the consequences of a 24-hour NaM bath exposure to 5-month old fry at concentrations of 0, 0.7 and 0.8ppm. Upon sampling at 14 months, the NaM treated fish exhibited an elevated liver somatic index and a dose-dependent severe hepatic pre-ductular stem cell hyperplasia heretofore un-described in this animal model. In order to determine if NaM causes DNA damage, we performed Comet assays on a rainbow trout hepatoma (RTH) cell line. RTH cells were exposed to NaM concentrations from 1ppm to 130ppm. A significant increase in tail moment was seen at the highest concentration. Based on these observations, we believe NaM exerts significant toxic effects on rainbow trout at concentrations of potential environmental relevance, with liver a primary target organ. Supported by NIH grants ES03850, ESS00210, ES07060.

2073 EVALUATION OF WHITE BLOOD CELL COUNT IN RAT SPLEEN AND THYMUS EXTRACTS USING THE ADVIA 120 HEMATOLOGY ANALYZER.

L. LeSauteur, J. McCartney, L. Huard and Y. Deschamps. *Immunology, CTBR, Senneville, QC, Canada.*

The objective of this work was to verify the use of the ADVIA 120 Hematology analyzer for measuring the total white blood cell count and differential count of spleen and thymus extracts from the Sprague-Dawley rat. Linearity of dilution was determined for the white blood cell count of spleen and thymus extracts. Smears of the spleen and thymus extracts were evaluated microscopically to qualitatively confirm the automated differential counts. Spleen extract samples from 4 rats and thymus extract samples from 5 rats were analyzed on the ADVIA 120 Hematology analyzer; samples were diluted with 0.9% saline to obtain at least 3 different levels of cell counts. Linearity of dilution results provided correlation coefficient values greater than 0.990 for all spleen and thymus samples and were considered acceptable since the correlation was greater than 0.900 and the slope was within 0.7500 and 1.2500. A 1:10 dilution of the extract samples was judged to provide maximal accuracy of the results and to avoid aspiration problems due to sample viscosity. Microscopic evaluation of the spleen and thymus extract smears qualitatively confirmed the automated differential results. In conclusion, the total white blood cell count and differential count obtained on the ADVIA 120 Hematology analyzer shows acceptable reactivity for testing rat spleen and thymus extracts.

2074 ESTABLISHING AN IMMUNOPHENOTYPING SPECIES SPECIFIC BIOLOGICAL CONTROL.

J. Van Ness, J. Sibley, J. Elliott and D. Walker. *Clinical Pathology, Wyeth, Chazy, NY.* Sponsor: M. Seme Nelson.

The FDA Immunotoxicology guidelines for preclinical drug safety testing suggest immune cell phenotyping be utilized as an indicator of immunosuppression. When conducting cell phenotyping in a GLP environment, one must consider the use of a preserved species specific biological control material to maintain interassay consistency and precision. However, currently there are no commercially available control material suitable for phenotyping non-human lymphocytes. For the purpose of generating rat-specific reference material for phenotyping blood and tissue lymphocytes, we excised rat spleens and homogenized the tissue to a single cell suspension. These spleenocytes were prepared for cryo-preservation (at a physiological equivalent density to whole blood) by mixing with culture media, fetal bovine serum, and dimethylsulfoxide. Aliquots of the suspension were placed at -80°C to freeze at a controlled rate. Fresh and thawed aliquots of the control material were stained and analyzed for lymphocyte subpopulation distribution of $CD4^+$, $CD45RA^+$, and $CD8a^+$ cells. To determine stability of, and establish ranges of each subtype within the control material, analysis was performed upon initial isolation, day one, week one, and monthly there after. Our results yielded a consistent recovery of $CD4^+$ (41.3 ± 6.0%), $CD45RA^+$ (23.8 ± 3.7%), and $CD8a^+$ (24.6 ± 3.5%) cells. The total recovery percentage over a 22 week period was 88.9 ± 5.5%. These results compared favorably with the spleenocytes analyzed before preservation. Stability tests are ongoing and will continue until an appreciable degradation is detected. For testing purposes, we generated the control material with and without the use of paraformaldyhide to determine the amount of interference the endogenous fluorescence of paraformadyhide would have. With this method, one can successfully create a suitable control material for rat lymphocyte phenotyping to meet the rigors of a GLP environment.

2075 THE MEASUREMENT OF RABBIT ANTI IDIO-TYPE ANTIBODY USING BIOSENSOR ASSAY.

K. Harada, N. Ohtake, S. Hayashi and J. Kawahara. *Toxicology, Pharmaceutical laboratory in Kirin Brewery Co.Ltd., Maebashi-shi, Japan.* Sponsor: F. Sagami.

The measurement of antibody titer in serum is recquired in preclinical and clinical studies of biotechnology pharmaceutical drugs, and Radioimmunoassay (RIA) and Enzyme immunoassay (EIA) are generally used in these studies. Because these methods can be used only in the limited facilities and/or are not adequate to some pharmaceutical drugs, the development of new method to measure the antibody titer based on the different strategy is needed. We studied in the usefulness of the measurement of the anti idio-type antibody using biosensor assay as one of the basic studies for the development of new method. Rabbits were immunized with Rituxan, pharmaceutical antibody, in complete and incomplete Freund' adjuvant, and antiserum were collected. Rituxan, as full length or Fab fragment, were immobilized to biosensore chip and rabbit anti-rituxan antibody titer were measured with Biacore 3000. We studied the specificity, the intra-assay reproducitibity, the inter-assay reproducibility and the dose-dependency of antiserum to confirm the usefulness of the method using biosensor assay. As results, these items were confirmed to be well. These results suggest that the method of the measurement of anti idio-type antibody in serum using assay is useful.

2076 CELL CHIP TECHNOLOGY - AN ALTERNATIVE METHOD FOR IMMUNOTOXICITY SCREENING.

T. Ringerike[1], E. Ulleras[2], G. Nilsson[2], R. J. Vandebriel[3], J. Dastych[4] and M. Lovik[1]. *[1]Department of Environmental Immunology, Norwegian Institute of Public Health, Oslo, Norway, [2]Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden, [3]Laboratory for Pathology and Immunobiology, National Institute of Public Health and the Environment, Bilthoven, Netherlands and [4]Laboratory of Molecular Immunology, International Institute of Molecular and Cell Biology, Warsaw, Poland.* Sponsor: E. Dybing.

It is important to find new technologies for high throughput immunotoxicity testing. Until recently, most tests for immunotoxicity have been developed and validated in experimental animals. The Cell Chip is an *in vitro* project that aims at using changes in cytokine expression upon exposure to chemicals as a measure of

immunotoxicity. The background for this approach is increasing evidence showing that at least certain types of immunotoxicity, like those leading to hypersensitivity and autoimmunity, are associated with modulation of cytokine production. Mouse T-cell lymphoma cells were transfected with the promoter region of different cytokines coupled to the sequence for green fluorescent protein (GFP). Hence, an increased expression of cytokine will result in an increased level of GFP fluorescence. To examine depression of cytokine expression, cells were incubated in the prescence of an induction mix of Ionomycin and PMA. Cells were incubated with different test compounds with already established immunotoxic potential, and GFP expression was measured by flow cytometry. Compounds were incubated with the cells for 24 hours and in 3 different concentrations. The highest dose tested was selected to result in 10% cytotoxicity, the two other doses were 10% and 1% of this dose. Our results indicate that the prototype immunotox Cell Chip is able to detect inhibition of cytokine expression following incubation with immunosuppressive chemicals such as Cyclosporin A, Rapamycin and Pentamidine. Use with other classes of chemicals (e.g. contact and respiratory allergens) will have to be explored further. (This project is A European Commision Shared-cost Research projct: QLK4-CT-2000-00787 and is patent pending).

2077 IN SEARCH OF A BIOMARKER FOR STRESS-INDUCED IMMUNOMODULATION.

P. Hebert, C. Schwab and S. B. Pruett. *Cellular Biology & Anatomy, LSUHSC-Shreveport, Shreveport, LA.*

Toxicological testing of compounds often is conducted at the maximum tolerated dose. These high doses can cause immunotoxicity. Gross changes indicating a generalized stress response have been used as evidence that immunosuppression is stress-mediated. Regulatory changes require objective evidence prior to claiming that these high doses are immunotoxic only due to the induction of a stress response. To separate immunomodulatory effects of stress (indirect) and chemical compounds (direct), a biomarker would be helpful. Serum corticosteroid (cort) levels in mice have been used to indicate stress levels, but often are obtained a single time during a study. Our lab has found that a good indication of stress that is predictive of immunosuppression in mice is the area under the curve (AUC) of cort *vs.* time as graphed from a time-course experiment. These experiments are time- and labor-intensive and require the use of many animals. We have found that the amount of MHC II expression on the surface of B cells correlates best with the AUC and can be used as a surrogate biomarker for stress-induced immunotoxicity. Our present objective is to find a similar biomarker for stress in rats. Female Sprague-Dawley rats, age 8 to 10 weeks, were subjected to various stressors (exogenous cort, restraint, EtOH). Serum cort data were collected for AUC analysis at 0, 1, 2, 4, 8, 12, and 24 h. Data were analyzed for body and organ (spleen and thymus) total and relative weights, organ cellularity, blood differential counts, cell surface expression of CD4/CD8 and MCH II, caspase activity (thymus), histology and immunohistochemistry. No definitive parameter has emerged as a reliable biomarker in acute (≦24 h) studies. However, 20 mg/kg cort given twice a day at 4 h intervals for 4 days resulted in a decrease of MHC II expression from 55.96 ± 1.90 percent (vehicle) to 50.34 ± 1.48 percent (cort) (p<0.05). These studies emphasize that rats are not just big mice and the search for a stress-related biomarker will be quite different in these rodents.

2078 ROLE OF STRESS-INDUCED IMMUNOTOXICITY IN 28-DAY EXPOSURE STUDIES: CHEMICAL-SPECIFIC HABITUATION AND LACK OF HABITUATION.

S. B. Pruett, Q. Zheng, R. Fan, C. Schwab and P. Hebert. *Cell. Biol. & Anatomy, LSU Health Sciences. Center, Shreveport, LA.*

We have previously documented that the immunotoxicity caused by acute exposure to some chemicals is mediated in part by a neuroendocrine stress response. Following daily exposure for various periods of time, rodents can become habituated to some stressors, leading to a diminished stress response. A few studies suggest that this may also be true for some chemical stressors, but these studies have not been done in a manner consistent with standard pre-clinical or safety testing. In the present study this was done by exposing female B6C3F1 mice to 28 days of daily administration of three compounds previously shown to induce a strong stress response after a single dose: atrazine (37.5, 75, 112.5, and 150 mg/kg, in corn oil i.p.); ethanol (4, 5, 6, and 7 g/kg, at 32 percent v/v in water by gavage); propanil (50, 75, 100, and 150 mg/kg in corn oil, i.p.). Serum corticosterone concentrations were determined at various times after the last dose of each compound, and a separate set of mice was used to determine total and differential blood leukocyte counts, total nucleated cells in the spleen, B cells, T cells, and MHC class II positive cells in the blood and spleen, and splenic NK cell activity. The results demonstrate that mice become habituated to ethanol and propanil (as indicated by minimal corticosterone responses and loss of stress-induced immunosuppression), but not to atrazine (as indicated by corticosterone responses and immunological changes very similar to those noted after a single dose). These results have implications for interpreting immunotoxicity data in pre-clinical and safety testing involving rodents. Supported by NIEHS grant R01 ES09158.

2079 EVALUATION OF ANTI-CD3 INDUCED T-CELL PROLIFERATION ASSAY FOR ASSESSING THE IMMUNOTOXICITY OF VERAPAMIL, NIFEDIPINE, CYCLOSPORIN A, AND FK506.

S. Mittelstadt, B. Hulette, G. Fadayel, M. Hare and F. Gerberick. *Procter & Gamble, Cincinnati, OH.*

It is well known that compounds that adversely effect calcium flux can inhibit T-cell proliferation *in vitro* and immune responses *in vivo*. The purpose of these experiments was to determine if inhibition of anti-CD3-induced T-cell proliferation could be used to rank order the immunosuppressive potential of Verapamil, Nifedipine, Cyclosporin A, and FK506. Cyclosporin A (CsA) and FK506 were chosen because they are known to be immunosuppressive in the clinic whereas evidence for Verapamil is that it is not immunosuppressive in the clinic. To do this, we deployed a T-cell blastogenesis assay that utilized aCD3 as the stimulus for T-cell proliferation. First, we determined the optimal dose of aCD3 to stimulate a robust T-cell proliferation using peripheral blood mononuclear cells (PBMC). PBMC were cultured in 96-well culture plates for 72 hrs in the presence of 4 ng/ml aCD3 with and without chemical treatment. The cultures were pulsed with 3H-thymidine (1 mCi/well) for 18 hrs, harvested and counts per minute (CPM) determined using a microplate scintillation counter. Next, we determined the IC50 concentrations for the above-mentioned chemicals using dose responses covering ranges over several orders of magnitude: FK506 0.05 mM, CsA 0.8 mM, Verapamil 18 mM, and Nifedipine > 50 mM. Our results indicated that FK506 > CsA > Verapamil > Nifedipine as inhibitors of aCD3-induced T-cell proliferation and these data correlate well with what is known about the immunosuppressive activity of these compounds. The IC50 concentrations from FK506 to Nifedipine spanned several orders of magnitude making this assay useful to predict the immunosuppressive potential of chemicals of similar class working through similar mechanisms.

2080 PERIPHERAL LEUKOCYTE PHENOTYPING AND SYSTEMIC ANTIBODY RESPONSE AS FIELD TESTS TO EVALUATE IMMUNOTOXICITY IN BEEF CATTLE.

D. Bechtel[1], C. Waldner[1], W. C. Davis[2] and M. Wickstrom[1]. *[1]University of Saskatchewan, Saskatoon, SK, Canada and [2]Washington State University, Pullman, WA.*

As part of an effort to develop tests for use in field studies of livestock immunotoxicology, a pilot study was conducted to evaluate the sensitivity of peripheral leukocyte phenotyping and systemic antibody response to vaccination in immunosuppressed cattle. Twenty four yearling heifers were divided randomly into two groups of 12 each. Cattle in the treatment group were given dexamethasone IM at 0.1 mg/kg on experiment days -3 through 3, and again on days 20 through 24, to suppress immune function. Cattle in the control group were given a representative dose of saline IM. Animals in both groups were inoculated with a commercial rabies vaccine (IMRAB Bovine Plus, Merial Canada Inc.) on days 0 and 21. Blood was collected from all animals prior to injection with dexamethasone or saline on day -3, and again on days 0, 21 and 42. Rabies antibody titre in day 0 (baseline), 21, and 42 samples was measured by the standardized Rapid Fluorescent Focus Inhibition Test (a rabies neutralizing antibody test). Sub-populations of peripheral blood leukocytes ($CD4^+$, $CD8^+$ and $\gamma\delta^+$ T-cells, B cells, and subsets of activated T-cells) were determined in day 0, 21 and 42 samples by flow cytometry. Average daily weight gain and body condition scores for all animals were incorporated into the statistical model as covariates. Peripheral blood leukocyte counts were significantly increased (P<0.05) in the dexamethasone treated animals. The increase is rationalized by the large increase in the circulating polymorphonuclear cells. Alternatively, $CD4^+$ and $CD8^+$ T-cells were significantly depressed in the treated group. Results of this study will be used to optimise our approach to assessing immune function in cattle chronically exposed to airborne contaminants from oil and gas production and processing facilities in western Canada.

2081 COMPARISON OF THE EFFECTS OF CYCLOPHOSPHAMIDE AND DEXAMETHASONE ON PFC ASSAY, ANTI-KLH AND ANTI-TETANUS TOXOID ELISA RESPONSES IN RATS.

F. Condevaux[1], J. Guichard[1], N. Eltschinger[1], C. Cretinon[1] and J. Descotes[2]. *[1]MDS Pharmacology Services, L'Arbresle, France and [2]Poison Center, Lyon, France.*

There is a consensus that a T-dependent antibody response assay is the best method for the early screening of the immunosuppressive potential of new molecular entities. However, the selection of the most appropriate antigen is a matter of debate. In

this study, groups of 10 SD rats treated with 5 mg/kg/day cyclophosphamide (CY) ip or 0.25 mg/kg/day dexamethasone (DEX) orally for 21 days were immunized either with SRBC on day 17, KLH on days 7 and 14 or tetanus toxoid (TTox) on days 7 and 14. Two groups treated with CY or DEX were not immunized. The PFC assay was performed on day 21. KLH and tetanus toxoid IgM and IgG levels were measured by ELISA on days 7, 14 and 21. Both CY and DEX induced a significant decrease in the PFC response. No changes whatsoever in anti-KLH IgM levels were seen, but IgG levels on days 7 and 14 were significantly decreased by CY and DEX. No anti-TTox antibodies were detected on day 14. There was a statistically significant decrease in both IgM and IgG levels on day 21 in animals treated with either CY or DEX. Although decreases in antibody responses were of a similar magnitude with either drug in the 3 assays, the suppressive effect of CY was moderately greater than that of DEX in the PFC and TTox assays, but not in the KLH assay. These results suggest that TTox is a valuable alternative to SRBC and KLH in a T-dependent antibody response assay. Importantly, unlike SRBC nor KLH, responses to TTox can be used as endpoints in clinical trials. Finally, there were no differences in the histology of the lymphoid organs or clinical pathology parameters following treatment with CY or DEX and/or immunization with SRBC, KLH or TTox. This lends support to the claim that immunization of animals is feasible in regulatory toxicity studies without compromising the evaluation of the toxicity profile of the tested compounds.

2082 VALIDATION OF A METHOD FOR THE DETECTION OF ANTI-KEYHOLE LIMPET HEMOCYANIN (KLH) IGM ANTIBODIES IN FISCHER-344 RAT SERUM BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA).

N. Rouleau, M. Boutet, P. Gauthier, V. Girard and L. LeSauteur. *CTBR, Senneville, QC, Canada.* Sponsor: L. LeSauteur.

Immunotoxicity testing guidance: (FDA) Final Guidance for Industry: "Immunotoxicology Evaluation of Investigational New Drugs", the European Agency for the Evaluation of Medicinal Products (EMEA): "Note for Guidance on Repeated Dose Toxicity" and the "Interim Draft Guidance for Immunotoxicity testing" (MHLW/JPMA) have recommended the T-cell dependent antibody response assay to monitor the immune response. In this study, the T-cell dependent primary antibody response in Fischer-344 rats was monitored using KLH as an antigen and a quantitative ELISA. Fischer-344 rats were immunized intra-peritoneally with 0.5 mL of KLH (0.2 mg/mL) and blood was collected pre- and post-KLH immunization (i.e. 5 to 7 days). The ELISA consisted of a plate coated with KLH, blocked with blocking buffer, loaded with control and study samples, then an antibody specific to rat IgM conjugated with peroxidase was added, followed by substrate, and the reaction was stopped and read at 450 nm. Validation of the ELISA, including specificity, linearity of dilution, intra- and inter-assay precision and accuracy, stability and prozone effect, met the acceptance criteria. Study samples can be diluted up to 1/12000, the validated range is 30.0 to 1000.0 ng/mL, the samples were shown to be stable at room temperature for up to 3 hours, at approximately 4°C for up to 7 hours, after 4 freeze-thaw cycles at approximately 70°C and after storage at approximately 70°C for up to 29 days. The anti-KLH IgM antibody response gave results ranging from below the lower limit of quantitation (LLOQ) to 198.0 µg/mL from 3 to 7 days post-KLH immunization. Assessment of the T-cell dependent antibody response using KLH as antigen, administered intra-peritoneally to Fischer-344 rats and the detection of anti-KLH IgM antibodies in serum using an ELISA was shown to be suitable for its intended use.

2083 PRIMARY ANTIBODY PRODUCTION TO A T CELL-DEPENDENT ANTIGEN: REPLACEMENT OF THE SRBC PLAQUE-FORMING CELL ASSAY BY A KLH-BASED PROTOCOL.

M. van Zijverden, E. I. Klein Koerkamp, P. T. van den Berg and A. H. Penninks. *Experimental Immunology, TNO Nutrition and Food Research, Zeist, Netherlands.* Sponsor: V. Feron.

To assess potential effects of pharmaceuticals on the immune system, a primary antibody response to a T cell-dependent antigen is suitable to complete the initial screening phase (EMEA guidance CPMP/SWP/1042/99, and FDA Guidance for Industry, Immunotoxicology Evaluation of Investigational New Drugs Oct 2002). The recommended standard protocol is based on immunization with sheep red blood cells (SRBC) as antigen during test compound treatment. The possible immunomodulation is determined four days later by means of the plaque-forming cell (PFC) assay, in which the number of SRBC-specific IgM-forming cells is assessed in spleen. However there are different disadvantages related to this protocol. First, the antigen (SRBC) is not standard and will differ between different labs. Second, the PFC assay is a terminal assay so it can only be performed at one time point, only IgM can be determined, and it is very laborious. The replacement of SRBC by a standard antigen (for example keyhole limpet haemocyanin, KLH) and of the PFC assay by an ELISA would greatly improve the assessment of T cell-dependent immunity. Therefore we assessed the response to KLH after using different dosing regimes in mice. Those were respectively single subcutaneous (s.c.) and intraperitoneal (i.p., with and without adjuvant) dosing, and repeated i.p. (6x) dosing. At day 0 (n=6), 7 (n=3) and 14 (n=3) blood was taken. Results showed optimal IgM titers were determined at day 7, and the i.p. regimes induced higher titers than s.c. injection. Addition of adjuvant prolonged the presence of IgM, as did repeated dosing. optimal IgG1 titers were determined at day 14, which was very well induced by i.p. adjuvant or the repeated i.p. injections. Single s.c. or i.p. injections induced less clear titers. Also for rat we are now comparing different routes of KLH immunization, during 28-day oral treatment with known immunosuppressants (cyclosporine A, cyclophosphamide) to validate the assay for the rat.

2084 T-CELL DEPENDENT ANTIBODY RESPONSES TO KEYHOLE LIMPET HEMOCYANIN (KLH) IN PRECLINICAL IMMUNOTOXICITY TESTING.

T. Bigwarfe, M. Abbott, D. DeVona, D. Gonchoroff, H. Haggerty, E. McAvoy, L. Phelps, K. Price and T. Reilly. *Immunotoxicology, Bristol-Myers Squibb, Syracuse, NY.*

Assessing the effects of drug candidates on immune function during preclinical toxicology studies is an integral part of drug safety evaluation. Recent guidelines set by the Food and Drug Administration and European Agency for the Evaluation of Medicinal Products have recommended the assessment of a T-cell dependent antibody response as part of the Tier 1 immunotoxicity testing strategy to assess the immunotoxic potential of human therapeutics. Keyhole limpet hemocyanin (KLH), a protein purified from the mollusk, Megathura crenulata, is capable of stimulating a T-cell dependent antibody response, which depends upon B-cells, T-cells, and macrophages for its development. Accordingly, antibody responses to KLH should serve as a surrogate for monitoring the potential effects of drug candidates on the immune system. To verify its utility, T-cell dependent humoral responses were assessed following administration of KLH to mice, rats, dogs, and monkeys *via* various routes of administration and over a range of doses (0.25 - 5 mg). Serum samples were collected at least weekly and KLH-specific IgM and IgG responses were monitored using a specific enzyme-linked immunosorbent assay to determine endpoint-titers. Robust anti-KLH antibody responses were observed in all species and optimized accordingly (e.g., 0.5 mg, subcutaneously in rats; peak IgM on day 7; peak IgG on day 21). In each species, the optimized methods were sensitive, specific, and reproducible. KLH antibody responses were significantly repressed by dexamethasone and cyclophosphamide and have since been used to evaluate the induction and reversibility of immunologic tolerance caused by immunomodulatory drugs with diverse mechanisms of action as well as the potential effect of non-immune-based therapeutics on immune function. These studies support the utility of using an anti-KLH antibody response to assess the pharmacologic and toxicologic effects of new drugs on immune function in multiple preclinical species.

2085 VALIDATING THE T-DEPENDENT ANTIBODY RESPONSE IN DOGS WITH A KNOWN IMMUNOSUPPRESSIVE AGENT (NEORAL).

D. L. Finco-Kent, J. Dugas, A. Hudson, R. Barnes and T. Kawabata. *WWSS, Pfizer, Groton, CT.*

The dog is commonly used for safety assessment of drugs in development. If standard toxicology studies indicate immunotoxicity, additional in depth testing may be required. The *in vivo* primary T-dependent antibody response (TDAR) has been recommended to assess the acquired immune response in mice and rats. A validated TDAR in the dog has not been reported. Our lab previously conducted studies to determine the appropriate dose of antigen (KLH), route and kinetics of the antibody response in the dog. An ELISA was developed to measure KLH-specific IgM and IgG in serum samples. To validate the sensitivity of this method, we investigated the TDAR to KLH in the dog with a known immunosuppressive drug, Neoral. In one study, Neoral was administered once/day for 17 days at 5 and 25 mg/kg. Dogs were immunized on day 5 with KLH and blood was drawn on days 12 and 17. A slight decrease in the IgM antibody response to KLH was observed in some dogs receiving either dose of Neoral. In contrast, the IgG antibody response to KLH was decreased considerably in the dogs that received Neoral. A subsequent study evaluated the IgM and IgG response in dogs receiving Neoral twice/day for 17 days at doses of 20 and 40 mg/kg. The design of this study was the same as before, however, additional blood samples were collected to evaluate cyclosporine trough levels and for hematology and serum chemistry. All dogs had normal hematology and serum chemistry results at the time points checked during the study. The low dose Neoral group had cyclosporine trough levels slightly below levels recommended for immunosuppression while the high dose group had levels at or above recommended immunosuppressive levels. IgM titers to KLH were slightly lower in the Neoral group compared to the controls, whereas IgG titers to KLH

were significantly lower in the Neoral-treated dogs. In conclusion, the results of this study demonstrate that the primary IgM and IgG response to KLH can be used to monitor the immune response in dogs. The IgG response appeared to be more sensitive to Neoral treatment than the IgM response.

2086 VALIDATION OF CYNOMOLGUS MONKEY IMMUNOPHENOTYPING BY FLOW CYTOMETRY.

D. Baker, D. Finco-Kent, W. Reagan and T. Kawabata. *WWSS, Pfizer, Groton, CT.*

Reports on the optimization and validation of immunophenotyping of lymphocytes subsets from Cynomolgus monkey peripheral blood for toxicology studies have been limited. Thus, studies were conducted to determine the optimal conditions for staining and sample processing and stability of peripheral blood samples. Validation studies of intra-assay reproducibility and technician-to-technician and lab-to-lab variability were evaluated. In addition, the day-to-day variability of lymphocyte subsets and a reference range were determined. Pan T cells (CD3+), T-helper (CD3+CD4+), NK cells (CD3-CD16+), T cytotoxic/suppressor (CD3+CD8+) and B cells (CD3-CD20+) were analyzed by flow cytometry. As a positive control for staining, processing and analysis, stabilized human blood (Streck, Inc.) was included in each sample run. Our studies showed that performing 4 color staining using a lyse wash method had good intra-assay precision (less than 5 % CV for all subsets) for cynomolgus peripheral blood. Inter-assay precision was determined using the stabilized human blood tested 18 times over a 3 month period and found to be less than 5% CV for all subsets. Variability between technicians and labs was minimal and reference ranges for the subsets were similar to other reports in the literature. The validated method is extremely robust and can be performed under good laboratory practice conditions.

2087 PRE- AND POSTNATAL THYMUS DEVELOPMENT IN THE CYNOMOLGUS MONKEY: RELEVANCE TO IMMUNOTOXICOLOGY.

E. Buse, G. Habermann, S. Friderichs-Gromoll, J. Kaspareit, F. Vogel and P. Thomas. *Pathology, Covance Laboratories GmbH, Muenster, Germany.*

Imunosuppression/-modulation have become highy relevant in toxicological research. Special attention is being paid to the ontogenetic development of the immune organs, and in peculiar the thymus as the primary lymphoid organ responsible for T-cell differentiation. Due to the complex function of its multiple cell types, receptors and mediating factors, the thymus represents an important target for drugs and drug related side effects. In order to better characterize development of thymic immune cell phenotypes in this species, this organ was studied from gd 35 to the stage of advanced involution in a 21-year old monkey. Special emphasis was placed on early thymus cell generation and cellular pattern formation during development. Histological standard stains and immunocytochemical methods were used to distinguish between thymic subpopulations. At gd 35 the epithelial bud of the thymus was visible in a sagital position at the level of the appertura thoracica. At gd 50 first lymphocyte-like cells and HLA-DR immunoreactivity of a few cells became obvious. The cortico-medullary differentiation, Hassall's body precursors and faint immmunoreactivity for T-lymphocytes were detected from gd 60 onward. First macrophages were apparent at day 70, first CD 20 immunoreactive cells (B lymphocyte-like cells) at gd 85, and M1014 immunoreactive cells (natural killer cells) at gd 100. At gd 100 all evaluated cell populations present in the adult cynomolgus thymus were in place as demonstrated by at least minimal immunoexpression. By contrast, there was never any indication of CD 34 and CD 117 and CD 35 immunoreactivity at this time. The gd 100 - immunoreactivities persisted, partly with changing distribution patterns, until the advanced age of 21 years with the exception of M1014 immunoreactive cells, which were found only until adult ages. The observed pattern of thymic development was similar to that described for human thymus. The cynomolgus monkey thus represents a suitable model for the study of thymic function in developmental toxicity studies.

2088 FLOW CYTOMETRY-BASED EVALUATION OF LYMPHOCYTE SUBSETS AND NATURAL KILLER CELL ACTIVITY IN DEVELOPING AND ADULT CYNOMOLGUS MONKEYS.

W. Frings and G. Weinbauer. *Covance Laboratories GmbH, Muenster, Germany.* Sponsor: P. Thomas.

Nonhuman primates are frequently the animal model of choice for immunotoxicologic evaluation of biologics functional peptides because of high protein-homology to humans, thus ob*via*ting the problems of non-responsiveness or induction of drug-clearance by neutralizing antibodies. The cynomolgus monkey (Macaca fascicularis) is currently the predominating primate model in use. We established a 4-colour immunophenotyping protocol using anti-human antibodies and investigated 75 adult animals of each sex in comparison to 10 human volunteers of each sex, and analysed the postnatal development of lymphocyte populations in infant animals. Natural killer cell activity was studied in adult animals and in infants up to 2 years of age. For the latter, K562 human myeloma cells were used as target cells (ORPEGEN Pharmacology Kit) and various E/T ratios were studied in the presence and absence of IL-2. Animals were also treated with 0.25 mg/kg iv dexamethasone. Our immunophenotoyping results indicate that identical staining protocols can be used for human and cynomolgus monkey blood, demonstrating good species comparability. Monkeys generally exhibited larger inter-species variations and had a slightly modified CD4/CD8 ratio. During early infant development in monkeys, a progressive decrease of CD4/CD8 ratio was seen which was similar to CD4/CD8 ratio change described for human postnatal development. Dexamethasone transiently suppressed natural killer cell activity. Overall, natural killer cell activity was comparable for adult and infant animals suggesting early activation of non-specific defense systems. In conclusion, adult and developing lymphocyte subset populations share similarities between cynomolgus monkey and human.

2089 EVALUATION OF T-DEPENDENT ANTIBODY RESPONSE IN CYNOMOLGUS MONKEYS.

J. D. Alvey and J. R. Piccotti. *Safety Sciences, Pfizer Global Research & Development, Ann Arbor, MI.* Sponsor: M. Bleavins.

The Committee for Proprietary Medicinal Products (CPMP) and the Food and Drug Administration (FDA) recommend evaluating a primary T-dependent antibody response when assessing the immunotoxicity potential of new drug candidates. We recently developed and validated an assay to measure a primary IgM response to keyhole limpet hemocyanin (KLH) in rats. The current study was designed to develop and test an IgM response to KLH in a non-rodent model. In the initial phase, 3 male cynomolgus monkeys were given a single intramuscular injection of KLH at 3, 10 or 30 mg per animal. Serum samples were collected prior to immunization, and on Days 3, 5, 7, 10, 14 and 21 post-immunization. IgM specific for KLH was measured by an ELISA developed in our lab, using a detection antibody with high reactivity to monkey IgM and no cross-reactivity with monkey IgG. In contrast to what was observed in the rat, low to moderate levels of anti-KLH IgM were detected in monkeys prior to KLH injection. The IgM in pretest sera was specific for KLH, since antibody was not detected in uncoated wells. Interestingly, anti-KLH IgM was detected in the majority of naive stock monkeys tested. Since cynomolgus monkeys eat crab and other shellfish, it is possible that animals are exposed to KLH in diet. Although IgM was detected in sera of monkeys predose, antibody concentrations were significantly elevated over pretest by Day 5 after KLH injection. The peak IgM responses occurred between Days 7 and 14, with antibody levels decreasing slightly by Day 21. The greatest antibody response was elicited by the monkey given 3 mg, which also was the animal with the highest pretest titer. Additional experiments are underway to increase the number of monkeys evaluated, and to better characterize the timing of the IgM response to KLH. Experiments evaluating alternate T-dependent antigens also are planned.

2090 INHIBITION OF IL-6 BUT NOT TNFα INHIBITS THE ANTIBODY RESPONSE TO KLH IN CYNOMOLGUS MACAQUES.

P. L. Martin[1], J. Cornacoff[1], P. Bugelski[1], S. Hersey[3], E. C. Martin[2], J. E. Sutherland[2] and G. Treacy[1]. *[1]Toxicology, Centocor, Malvern, PA, [2]Charles River Laboratories, Worchester, MA and [3]Charles River Laboratories, Sparks, NV.*

Tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) are proinflammatory cytokines believed to play a role in a variety of autoimmune diseases. Inhibitors of TNFα and IL-6 have been shown to be effective therapeutically in a number of animal disease models. However, the role of these cytokines in immune surveillance is not well understood. In this study, the effects of TNFα and IL-6 monoclonal antibodies (mAbs) on the humoral immune response (IgG and IgM) to keyhole limpet hemocyanin (KLH), a T-dependent antigen were evaluated in cynomolgus macaques. Male and female macaques were administered 0.1 mg of KLH in 1 mL of Incomplete Freund's Adjuvant (IFA) intramuscularly. KLH/adjuvant was administered on two occasions separated by 14 to 18 days. Treatment with the cytokine inhibitors was initiated 9 - 11 days prior to the first KLH/adjuvant administration. Serum samples for measurement of anti-KLH antibodies were collected prior to and for up to six months during anti-cytokine treatment. Titers of anti-KLH antibodies (IgG and IgM) were measured using an ELISA method. Anti-KLH antibodies were detectable 12-13 days after the first KLH administration. KLH antibody titers continued to rise following the second KLH administration and peaked at 23

to 26-days post challenge. The TNFα inhibitor had no effect on anti-KLH antibody development. The IL-6 inhibitor produced a modest (~20%) reduction in anti-KLH antibody titers. This study shows that KLH can be used to evaluate the immune competence of cynomolgus macaques treated with cytokine inhibitors. The results suggest that IL-6 but not TNFα is involved in the development of the antibody response to KLH/IFA in cynomolgus macaques. These results in non-human primates are consistent with published data obtained in mice.

2091 DEVELOPMENT AND IMMUNOMODULATION OF DELAYED-TYPE HYPERSENSITIVITY (DTH) IN CYNOMOLGUS MONKEYS.

K. D. Price, L. Mezza, R. Diters, S. Wells, D. DeVona, Z. Tzogas and H. Haggerty. *Bristol-Myers Squibb, Syracuse, NY.*

DTH is a measure of cellular immunity useful in assessing multiple aspects of the immune response. Although others have described monkey DTH models, clinical reactions have not been robust or consistent in our hands. To address this issue, monkeys were given s.c. injections of an Ag cocktail containing diphtheria (Dp;15 Lf), trichophyton and *C. albicans* (6375 PNU) toxoids in IFA on days 1, 4, 7, 10, and/or 13. At 2-24 wk postsensitization, monkeys received i.d. injections of each Ag (4-10X lower amounts), Ag cocktail, or diluent. Dermal responses (reaction diameter, induration, erythema) were measured at 24-96h postchallenge and skin biopsies were obtained for histology and immunohistochemistry. Elicitation of DTH responses at 2 weeks post-sensitization was unsuccessful, complicated by a nonprogressive neutrophilic response that was only distinguishable microscopically. Classical DTH was observed at ≥4wk post-sensitization and sustained for >24wk. Erythema preceded induration, which peaked at 72h. DTH cellularity was observed microscopically even if a clinical reaction was not, and was preceded by an influx of neutrophils and some macrophages at 24h (subacute), followed by angiocentric distribution of lymphocytes and macrophages (chronic inflammation) at 48-96h. Immunohistochemistry revealed an influx of macrophages and T-cells (CD8>CD4) but not B-cells. Clinical reactions following oral (1 week during challenge) dexamethasone (Dex, 2 or 20 mg/kg) or cyclosporin A (120 mg/kg) were not diminished and humoral immunity to Dp was not suppressed. However, dose-related decreases in cellularity and subacute rather than chronic inflammation was observed in monkeys given Dex. Humoral immunity to Dp was augmented in monkeys receiving 1 mg of oligo CpG (during sensitization), but DTH was not enhanced. Thus, clinical reactions may not always predict classical DTH and microscopic confirmation is needed, particularly to monitor immunomodulation.

2092 IMMUNOLOGICAL, HEMATOLOGICAL AND CLINICAL PATHOLOGY PARAMETERS DURING POSTNATAL DEVELOPMENT IN THE CYNOMOLGUS MONKEY: PRELIMINARY FINDINGS.

G. Weinbauer, G. Habermann, I. Osterburg, J. Kaspareit, S. Friderichs-Gromoll, S. Srivastav, W. Mueller and F. Vogel. *Covance Laboratories GmbH, Muenster, Germany.* Sponsor: P. Thomas.

Nonhuman primates are gaining increasing importance as preclinical animal model for the study of developmental and reproductive toxicology. A major reason for the increased need of nonhuman primates is the fact that many immunotherapeutics and biotechnology products do not interact with rodent tissues and receptors or are immunogenic in rodents and frequently only cross-react with primate tissue. This and the issuance of new guidelines mandating immunotoxicity screening of new drugs has led to increased demands of using the cynomolgus monkey (Macaca fascicularis) in pre-/postnatal development studies. This investigation compiles developmental control data from more than 80 infant cynomolgus monkeys over the age of one month to two years. Wherever possible a comparison to published human pediatric data was attempted. For the study of B cell- and T cell-mediated immune response, keyhole-limpet-hemocyanin (KLH) and delayed-type-hypersensitivity (DTH) testing were used. Antibody formation was seen by 6-7 months of age and lymphocyte subset development was similar for monkey and human. Hematological parameters such as red and white blood cell count, hematocrit and platelet count showed a comparable developmental pattern relative to human data. Although highly variable across animals, the average pattern of immunoglobulins A, G and M appeared comparable to that described for human. Data for selected clinical pathology parameters, e.g. glucose, potassium, cholesterol, triglycerides, total protein and liver enzymes, also revealed close similarity to human reference data. Our preliminary evaluation suggests that the postnatal development of various cynomolgus monkey organ systems shares similarities with that in humans. These observations support the use of the cynomolgus monkey for toxicity evaluation in pre-/postnatal development studies.

2093 EFFECT OF CHRONIC EXPOSURE TO ISOFLAVONE ON POSTNATAL DEVELOPMENT OF MICE.

K. Takashima[1, 2], H. Fukata[2], H. Kato[3], T. Iguchi[4, 5], M. Komiyama[6] and C. Mori[1, 5]. *[1]Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan, [2]Department of Environmental Medical Science (SRL), Graduate School of Medicine, Chiba University, Chiba, Japan, [3]Nihon Bioresearch Inc., Hashima, Japan, [4]Center for Integrative bioscience, Okazaki National Research Institutes, Okazaki, Japan, [5]CREST, JST, Kawaguchi, Japan and [6]Center for Environment, Health and Field Sciences, Health and Field Sciences, Chiba University, Chiba, Japan.*

We have investigated the effect of chronic exposure to isoflavone (IF) on postnatal development of C57BL/Cr Slc mice. Mother mice were fed with an IF-free purified diet (IF-free group) or the purified diet supplemented with 0.05% IF (0.05%-IF group) from 1, 3 or 5 weeks before gestation to the end of lactation, and their pups were fed with the same diets after weaning until sacrifice at postnatal day (PND) 31. The IF-free diet was composed of 24.5% milk casein, 45.5% corn starch, 10% granulated sugar, 6% corn oil, 5% cellulose powder, 1% α-starch, 1% vitamine mix and 7% mineral mix. The dose of 0.05% IF is comparable to a daily intake of IF for some of Asian people and that for neonates who are fed on soy-based formula. Thus, the mice examined were chronically exposed to isoflavone *in utero* and during postnatal period. Body weight and other developmental landmarks of the neonatal mice were monitored at evry seven days till PND 31. Regarding the body weight and anogenital distance, there was no significant difference between IF-free group and 0.05%-IF group. However, viginal opening in 0.05%-IF group was several days earlier than that in IF-free group, significantly. This result suggests that chronic exposure to IF causes earlier puberty to female neonates.

2094 PRACTICAL ASPECTS OF JUVENILE SAFETY STUDIES IN MINIPIGS.

A. Makin and A. Christensen. *Toxicology and Pharmacology, Scantox A/S, Lille Skensved, Denmark.* Sponsor: R. Harling.

Toxicity testing of pharmaceuticals for regulatory purposes should be suited to the target population. Standard non-clinical testing requirements cover, usually, adult animals and also address the subject of reproductive function. The reproductive studies cover the full reproductive cycle from mating performance of the parents through to the same stage in the F1 generation. However, at no stage is there a requirement for direct dosing of the offspring. There is a recognition that this leaves a gap in the safety evaluation of a pharmaceutical whereby the period from birth to adulthood is incompletely studied. In 2003, a draft guidance on non-clinical safety evaluation of pediatric drug products was issued. However, studies in young animals present many practical challenges, particularly when using non-rodent species. Long gestation periods, small litter sizes and a general lack of background data for young animals present difficulties. Because of the relatively short period from birth to sexual maturity in laboratory species compared to humans, it is important that testing in the animal species should commence as soon after birth as is practicably possible. This is particularly important where drugs are intended for use in neonatal humans. Particularly for non-rodent species the arrangement of pre-weaned animals into experimental groups is a key consideration. A practical challenge is having the birth of the experimental animals synchronised in order to have a study design that is optimum. At Scantox we have performed studies to establish the minipig as a suitable non-rodent model for non-clinical testing of pharmaceuticals for use in pediatric human populations. We demonstrate the practical feasibility of randomised cross-fostering, and we have established the possibility of performing the standard techniques e.g. for parenteral administration of drugs, and repeated collection of blood samples for TK. We have a growing background database of clinical pathology, and pathology parameters in relation to animal age. This poster describes the practicalities of performing such studies and presents background data.

2095 PRENATAL DOSE LEVEL OF 3, 3', 4, 4', 5-PENTACHLORBIPHENYL (PCB 126) TO INDUCE HYPOSPADIAS IN FEMALE RATS.

M. Shirota[1, 2], Y. Sakurada[2], K. Hayasaka[2], K. Inoue[2] and K. Shirota[2]. *[1]Hatano Research Institute, Food and Drug Safety Center, Hadano, Kanagawa, Japan and [2]Research Institute of Biosciences, Azabu University, Sagamihara, Kanagawa, Japan.* Sponsor: H. Ono.

We have shown that prenatal but not postnatal exposure to PCB 126 induces genital dysmorphogenesis, such as hypospadias, in female rats. In the present study, we examined a relation between prenatal dosage of PCB 126 and incidence and severity of the malformation. Each six pregnant Sprague-Dawley rats were orally given

PCB 126 at a dose level of 0 (corn oil, control), 0.3, 1, 3, 10 or 30 μg/kg on gestational day 15, and were allowed to deliver spontaneously. One day after the delivery, their litter size was examined and then was standardized to 8. The female offspring (35-41 offspring/group) were examined for external appearance on postnatal day (PND) 1 and at necropsy (on PNDs 25-28), and were determined body weight on PND 1 and at weaning (PND 21) and the necropsy. External genital malformations were classified based on the grossly appearance as slight (partial cleft formation between urethral orifice and vaginal orifice), moderate (more severe cleft formation between urethral orifice and vaginal orifice) and severe (hypospadias). Levels of statistical significance were analyzed by ANOVA, followed by a Dunnett-test. No adverse effects of the treatment were observed in any dam. Whereas *via*bilities of the offspring were comparable among the groups, body weight of the offspring in the groups given PCB 126 at 3 μg/kg and more was significantly lower than that of age-matched control. Abnormal external appearance was not observed on PND 1, however, genital anomalies became evident at the necropsy in the groups given PCB 126 at 3 μg/kg and more. Severity of the anomalies increased in relation to the prenatal doses of PCB 126, and hypospadias was developed in 2.5, 2.8 and 27.3 percent of the offspring in the 3, 10 and 30 μg/kg-treated groups, respectively. From these results, the lowest oral dose of PCB 126 to induce genital dysmorphogenesis in rats is concluded to be 3 μg/kg on gestational day 15.

2096 ECOLOGICAL INDICATORS, ENVIRONMENTAL CONTAMINANTS, AND AUTISM: PRELIMINARY FINDINGS FROM MINNESOTA.

M. G. Opler[1], L. Moy[2], K. Eng[2] and M. Bresnahan[3]. [1]*Psychiatry, Columbia University, New York, NY,* [2]*Bronx High School of Science, Bronx, NY and* [3]*Epidemiology, Columbia University, New York, NY.* Sponsor: W. Zheng.

Autism, a developmental disorder of unknown etiology is characterized by marked difficulties in social relations and communication during early childhood and throughout the lifecourse. Prevalence data collected by the Department of Education of the State of Minnesota suggests that rates are increasing rapidly. While no specific genetic or environmental risk factor has been conclusively linked to autism, several ecological and environmental indicators may help to guide investigations, including developmental malformations in free-living amphibian populations, timber damage, and various pollutant levels. Data was taken from studies conducted by the Minnesota Pollution Control Agency, the USEPA, and the US Biological Service. This data was dichotomized to high and low categories, using the median as a cutoff for each indicator. Annual increases in autism rates in children aged 6-11 in the public school system were calculated, and group differences were measured using different indicators. When county-level rates of amphibian malformations were examined, autism rates in children aged 6-11 were significantly higher in those counties with malformation rates$\propto$ 2% (p=0.016). Reported airborn releases of toxins, including heavy metals such as cadmium, mercury, and lead were also tested. Autism rates were higher in counties with total airborn pollutant levels above the median. These differences were not statistically significant. In counties with high aggregate timber loss per square mile due to fire, logging, drought, etc., autism rates increased more quickly and demonstrated a trend towards significance($p<0.1$). When the results were stratified by population, the results were not measurably altered, excepting the case of total air pollutant levels, which demonstrates a statistical trend when the most populous counties (top 10%) are excluded. Further analyses are required to expand these findings, using more complete data, specific exposures, and rigorous analyses.

2097 A TERATOLOGY STUDY OF ZICONOTIDE UTILIZING DOUBLE-STAINING AND POST NATAL EXAMINATIONS TO ELUCIDATE DELAYED OSSIFICATION.

M. J. Skov[1], G. M. Shopp[1], L. Pouliot[2], K. J. Robinson[2] and J. C. Beck[3]. [1]*Safety Evaluation, Elan Pharmaceuticals, Inc., South San Francisco, CA,* [2]*CTBR Bio-Research Inc., Senneville, QC, Canada and* [3]*Roche, Palo Alto, CA.*

Ziconotide (PRIALT™) is the synthetic equivalent of a 25-amino acid, polybasic peptide found in the venom of the marine snail, *Conus magus.* Ziconotide is a potent and selective N-type calcium channel blocker being developed as an intrathecal (IT) analgesic agent for severe, chronic pain. The effects of ziconotide on rat embryo-fetal development were evaluated by continuous intravenous (IV) infusion from gestation day (GD) 6 through 15, at doses of 1.5, 4.5 and 15 mg/kg/day. Fetal abnormalities were limited to a significant increase in the incidence of incompletely ossified or unossified pelvic bones in the fetuses and litters of the high-dose dams compared to vehicle. These effects were seen at a dose where maternal toxicity coexisted and consisted of significant weight loss and reduced food consumption from GD 6 to 9. In an effort to show that the incomplete ossification was a transient effect, a repeat study was performed utilizing double-staining with Alizarin red S for bone and Alcian blue for cartilage. A littering phase was incorporated to assess the post-natal skeletal development of the pelvic girdle in Day 4 post-partum pups. Consistent with the first study, significant weight loss and reduced food consumption were noted from GD 6 to 9 in dams treated with 15 mg/kg/day ziconotide and the incidence of incomplete ossification of the pubic bone(s) was increased for fetuses and litters examined at GD 20. Pelvic bones were normal for all Day 4 post-partum pups. These findings indicate that the reduced bone formation of the pelvic girdle in fetuses at GD 20 was due to a transitory delay in ossification, and not a permanent effect. In conclusion, ziconotide was not teratogenic in a study of rat prenatal development when administered by continuous IV infusion from GD 6 through 15 at systemic exposures producing maternal toxicity and greater than 2500-times the maximum measured human systemic exposure after IT administration.

2098 NEONATAL EXPOSURE TO GENISTEIN, A SOY PHYTOESTROGEN, ALTERS MAMMARY GLAND DIFFERENTIATION.

E. Padilla-Banks, W. Jefferson and R. Newbold. *NIEHS, Research Triangle Park, NC.*

Some reports suggest developmental exposure to genistein (Gen) protects against mammary carcinogens later in life; however, *in vitro* studies suggest the opposite, i.e., that Gen stimulates breast cancer cells to proliferate. Further, exposure to estrogens, including the period of fetal development, is a well-known risk factor for the development of breast cancer. Numerous experimental studies involving the mammary gland and the reproductive tract attest to the proliferative and carcinogenic potency of estrogens. Since soy products including soy based infant formulas are increasingly being marketed to children, and to adults for potential health benefits and hormone replacement therapy, we investigated the effects of early exposure to genistein on the mammary gland. Neonatal CD-1 mice were treated on days 1-5 with Gen 0.5-50 mg/kg/day; these doses span the range for human exposures. At 5 and 6 wks, mammary glands (#4) were examined by whole mount analysis. Estrogen receptor (ER)α, ERβ and progesterone receptor (PR) were studied by immunohistochemistry and/or Western blots. Control mice exhibited an increase in terminal end buds (TEBs) from 5 to 6 wks of age indicating active ductal morphogenesis. Mice treated with Gen-0.5 showed similar increase in the number of TEBs from 5 to 6 wks but the numbers were higher than controls at both ages. Gen-5 showed a different pattern of mammary gland development with more TEBs present at 5 wks but less at 6 wks of age suggesting an advance in differentiation at this dose. The highest dose of Gen showed similar numbers of TEBs as controls, but mammary gland development was inhibited with smaller ducts and less ductal branching. As another indication of altered development, the length of the furthest elongated duct was measured; the expanding mammary ductal mass increased after exposure to Gen-0.5 at 5 weeks, and the 0.5 and 5 doses at 6 wks. In contrast, mammary glands from Gen-50 showed growth inhibition of the genistein exposed gland; this stunted development persisted throughout life.

2099 FEASIBILITY STUDY TO ASSESS SKELETAL DEVELOPMENT IN NON-CLINICAL PEDIATRIC STUDIES.

K. Robinson, A. Varela, N. Doyle, J. Jolette, C. Luc, M. Sabourin, C. Chevrier and S. Smith. *CTBR, Senneville, QC, Canada.* Sponsor: M. Vezina.

The increased emphasis placed upon non-clinical pediatric testing in recent years and the focus upon the development of several organ systems including the skeleton has lead to the adaptation of a variety of techniques for these evaluations. The pediatric use of drug classes such as the quinolones, glucocorticoids and bisphosphonates and their effects on the skeleton, highlights the need to evaluate new test agents on the immature skeleton during non-clinical testing. Morphological abnormalities, longitudinal bone growth and epiphyseal closure is effectively monitored using standard radiography (X-rays) while the more specialized technique of Dual Energy X-ray Absorptiometry (DXA) provides information on bone area, bone mineral content and bone mineral density for the whole body, axial and appendicular skeleton. Peripheral quantitative computed tomography (pQCT) provides information on the trabecular and cortical bone compartments and bone geometry at the proximal tibia and/or distal radius. These techniques can be performed repeatedly on the same animals at a range of ages, from neonatal pups to juvenile animals of several months of age. DXA scans have been performed successfully in our laboratory on dog pups from 1 day of age up to adulthood. Histopathological and histomorphometric techniques further evaluate treatment effects on bone and carti-

lage. Bone structural variables were accurately measured on 29-day old rat pups. Growth plate width was measured to assess the potential for effects on bone mineralization. The articular cartilage structural integrity was assessed using Safranin-O stained sections to target the proteoglycan content and chondrocyte population. We have shown that DXA provided reproducible and repeated non-invasive measurements of bone density *in vivo* in skeletally immature animals, and that radiography was a useful tool to assess bone growth. Post-mortem evaluations using morphometry and histology techniques were used to further characterize the structural changes of bone and/or cartilage.

2100 SAFETY ASSESSMENT OF CARGLUMIC ACID IN JUVENILE RATS.

R. Forster[1], G. Chevalier[1], M. Attia[1], L. Martin[2] and M. Fortun[2]. *[1]CIT, Evreux, France and [2]Orphan Europe, Paris, France.*

Carglumic acid (N-carbamoyl-L-glutamic acid) is currently the only specific drug therapy for N-acetylglutamate (NAG) synthase deficiency, a very rare congenital disorder of the urea cycle. Sufferers from this condition are unable to eliminate nitrogen which accumulates as ammonia in the blood, with consequent harmful effects, in particular for the brain, and rapid lethal course in most cases. Carglumic acid can substitute for the lacking NAG in activating the first enzyme of the urea cycle (carbamoyl phosphate synthetase) and thus re-establishing nitrogen elimination. As part of a package of regulatory studies to support registration of the pharmaceutical grade carglumic acid supplied by Orphan Europe as an orphan drug, a chronic toxicity study was performed in rats. Since childhood therapy is often required, the study included an evaluation of growth and development during the juvenile period. Carglumic acid was administered daily to rats from age 4 weeks for a period of 6 months. In addition to standard parameters (body weight, clinical observations, ophthalmology, haematology, blood biochemistry, urinalysis and histopathology) the study included evaluation of the development of bone (by DXA *in vivo* and *ex vivo*), teeth and body length. Drug levels in plasma and urine samples were determined in order to investigate systemic exposure and elimination of the compound. On completion of 26 weeks treatment, potential effects on the immune system were assessed through histopathology of lymphoid organs and lymphocyte subset determinations. Cell proliferation in selected tissues was quantified by PCNA staining (kidneys, liver and testes). Potential effects on reproductive functions were evaluated in males (by mating trial with untreated females and seminology) and in females (estrus cycle). Treatment with carglumic acid was well tolerated at the selected dose-levels, which represents appropriate multiples of the clinical long-term treatment-levels, supporting the safety of the proposed clinical use in NAGS deficiency.

2101 COMPARATIVE TOXICITY STUDY OF PHENYTOIN IN JUVENILE AND ADULT CYNOMOLGUS MONKEYS.

R. M. Perez[1], F. P. de Villa[1], L. S. Antonio[1], T. Hayashi[1], N. Muto[2], E. Suzuki[2] and M. Nomura[2]. *[1]INA RESEARCH PHILIPPINES, INC., Laguna, Philippines and [2]Ina Research Inc., Nagano, Japan.*

The decision to utilize particular animal species as test systems is based on similarities in the drug metabolic profile and the developmental status of the target organs between such species and humans. The 2C enzymes of the adult monkey are known to be comparable to their human counterpart. Juvenile monkeys exhibit developmental characteristics similar to pediatric patients making them potentially excellent test systems for assessing toxicity in the pediatric population. In this study, the comparative toxicity of oral phenytoin (PHT), a drug known to be largely metabolized by the CYP2C9 and CYP2C19 isoforms of the cytochrome P450 enzyme system, at dose levels of 15 and 30 mg/kg/day for 14 days was evaluated in juvenile and adult cynomolgus monkeys. Six- and 18-month old male and female juvenile monkeys were randomly divided into control and treatment groups. Adult (4.5-year old) male and female monkeys were included in the treatment group. The treatment group initially received 15 mg/kg/day for 2 weeks. No remarkable changes in the neurobehavioral scores, body weights, food consumption, hematology and clinical chemistry parameters, ECG or EEG measurements were observed among the animals given 15 mg/kg/day. PHT plasma concentrations were comparable in all age groups of both sexes on Day 1 at 24 hours after administration. However, the PHT plasma concentration was apparently higher in the 6-month old animals compared to those in the 18-month old and adult monkeys. The findings were suggestive of the slower metabolic rate in the 6-month old monkeys compared to the relatively older animals. The results are explained in terms of the age-related differences in the development of toxicity and their relationship with CYP enzyme activities. Our results support the necessity of conducting nonclinical toxicological evaluation of drugs intended for the pediatric population.

2102 GLOBAL ANALYSIS OF ABERRANT DNA METHYLATION INDUCED BY NEONATAL EXPOSURE TO DIETHYLSTILBESTROL USING RESTRICTION LANDMARK GENOMIC SCANNING (RLGS).

K. Sato[1], H. Fukata[2], Y. Kogo[3], J. Ohgane[3], K. Shiota[3] and C. Mori[1, 4]. *[1]Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan, [2]Department of Environmental Medical Science (SRL), Graduate School of Medicine, Chiba University, Chiba, Japan, [3]Laboratory of Cellular Biochemistry, Veterinary Medical Science/Animal Resource Science, The University of Tokyo, Tokyo, Japan and [4]Core Research for Evolutional Science of Technology (CREST), Japan Science and Technology Corporation (JST), Kawaguchi, Japan.*

Exposure to endocrine disruptors during the critical periods of development causes persistent induction or suppression of certain genes, and is supposed to result in abnormality of reproductive organs and cancer. In these processes, epigenetic changes such as DNA methylation and histone modification (acetylation, methylation, or phosphorylation) suggested to be involved. In the present study, we examined the effect of an endocrine disruptor on global DNA methylation. Synthetic estrogen, diethylstilbestrol (DES) was injected into C57BL/6 mice from neonatal day 1 to 5 (3 μg/day). At day 30, genomic DNA was collected from epididymis or uterus. Restriction landmark genomic scanning (RLGS) is a two-dimensional gel electrophoresis method that allows detection of DNA methylation if a methylation-sensitive landmark enzyme such as NotI is used. Using RLGS method, DNA methylation profiles of collected samples were analyzed. In the epididymis of DES-treated group, 6 loci were demethylated and 1 locus was methylated in about 1, 000 loci, as compared to untreated group, and in the uterus, 6 loci were demethylated and 5 loci were methylated. Furthermore, effects of lower dose of DES (from 0.00003 to 0.3 μg) in uterus were examined. The number of loci which status of methylation changed was increased dose-dependently. We suggest that global analysis of DNA methylation using RLGS is an effective approach to assess the toxicity of exogenous agents including endocrine disruptors.

2103 DETERMINING THE RESPIRATORY BIOAVAILABILITY OF WATER-SOLUBLE VAPORS USING PBPK MODELING AND A COMBINED GAS UPTAKE INHALATION, PLETHYSMOGRAPHY, AND BLOOD SAMPLING SYSTEM.

A. Woodstock[1], T. S. Poet[1], J. J. Soelberg[1], H. Wu[1], J. G. Teeguarden[2], W. Faber[3], B. Francis[4] and R. A. Corley[1]. *[1]Pacific Northwest National Laboratory, Richland, WA, [2]Environ, Collegeville, PA, [3]WFTC, LLC, Victor, NY and [4]American Chemistry Council, Arlington, VA.*

A novel approach involving PBPK modeling and a combined gas uptake inhalation, plethysmography and blood sampling system was developed to determine the respiratory bioavailability of a series of ethyl, propyl, isopropyl, butyl, isobutyl and amyl alcohols and acetate esters in rats. Six male SD rats, with jugular cannulas exteriorized through a gas uptake chamber, were individually exposed for 2 hr to initial chamber loading concentrations of 2000 ppm for each chemical. Chamber concentrations and respiratory rates were determined simultaneously while blood samples were collected for analysis of each parent chemical and its key metabolites. Respiratory rates rapidly decreased at the start of each exposure then increased linearly as chamber concentrations decreased. A generic gas uptake PBPK model using published partition coefficients and metabolism rates, was modified to incorporate measured, time-dependent respiration rates to analyze the data. For these highly water-soluble vapors, chamber concentration data were highly sensitive to respiratory rates and bioavailability; thus, incorporating plethysmography in the gas uptake system was critical to the determination of respiratory bioavailability. In contrast, chamber concentration data were not sensitive to either metabolic rates or blood:air partition coefficients >10. However, the concentrations of parent chemical in blood during each exposure were highly sensitive to metabolism as well as ventilation rates and fractional absorption. Thus, the blood data served as an internal validation of the bioavailability estimated from the chamber concentration data. The resulting respiratory bioavailabilities averaged ~40-50% of total ventilation for the alcohols and ~50-70% for the acetates. (Sponsored by the Oxo Process Panel of the American Chemistry Council).

2104 ROLE OF TUMOR NECROSIS FACTOR ALPHA AND INTERLEUKIN-1 BETA IN THE DEVELOPMENT OF PULMONARY TOXICITY FROM EXPOSURE TO ADVANCED COMPOSITE MATERIAL COMBUSTION ATMOSPHERES.

P. G. Reinhart[1], D. L. Courson[2], J. E. Reboulet[3] and E. C. Kimmel[3]. *[1]Naval Health Research Center (Toxicology Detachment), Wright-Patterson AFB, OH, [2]Man Tech Environmental Technology Inc., Wright-Patterson AFB, OH and [3]Geo-Centers Inc., Wright-Patterson AFB, OH.*

Exposure to advanced composite material (ACM) combustion atmospheres results in a significant release of inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) as well as an influx of inflammatory cells entering the airways and an increase in pulmonary epithelial permeability. The purpose of this study was to evaluate the role of these cytokines in the development of ACM smoke-induced pulmonary toxicity. Genetically altered mice (TNF-α -/-, and IL-1β -/-) and their genetic controls were exposed to ACM combustion atmospheres for 1 hour. Twenty four hours later the lungs were lavaged and the lavage cells counted and identified. The lavage fluid was analyzed for inflammatory cytokines and albumin. To determine the effectiveness of pre-treatment, antibodies against TNF-α as well as IL-1β were administered immediately prior to smoke exposure. The results revealed significant reductions in total lavage cells, percentage of neutrophils, IL-1β, and IL-6 in the TNF-α -/- group when compared to controls. In the IL-1β study, significant reductions in total lavage cells and percentage of neutrophils were noted. The use of antibodies failed to protect against changes in epithelial permeability based on lavage fluid albumin levels. No changes were noted in the levels of TNF-α or IL-1β in antibody treated groups as compared to controls. Anti-TNF-α resulted in a significant reduction in the percentage of PMNs in the lavage fluid while anti-IL-1β resulted in a higher percentage. Both antibody treatments resulted in elevated levels of IL-6. In conclusion, silencing of either TNF-α or IL-1β gene results in a significant reduction in ACM smoke induced inflammatory response while pretreatment with the appropriate antibody failed to prevent the inflammatory responses.

2105 TIME COURSE OF PULMONARY EFFECTS FROM EXPOSURE TO ADVANCED COMPOSITE MATERIAL COMBUSTION ATMOSPHERES.

D. L. Courson[1], P. G. Reinhart[2], J. E. Reboulet[3] and E. C. Kimmel[3]. *[1]Man Tech Environmental Technology Inc., Wright-Patterson AFB, OH, [2]Naval Health Research Center (Toxicology Detachment), Wright-Patterson AFB, OH and [3]Geo-Centers Inc., Wright-Patterson AFB, OH.*

While the use of advanced composite materials (ACM) provides a significant benefit as a lighter alternative to metallic components, their downside is their combustibility. The effects of exposure to such atmospheres are not completely known. To address this data gap, we exposed Fischer 344 rats to ACM combustion atmospheres. Rats were exposed for 1 hour in whole body chambers to smoke produced by the pyrollysis of 60 g of carbon/graphite/epoxy material. Controls were exposed in a similar fashion to filtered air. After 1, 3, 7, 21, and 156 days post-exposure, the animals were evaluated for pulmonary toxicity. Bronchoalveolar lavage was performed and the cellular components identified and quantified. The lavage fluid was analyzed for tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and albumin. Significant increases in TNF-α were noted in all of the smoke-exposed groups. Significant increases in IL-1β were noted on days 1 and 21 while IL-6 was significantly elevated on day 1 only. Albumin levels in the lavage fluid were significantly greater in the smoke exposed groups for all time points examined, with the exception of day 156. Cell differentials in the smoke-exposed animals revealed significant increases in neutrophils on days 1 and 3 while total lavage cell counts were significantly higher for all time points with the exception of day 7. In conclusion, exposure to ACM combustion atmospheres results in a time dependent release of inflammatory cytokines with a sustained influx of inflammatory cells, and changes in epithelial permeability.

2106 FUNCTIONAL ROLE OF TRANSFERRIN IN THE UPTAKE OF METALS TO THE BRAIN VIA THE OLFACTORY PATHWAY.

D. C. Dorman, B. A. Wong, M. Sar, B. E. McManus, A. M. McElveen, D. G. Wallace, E. E. Gross and D. B. Rao. *CIIT Centers for Health REsearch, Research Triangle Park, NC.*

Environmental and occupational exposures to metals *via* inhalation are associated with several neurodegenerative diseases. Although inhaled or instilled Mn can be directly transported from the nasal olfactory mucosa (OM) to the brain, the exact mechanism of uptake and transport to the brain remains undefined. Manganese and iron share structural, chemical and physiological similarities and studies with both metals have alluded to a common transport protein such as transferrin (Tf). This study investigates the role of Tf with respect to inhaled metals in the rat nose. Initial experiments demonstrate for the first time, the presence of Tf and Tf receptor in the rat nasal mucosa (using immunohistochemistry and western blots). Subsequent experiments involving nasal instillations with 59FeSO4 show that iron is co-localized with Tf in the nasal mucosa (cellular autoradiography plus immunohistochemistry). Results from the above experiments suggest that inhaled iron deposited in the nose may not be available for transport to the brain. To test the latter hypothesis, rats were exposed to 59FeSO4 for 90 min *via* inhalation and then euthanized at various time-points postexposure. Tissues from the nose (olfactory mucosa and nonolfactory mucosa) and brain (olfactory bulb, olfactory tract plus tubercle, and striatum) were collected. Tissue 59Fe concentrations were determined by gamma spectrometry, and heads were collected for autoradiography to visualize the localization of 59Fe from the nasal mucosa across to the brain. Data obtained from both gamma spectrometry and autoradiography reveal that iron remained in the nasal regions of the olfactory system. Analysis of olfactory mucosa by combined HPLC and gamma spectroscopy revealed that iron was associated with Tf. In conclusion, unlike manganese, inhaled iron remains in the nose and is not transported to the brain. Differences in stability of different valence states of Mn (Mn2+) versus Fe (Fe3+) may permit diffusion of soluble Mn in nervous tissue while limiting Fe to the mucosa.

2107 CORRELATION OF NASAL SURFACE-AREA-TO-VOLUME RATIO WITH PREDICTED INHALED GAS UPTAKE EFFICIENCY IN HUMANS.

R. Segal[1], G. M. Kepler[2], D. L. Kalisak[1], R. B. Richardson[1] and J. S. Kimbell[1]. *[1]CIIT Centers for Health Research, Research Triangle Park, NC and [2]Consultant, Chapel Hill, NC.*

Variances in nasal anatomy, airflow, and patterns of inhaled gas uptake among human individuals may cause significant differences in the regional dose of inhaled gases or vapors in the nasal passages and subsequently to the lung. Computational fluid dynamics (CFD) models can quantify the contribution of anatomical variation to risk assessment uncertainty and reduce the reliance on default uncertainty values. For water-soluble, reactive gases, nasal surface area and volume may be predictors of nasal scrubbing efficiency. To test this hypothesis, nasal surface area and volume from the nostrils to the posterior end of the nasal septum were calculated from digitized MRI scans of the head for a number of adult individuals provided to us by Dr. Ray Guilmette and the Lovelace Respiratory Research Institute. Four subjects were selected whose ratio of nasal surface area to volume (SAVR) represented a range of values: 1.07, 1.03, 0.94, and 0.85 mm^{-1} for Subjects A (male), 12 (female), 14 (male) and 18 (female), respectively. Anatomically-realistic CFD models for the nasal passages of these individuals were constructed by a semi-automated process that provided input to a commercial mesh generator (Gambit, Fluent, Inc., Lebanon, NH). Allometrically-scaled minute volume flow rates were calculated to be 16.1, 12.9, 16.2, and 13.9 L/min for Subjects A, 12, 14, and 18, respectively. Steady-state inspiratory airflow at individualized flow rates and inhaled gas uptake were simulated for a reactive, water-soluble, low-molecular-weight gas using commercial CFD software (FIDAP, Fluent, Inc., Lebanon, NH). Total nasal uptake was estimated to be 90, 89, 85, and 84% for Subjects A, 12, 14, and 18, respectively, suggesting a correlation with SAVR. These studies and their extension to additional individuals form a basis for developing an empirical means of characterizing populations that may be at a higher risk for developing respiratory tract effects than the general population.

2108 EVALUATION OF THREE COMMERCIALLY AVAILABLE AEROSOL GENERATORS FOR ANIMAL BASED RESEARCH STUDIES.

M. Eifrid, M. J. Brooker and R. Moutvic. *Toxicology, Battelle, Columbus, OH.*

The purpose of this study was to compare the aerosol output and particle size distribution of three different aerosol generators and to evaluate their use for animal based research studies. The generators tested were the Aeroneb® Pro, Omron MicroAir®, and the Pari LC® Plus. The study was performed using a simple matrix approach. Three different concentrations of saline (9, 30, and 70 mg/ml) were used as the testing solutions. Airflow rates of 5, 10, and 15 L/min were used for the collection of aerosol filter samples. Particle size distributions were sampled using 5 L/min In-Toxicology cascade impactors. The generation system consisted of a 2-inch inner diameter (I.D.) acrylic tube, two conical delrin end caps, and an anesthetic wye piece(s). The total volume of the system was approximately 400 ml. The nebulizer being tested was connected to one limb of an anesthetic wye piece, and the common limb of the wye piece was connected to one of the end caps. The remaining limb of the wye piece was capped or used for the addition of positive pressure air. All sampling was performed downstream of the nebulizer. All nebulizer-testing systems were composed of the same components with exception of any

special fittings needed to attach the nebulizer to the anesthetic wye piece. All generators produced similar aerosol concentrations with each concentration of saline. Particle size distribution measurements for all nebulizers resulted in mass median aerodynamic diameters (MMAD) that were suitable for animal based research studies. However, differences in the MMAD were observed between the nebulizers were a deciding factor depending on species of animal being used. The Omron MicroAir® was the only device which needed special adaptations to be used in an animal exposure system. The overall results show that all 3-aerosol generators were suitable for animal based research studies.

2109 AIRFLOW DISTRIBUTION AND PARTICLE DEPOSITION IN DIFFERENT LOBES OF THE LUNG.

B. Asgharian and O. T. Price. *Biomathematics and Physical Sciences, CIIT Centers for Health Research, Durham, NC.* Sponsor: B. Wong.

Preferential, site-specific lung injury may be the result of nonuniform particle deposition on lung airway surfaces. Existing predictive models of particle deposition in the lung are based on simplified lung structures such as a typical path model and are unable to accurately predict dose inhomogeneity throughout the lung. A multiple-path dosimetry model was developed previously that allowed the prediction of particle losses due to the actual asymmetric nature of the lung (Anjilvel and Asgharian, 1995, Fundam. Appl. Toxicol., 28:41-50). Lung airways were assumed to expand and contract uniformly. As a result, airflow rate in an airway was proportional to the lung volume distal to that airway. Calculations of particle losses indicated that a major determinant of local particle deposition distribution was the volume of air going to that location of the lung. Thus, formulation of a realistic model of deposited dose variation requires accurate capture of airflow distribution in the lung. Based on physiological and physical considerations, three models of airflow distribution in the lungs of humans are presented and used in a dosimetry model of particles to calculate deposition in the lung. The airflow models examined were based on lung compliance and resistance, uniform expansion and contraction of lung airways, and conservation of the air mass and momentum transport in a lung with fixed airway dimensions during breathing. Under resting breathing conditions, the lung compliance and resistance model predicts up to 4% higher deposition fraction than the uniform expansion model. The difference in deposition diminished with increasing minute ventilation. While the total deposition between the two models remained relatively the same, the distribution of the deposited particles in different lobes of the lung varied. For example, the ratio of particles deposited in the upper to that in the lower lobes was 15% higher in the compliance and resistance model. The two models predict significantly different deposition values compared with the third model based on fixed lung geometry.

2110 COMPARISON OF EXPERIMENTAL MEASUREMENTS WITH MODEL CALCULATIONS OF PARTICLE DEPOSITION EFFICIENCIES IN MONKEY AND RAT NASAL AIRWAYS.

B. A. Wong[1], J. T. Kelly[2], B. Asgharian[1] and J. S. Kimbell[1]. *[1]CIIT CHR, Research Triangle Park, NC and [2]UC Davis, Davis, CA.*

One function of the nasal airways is to filter inspired air to prevent toxic particles from reaching the lungs. Particles in inspired air may deposit in the upper respiratory tract (URT) before reaching the lung. Experimental measurements of URT particle deposition efficiency involving hollow molds have been developed. In this study, acrylic molds of F344 rat and rhesus monkey nasal airways were produced using a lost-wax casting technique. The deposition of aerosols from 1 to 10 μm was determined by measuring the concentration of a monodisperse aerosol at the inlet and the outlet of the molds. Air flow was maintained at constant inspiratory flow rates. Computational fluid dynamics (CFD) models of the nasal airways were also developed for the F344 rat and rhesus monkey. A numerical mesh describing the nasal airways was generated by digitizing images of tissue sections. Air flow patterns were calculated using CFD techniques to solve the Navier-Stokes equations governing the behavior of fluid flow. The deposition of particles as a function of size was determined from the air flow patterns using a computer model of particle dynamics developed in house. The experimental measurements of particle deposition efficiency resulted in a sigmoidally-shaped deposition curve in which larger particles deposited with approximately 100% efficiency, while smaller particles had a low deposition efficiency. The computer model calculations of particle deposition efficiency showed a similar trend. The predicted deposition of larger particles matched experimental data reasonably well. However, the computer model calculations over-predicted the deposition of smaller particles significantly. The reasons for the discrepancy at smaller particle sizes may be attributed in part to the density of the numerical mesh and to the initial conditions used in the CFD and particle computer model calculations. The comparison with experimental measurements helps evaluate model predictions to enable model refinements to reduce numerical error.

2111 NASAL TOXICITY OF CARBON TETRACHLORIDE IN RATS: DOSE RESPONSE AND TIME COURSE STUDIES.

C. Reed[1], S. Simpson[1] and J. Foster[2]. *[1]School of Biomolecular Sciences, Liverpool John Moores University, Liverpool, United Kingdom and [2]Pathology Department, AstraZeneca, Macclesfield, United Kingdom.* Sponsor: E. Lock.

Carbon tetrachloride (CCl_4) is known to be metabolised within the olfactory epithelium of rats, and its nasal toxicity has been alluded to. The aim of this work was to fully characterise the toxicity of CCl_4 in the rat nasal cavity. Adult male Han Wistar rats (dose response, n = 4; time course, n = 6) were administered a single oral dose of CCl_4 in corn oil (10ml/kg; dose response, 0-2400mg/kg; time course, 1600 or 2400mg/kg). Animals were killed at 24hr (dose response) or at intervals between 4 and 96hr (time course). Heads and samples of liver were processed for morphological assessment. As expected, dose-dependent centrilobular degeneration was observed in the liver. Within the nasal cavity, only the olfactory epithelium was affected and the lesion was restricted to the septum and the medial aspects of endoturbinates 1-3. Both the extent and severity of the mucosal degeneration were dose-dependent. The severity of the mucosal degeneration increased from epithelial vacuolation (800mg/kg) to undulation and stripping (1600mg/kg). At higher doses CCl_4 appeared less toxic and at 2400mg/kg there was no evidence of epithelial stripping, only vacuolation and undulation. During the time course, hepatic degeneration was evident 4hr after dosing and was both dose- and time-dependent, with some evidence of recovery at 96hr. In the nasal cavity, degeneration of the olfactory mucosa was not apparent until 16hr after dosing, increased in severity up to 48hr and was less severe at 96hr. At all time points olfactory degeneration was more marked in the 1600mg/kg group compared to the 2400mg/kg group. Thus CCl_4 was found to be toxic to those regions of the nasal cavity that express high levels of cytochromes P450, suggesting *in situ* bioactivation. The reasons for the decrease in olfactory toxicity at high doses are unknown, but may include altered toxicokinetics due to damage to other organs such as the liver and gastrointestinal tract.

2112 APPLICATION OF MAGNETIC RESONANCE IMAGING IN THE DEVELOPMENT AND VALIDATION OF 3D COMPUTATIONAL MODELS OF THE RESPIRATORY SYSTEM.

R. A. Corley[1], K. R. Minard[1], B. Saam[2], C. Timchalk[1], H. E. Trease[1], L. L. Trease[1], C. G. Plopper[3], J. Fowler[4], E. M. Postlethwait[5] and J. R. Harkema[6]. *[1]PNNL, Richland, WA, [2]University of Utah, Salt Lake City, UT, [3]University of California, Davis, CA, [4]CGC, Los Alamos, NM, [5]UAB, Birmingham, AL and [6]MSU, Lansing, MI.*

The development and validation of detailed 3D biologically based models of the respiratory system requires new techniques for measuring localized airflows, particle deposition, airway geometry and tissue mechanics. To obtain accurate 3D geometry datasets on nasal and pulmonary airways of rats and monkeys, several magnetic resonance imaging (MRI) techniques were developed including perfusion of airways with contrast agents to enhance imaging, intravenous infusion of contrast agents to enhance the imaging of tissues surrounding airways and the imaging of silicone casts of airways. Each technique provided important geometric information on the structures of the respiratory system with resolutions ranging from 50-200 μm. Geometry phantoms and correction algorithms were developed to correct for image distortions over the field of view. New, semi-automated digital data processing methods were refined to significantly reduce the time airway segmentation and generation of 3D computational meshes from imaging data. This will facilitate the processing of multiple data sets from a variety of species for use in computational fluid dynamics (CFD) airflow simulations. The data processing techniques were also applicable to spiral-CT scans of human and sheep lungs and confocal images of cellular structures lining airways of normal and asthmatic monkeys. In addition to reconstructing airway surface geometries, MRI techniques were developed to exploit the physical properties of hyperpolarized 3He. Hyperpolarized 3He imaging coupled with 1H imaging shows considerable promise for imaging the dynamic properties of respiratory system, localized airflows and particle deposition. Such data will be necessary for validating 3D, CFD models of the respiratory system. (Supported by DOE LDRD, DOE Contract DE-AC06-76RLO 1830 and NIEHS grant no. 1 P01 ES011617-01A1)

2113 INHALED SOLID ULTRAFINE PARTICLES (UFP) ARE EFFICIENTLY TRANSLOCATED VIA NEURONAL NASO-OLFACTORY PATHWAYS.

T. Feikert[1], P. Mercer[1], N. Corson[1], R. Gelein[1], L. Opanashuk[1], A. Elder[1], V. Silva[1], J. Carter[2], A. Maynard[3], J. Finkelstein[4] and G. Oberdorster[1]. *[1]Environmental Medicine, University of Rochester, Rochester, NY, [2]Procter & Gamble Co., Cincinnati, OH, [3]NIOSH, Cincinnati, OH and [4]Pediatrics, University of Rochester, Rochester, NY.*

Clearance pathways for inhaled solid UFP (<100 nm) depositing in the respiratory tract include their translocation to extrapulmonary sites. Our previous results in rats with inhaled ultrafine carbon particles suggested that nasally-deposited solid

UFP may be taken up by olfactory rods in the olfactory nasal mucosa and translocated along the olfactory dendrites and axons. Olfactory nerve translocation has been well demonstrated for inhaled soluble metal compounds. In order to test this hypothesis for solid UFP and determine the significance for causing health effects we exposed groups of rats to ultrafine MnO_2 particles (31 nm) in whole-body exposure chambers for up to 11 days, with either both nostrils patent or the right nostril occluded. AAS analysis of Mn in lung, liver, kidney, olfactory bulb and several other brain regions as well as Atlas gene array and antibody protein array analyses of these brain regions were performed. After 11 days of exposure, Mn concentrations in the olfactory bulb were 3.6-fold higher whereas lung Mn concentrations only doubled, and there were also significant increases of Mn in striatum and frontal cortex and cerebellum. With the right nostril occluded, Mn accumulated only in the olfactory bulb of the side with the patent nostril. TNFα mRNA and protein were significantly increased about 30-fold in the olfactory bulb and showed also increases in the other brain regions associated with increased Mn levels. MIP-2 and NCAM mRNA were also significantly increased. We conclude that the olfactory neuronal pathway is very efficient for translocating inhaled solid UFP to the CNS and that translocated UFP, depending on their chemistry, can cause serious adverse effects. We suggest further that despite differences between the human and rodent olfactory systems this pathway is likely to be operative in humans as well.

2114 CARBON BLACK-INDUCED NASAL LESIONS IN LABORATORY RODENTS: A SPECIES COMPARISON.

P. Santhanam[1], J. Wagner[1], L. Bramble[1], A. Elder[2], G. Oberdorster[2] and J. Harkema[1]. *[1]Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, MI and [2]Environmental Medicine, University of Rochester, Rochester, NY.*

Previously we demonstrated that rats repeatedly exposed to airborne carbon black particles (Cb) develop chronic rhinitis and persistent alterations in the nasal epithelium. The purpose of the present study was to determine if mice and hamsters subjected to similar long-term inhalation exposures to Cb would develop nasal lesions comparable to those in rats. Female F344 rats, B6C3F1 mice, and Syrian golden hamsters were exposed to filtered air alone or nominal concentrations of 1, 7 or 50 mg/m3 Cb (primary particle size 17 nm; airborne agglomerate 0.9 micron) for 6 h/day, 5 days/wk for 13 wk. Rodents were sacrificed 1 day, 13 wk, or 11 mo post-exposure (PE). Nasal airways were processed for light microscopy. Volume densities of intraepithelial mucosubstances (IM) and numeric densities of airway epithelial cells were estimated using image analysis and morphometry. Rats exposed to 7 and 50 mg/m3 Cb had chronic rhinitis and mucous cell metaplasia (MCM) and hyperplasia (MCH) with increased mucous cells and IM. Mice exposed to these same concentrations of Cb showed similar but less severe rhinitis, MCM, and MCH. In contrast, Cb-exposed hamsters did not develop rhinitis or MCM, but rather only mild MCH in the nasopharynx. The magnitude of the lesions in all the rodents was dependent on dose and time PE. At 1 day PE, rats exposed to 50 mg/m3 Cb had 14, 22, and 11x more IM in the proximal, middle, and distal nasal airways, respectively, than did mice exposed to the same concentration of Cb. Hamsters exposed to 50 mg/m3 Cb had only minimal IM in these intranasal sites. Cb-induced lesions persisted in rats at 11 mo PE, but the lesions in mice and hamsters were resolved by 13 wk PE. These results indicate that Cb-induced nasal injury, like previously reported Cb-induced lung injury, is more severe in rats than in mice or hamsters. (Research sponsored in part by the International Carbon Black Association).

2115 THE BIOPERSISTENCE OF CANADIAN CHRYSOTILE ASBESTOS FOLLOWING INHALATION.

R. Rogers[2], D. Bernstein[1] and P. Smith[3]. *[1]Consultant in Toxicology, Geneva, Switzerland, [2]Rogers Imaging Corporation, Needham, MA and [3]Research & Consulting Company Ltd., Fullinsdorf, Switzerland.*

Chrysotile asbestos is often included with other asbestos materials in evaluation and classification. However, chrysotile is a serpentine with markedly different physical and chemical characteristics in comparison to amphiboles (eg tremolite). To quantify the dynamics and rate of chrysotile removal from the lung the biopersistence of commercial "QS Grade 3-F" textile grade chrysotile (Eastern Townships - Quebec) was studied. As long fibers have been shown to have the greatest potential for pathogenicity, the chrysotile was chosen to have more than 200 fibers/cm3 L > 20 um in the exposure aerosol. Study Design: 1) Fiber clearance (lung digestions): At 1, 2, 7, 14 d, 1, 3, and 12m following a 5 d (6 h/d) inhalation exposure lungs were digested by low temperature plasma ashing and analysed by TEM for total chrysotile fiber number and size. 2) Fiber distribution (confocal microscopy): At 1, 2, 7, 14-d, 1 & 3 m post exp. lungs of animals were analysed by confocal microscopy to determine the anatomic fate, orientation and distribution of the chrysotile fibrils deposited on airways and in the parenchymal region. Chrysotile was rapidly removed from the lung. Fibers L > 20 um were cleared with a T1/2 = 16 days, most likely by dissolution and disintegration into shorter fibers. The shorter fibers also rapidly cleared with fibers 5-20 um clearing even faster (T1/2 = 29.4 d) than those < 5 um in length (T1/2 = 107 d). This is within the range of clearance for insoluble nuisance dusts. The short fibers appeared as separate, fine fibrils occasionally unwound at one end. Short free fibers appeared in the corners of alveolar septa, and fibers or their fragments were found within alveolar macrophages. Neutrophil-mediated inflammatory response did not occur in the presence of chrysotile fibers at the time points examined. Taken in context with the scientific literature to date, this report provides new robust data which clearly supports the difference seen epidemiologically between chrysotile and amphibole asbestos.

2116 COMPARISON OF CALIDRIA CHRYSOTILE ASBESTOS TO PURE TREMOLITE: INHALATION BIOPERSISTENCE AND HISTOPATHOLOGY FOLLOWING SHORT TERM EXPOSURE.

D. Bernstein[1], J. Chevalier[2] and P. Smith[3]. *[1]Consultant, Geneva, Switzerland, [2]EPS, Muttenz, Switzerland and [3]RCC Ltd., Fullinsdorf, Switzerland.*

The differences between chrysotile, a serpentine mineral, and amphibole asbestos have been debated extensively. This study was designed to quantify the comparative clearance amd histopathological response of chrysotile and the amphibole asbestos tremolite. The inhalation biopersistence of of commercial grade Calidria RG144 chrysotile (Coalinga mine, New Idria, California) and of a long fiber tremolite were studied. For synthetic vitreous fibers, the biopersistence of the fibers longer than 20 μm has been shown to be directly related to their potential to cause disease. The chrysotile sample was specially prepared to have 200 fibers/cm3 L>20 μm in the exposure aerosol. These longer fibers were found to be largely composed of multiple shorter fibrils. The tremolite sample was chosen to have 100 fibers/cm3 L>20 um in the exposure aerosol. Calidria chrysotile fibers clear from the lung more rapidly (T1/2 fibers L>20 μm = 7 hours) than any other commercial fiber tested including synthetic vitreous fibers. The histopathological examination showed no sign of inflammation or pathology and was no different than the air control lungs. Following this 5-day exposure to tremolite, the tremolite fibers once deposited in the lung parenchyma do not clear and almost immediately result in inflammation and a pathological response in the lung. At 1d after cessation of exposure inflammation was observed and granulomas were already formed. By 14d these microgranulomas had turned fibrotic; and by 90d the severity of the collagen deposits had increased and interstitial fibrosis was observed in one rat. These findings provide an important basis for substantiating both kinetically and pathologically the differences between chrysotile and the amphibole tremolite. As Calidria chrysotile has been certified to have no tremolite fiber, the results of the current study together with the results from toxicological and epidemiological studies indicate that this fiber is not associated with lung disease.

2117 TOXICOLOGICAL AND MINERALOGICAL ANALYSIS OF RICHTERITE-WINCHITE ASBESTOS.

B. S. Van Gosen[1], H. A. Lowers[1], J. D. Hyde[2], G. P. Meeker[1], A. M. Bern[1], S. J. Sutley[1], M. L. Witten[2] and T. L. Ziegler[1]. *[1]US Geological Survey, Denver, CO and [2]The University of Arizona, Tucson, AZ.*

Inhalation of amphibole mineral fibers has been linked to respiratory disease, such as asbestosis and mesothelioma. As a result, the USEPA and OSHA chose to regulate occupational exposures to the asbestiform varieties of five amphiboles most commonly used in commercial asbestos applications, including grunerite (amosite), riebeckite (crocidolite), anthophyllite, tremolite, and actinolite. Fibrous varieties of other amphibole species have been increasingly recognized as toxic to the respiratory system. For example, high rates of respiratory disease in miners, mill workers, and residents of Libby, MT, are suspected to be due to fibrous sodic-calcic amphiboles that occur as accessories of the vermiculite ores mined and milled near Libby from 1923 to 1990. The Libby amphiboles may be classified compositionally as mainly winchite, with lesser amounts of richterite, tremolite, and magnesioriebeckite, and occur in a variety of particle shapes ranging from prismatic crystals to asbestiform fibers. For a comparative study, samples of amphibole asbestos were collected by the USGS from an abandoned mine site in CO. The samples display several properties typical of asbestos, such as bundles of separable, long, thin, hair-like fibers, which are <1 μm in width, tens to thousands of μm in length, curved, and bendable. Our study of the samples included x-ray diffraction, x-ray fluorescence spectrometry, and scanning electron microscopy accompanied by energy dispersive x-ray spectrometry. These techniques determined that the fibers are sodic-calcic amphiboles, exhibiting chemical compositions consistent with the richterite-winchite solid-solution series. Toxicological analysis of this richterite-winchite asbestos material using an rat lung cell model is in progress. This study will compare the toxicity of the CO richterite-winchite asbestos to the toxicity of Libby amphiboles with similar composition but differing morphologies, supporting efforts to understand the roles of amphibole composition and morphology in toxic response.

2118 TOXICITY TESTING OF FIBROUS PARTICLES: THE APPROPRIATE USE OF SHORT-TERM ASSAYS.

S. Olin and . ILSI RSI Working Group. *Risk Science Institute, International Life Sciences Institute, Washington, DC.* Sponsor: P. Fenner-Crisp.

Natural and synthetic fibers are a diverse group of substances of potential toxicological and public health concern. While many of these fibers have wide industrial and commercial applications, information regarding their potential impact on human health is often limited. The ILSI Risk Science Institute convened an expert working group to review and evaluate the available short-term assay systems for assessing the toxic and carcinogenic potential of both inorganic and organic fibers. Short-term assay systems are important, at least in part, because the costs of carrying out chronic tests on all new and existing fibers are prohibitive. In addition, short-term assays may help to minimize the use of animals in toxicity testing. This poster presents the perspectives of the working group on the strengths and limitations of the various short-term assay systems and will lay out a strategy for the use of these assays in prioritizing fibers for long-term testing, distinguishing those that are unlikely to present a hazard from those that warrant further investigation. Assay systems include those that determine physical and chemical characteristics, those that measure biodurability *in vitro* or biopersistence *in vivo*, as well as *in vitro* and *in vivo* toxicity assays of varying duration with a wide range of endpoints. (Supported by EPA Cooperative Agreement X-82916701 and the ILSI Risk Science Institute.)

2119 KINETICS OF ABSORPTION OF INHALED BENZO(A)PYRENE IN THE ISOLATED PERFUSED RAT LUNG.

P. Ewing, A. Ryrfeldt and P. Gerde. *Environmental medicine, Karolinska Institute, Stockholm, Sweden.*

Recent data in the dog has shown that the inhalation and rapid absorption of polycyclic aromatic hydrocarbons (PAHs) through the alveolar epithelium is an important route of entry to the circulation of unmetabolized PAHs. In this study, the absorption of inhaled B(a)P through the alveolar epithelium was investigated in the *in vitro* isolated perfused lung (IPL) model. The IPL of the rat, perfused with Krebs-Ringer bicarbonate 2% BSA medium, was exposed to B(a)P as a dry powder aerosol. Silica particles, (3 μm in diameter), were coated with tritium-labelled B(a)P. This method allowed us to deliver a reproducible amount of soluble B(a)P to the deep lung. The bioavailability of the silica-bound B(a)P, simulated *in vitro* in a 1-Octanol assay, was ≥ 85 % within 10 min. Concentration of ^{3}HB(a)P in perfusate samples and lung tissue was measured by liquid scintillation counting. A minute-long inhalation exposure of the deep lung to a bolus of B(a)P-coated silica resulted in a deposited lung burden of ∽ 8 ng of B(a)P. Desorbing B(a)P appeared rapidly in the lung perfusate. One third of the deposited dose was absorbed through the alveolar epithelium within 30 minutes. The B(a)P concentration in the perfusate peaked after 2, 5 min at a concentration of 7, 5 pM and decreased subsequently to 2 pM after 20 min. Preliminary data indicates an absorption kinetics of inhaled B(a)P in the IPL similar to that observed in BaP-exposed dogs. However, in the IPL the concentration peaked within 150 s of exposure compared to 90 s in the dog, suggesting that canine blood is more effective in extracting PAHs than the perfusion medium. Presently we are investigating different medium compositions to better simulate absorption kinetics *in vivo*. This study demonstrates the usefulness of combining our novel dry aerosol generating system with the IPL model to expose rodent lungs by inhalation. The system could be used as a labor- and cost-efficient alternative to *in vivo* inhalation exposures in studying the absorption and metabolic interaction of substances with a route-of-entry in the respiratory tract.

2120 TRACHEOBRONCHIAL AND NASAL CLEARANCE OF 1.0 MICRON PARTICLES IN THE RAT: COMPARISON WITH A TYPICAL PATH TRACHEOBRONCHIAL CLEARANCE MODEL.

E. C. Kimmel[1, 2], S. L. Prues[1, 2], J. E. Reboulet[1, 2] and D. L. Courson[1, 3]. [1]*Inhalation/Pulmonary Effects Laboratory, Naval Health Research Center (Toxicology), Wright-Patterson AFB, OH,* [2]*Geo-Centers, Inc., Wright-Patterson AFB, OH and* [3]*ManTech Environmental, Wright-Patterson AFB, OH.*

A typical path tracheobronchial (TPTB) model of particle clearance in rats based on application of flow continuity equations was recently developed (Kimmel et al., 2002, 2003). Based on a survey of the literature, TPTB predictions and reported particle retention at 24 hrs post exposure differed by -18 to + 7% for a variety of aerosols (MMADs = 0.1 to 4.18 μm, σgs = 1.0 to 1.4) giving an average agreement of 95.1%. Experimental studies were undertaken to refine the TPTB by incorporation of non-uniform mucus coverage of the airways and to account for its effect on conservation of mucus mass and estimated transport velocity; and to characterize an extrathoracic clearance component of the model. Forty-eight F-244 rats received a brief single, nose-only exposure to 1.0 μm monodisperse, stable isotope labeled latex particles. Respiratory tract tissues were harvested (0, 6, 15, and 24 hrs post exposure), neutron activated, and tissue particle burden was determined by beta and gamma emissions counting. Initial TPTB predictions of particle clearance from the tracheobronchial and extrathoracic compartments were comparable to experimental observations, with differences between predicted and observed fractional clearance ranging from -8 to + 22%. Normalization of initial particle burden for differences in ventilation (measured continuously during exposure) significantly improved agreement between unmodified TPTB predictions and observed clearance/retention to an average 92% accross observed time points. Modifications of the TPTB that account for non-uniform mucus coverage as a function of airway generation are currently in progress and the impact on model performance will be reported.

2121 COMPARATIVE ANALYSIS OF PULMONARY IRRITATION BY FUNCTIONAL MEASUREMENTS (PENH) AND PROTEIN IN BRONCHOALVEOLAR LAVAGE FLUID IN BROWN NORWAY RATS AND WISTAR RATS EXPOSED TO POLYISOCYANATE AEROSOL.

J. Pauluhn. *Toxicology, Bayer HealthCare, Wuppertal, Germany.*

Previous acute inhalation studies with polymeric methylenediphenyl-4, 4'-diisocyanate (MDI) aerosol provided experimental evidence that the effects related to lower respiratory tract irritation constitute the most sensitive changes and that they can be probed best by the measurement of protein in bronchoalveolar lavage (BAL). The objective of this study was to extend previous acute dose-response and time-course studies in Wistar rats by determining both the concentration and temporal effects following a single 6-hour MDI aerosol exposure on the breathing dynamics with selected endpoints in BAL. Results show that total protein in BALF was amongst the most sensitive endpoints to probe early effects caused by exposure to irritant polyisocyanate aerosol. Baseline Penh increased in a concentration-dependent manner and paralleled closely the magnitude of increase in BAL-protein on postexposure day 1. The time-course of changes in Penh complemented those of BAL-protein observed in previous studies. The incremental change of Penh during a stepped MCh-challenge showed decreased responsiveness with increasing baseline Penh values. In summary, these data show that the exposure to irritant concentrations of aerosolized poly-isocyanate caused a transient perturbation of the blood/air-barrier which can suitably be probed by measurements of protein in BALF. Penh paralleled this change. This suggests that Penh mirrors changes occurring at the alveolar rather than airway level. Accordingly, Penh might be viewed to be a sensitive, although non-specific functional endpoint suitable to probe changes in breathing patterns indicative of effects occurring at the parenchymal level. In regard to Penh and selected BAL-endpoints both strains of rats were equally susceptible, although some endpoints displayed a higher variability in Brown Norway (BN) rats when compared to Wistar rats. This supports the conclusion that Wistar rats afford a higher diagnostic resolution than BN rats.

2122 EXHALED BREATH PROTEIN SAMPLING IN UNANESTHETIZED PIGS.

O. R. Moss[1], N. Boggs[2] and J. Jackman[2]. [1]*Biomathematics and Physical Sciences, CIIT Centers for Health Research, Research Triangle Park, NC and* [2]*Research & Technology Dev. Center, APL/JHU, Laurel, MD.*

The early inflammatory response of the lung is useful for the identification of environmental, industrial, or biological warfare exposure agents and the evaluation of exposed individual response so as to expedite treatment. Exhaled breath has been used to assess the physiological state of humans by examining the levels of recoverable volatile compounds. More recently, non-volatile compounds, including proteins such as IL-4 and tumor necrosis factor, have been successfully recovered from human exhaled breath. In order to apply this method to rapid and specific identification of exposed individuals there is a need for enhanced analytical capability as well as the development of animal models. The work described here focuses on the development of a porcine model and evaluation of two techniques (a face mask and a "virtual" mask system) for obtaining exhaled breath from pigs. The face mask system is based on using a vacuum collection system and a modified anesthesia cone with one way valve. Each piglet (8 to 12 kg) is individually held during a half hour collection period. The "virtual" mask system is based on two funnels, one inside the other. In this system dry air is supplied in the annular passages between cones and is directed towards the head of the pig in such a way that the air surrounds the pigs snout, forming a virtual mask. This air flow turns back on itself into the inner funnel centered 5 to 8 cm from the nostrils of the animal. Exhaled breath is carried along in this sampling flow. Each pig is suspended in a standard restraint sling during sampling. Breath collections of approximately 45 piglets were initiated at a local farm in order to obtain real world samples of exhaled breath. In the field, the face

mask and virtual mask systems produced similar results of 3 to 5 ml of exhaled breath condensate collected per hour per pig for pigs weighing approximately 8 kg. (This work was performed under a subcontract to Applied Physics Laboratory, APL No 864736, as part of a DARPA funded project MDA972-01-D-005)

2123 EFFECTS OF 1, 3-BUTADIENE, ISOPRENE, AND THEIR PHOTOCHEMICAL DEGRADATION PRODUCTS ON HUMAN LUNG CELLS.

M. Doyle[1], K. Sexton[1], I. Jaspers[2, 1], K. Bridge[1] and H. Jeffries[1]. *[1]ESE, University of North Carolina, Chapel Hill, NC and [2]CEMALB, University of North Carolina, Chapel Hill, NC.*

Due to potential exposure in both workplace and ambient air, the known carcinogen 1, 3-Butadiene (BT) is considered to be a priority hazardous air pollutant (HAPS). Most of the studies investigating BT toxicity have examined carcinogenic endpoints. BT and its 2-methyl analog, Isoprene (ISO) are chemically similar but have very different toxicity, with ISO showing no significant carcinogenesis. The degradation chemistry of these compounds induced by sunlight is not fully understood, resulting in many unknown degradation products, which makes it difficult to identify their full toxic potential. In this study, we determined the relative toxicity and inflammatory gene expression induced by BT, ISO, and their photochemical, degradation products in A549 cells, a human alveolar type II-like cell line. A concentration of 200 ppb BT or ISO in the presence of 50 ppb NO were injected into separate photochemical smog chambers and allowed to photochemically react for approximately 4 hours. At sundown, A549 cells grown on membraneous support were exposed for 5 hours to either the gaseous precursor mixtures, i.e. BT + NO or ISO + NO, or their gaseous photochemical reaction products. GC and GC/MS initial analysis indicates that the primary photochemical products produced during these experiments for BT are acrolein, acetaldehyde, and formaldehyde, while photochemical reactions of ISO generated methacrolein, methylvinylketone, and formaldehyde (both formed ozone ≤ 200ppb). Approximately 9 hours post-exposure, the cells were examined for cytotoxicity and IL-8 gene expression. BT induced a 2-3 fold greater cytotoxicity and IL-8 gene expression as compared to its photochemical reaction products. In contrast, ISO photochemical reaction products caused a significantly greater cytotoxicity and IL-8 gene expression as compared to the unreacted ISO precursor. Taken together, these data suggest that biogenic ISO becomes increasingly more toxic as it reacts within the natural atmosphere, unlike BT, which appears to become less toxic as it reacts with sunlight.

2124 ACUTE, FOUR-WEEK, AND MICRONUCLEUS INHALATION STUDIES IN RATS WITH HEXAFLUOROISOBUTENE EPOXIDE.

D. P. Kelly[1], M. Donner[1], L. A. Malley[1], J. F. Hansen[1], V. O. Wagner, III[2] and N. E. Everds[1]. *[1]Haskell Laboratory, DuPont Company, Newark, DE and [2]BioReliance, Rockville, MD.*

Hexafluoroisobutene epoxide (HFIBO) is an intermediate used in the preparation of perfluorinated compounds and it is considered to be moderately toxic following inhalation. A single 4-hr exposure to 820 ppm was lethal in 3 of 6 male (m) rats; ataxia was noted at 600 ppm, and weight loss at 600 and 200 ppm. After positive findings (strains TA 100 and WP2 uvrA) in an Ames Salmonella assay, HFIBO was found to be negative in an *in-vivo* bone marrow micronucleus study where m and f rats were exposed to 25, 125 or 350 ppm of HFIBO for 6 hr/day for 2 days. However, at 350 ppm, 3 of 12 f rats died on the 2nd exposure day. In a 4 wk study, groups of 20 m and 20 f rats were exposed for 6 hrs/day, 5 days/wk to 5, 25, or 125 ppm of HFIBO followed by a 4 wk recovery. No deleterious effects were observed in clinical observations or body weights during the exposure or recovery periods. Neurobehavioral tests (including grip strength, FOB, and motor activity tests) at the end of the 4-week exposure period showed no effects. Clinical pathology evaluation showed minimal changes in rats exposed to 125 ppm, including decreased platelets (m only), decreased mean cell hemoglobin concentration and red cell distribution width (f only), increased serum bilirubin and urea nitrogen (f only), and increased urine volume/decreased urine osmolality (m only). Urinary fluoride was increased only at 125 ppm, indicating metabolism of HFIBO. None of the clinical pathology findings were considered necessarily adverse; rather the changes were minimal, likely unrelated to the test substance, and within the range of normal values. A slight reversible increase in heart weights (but no corresponding histological observations) was seen in m and f rats in the 125 ppm groups. No other anatomic pathologic effects were observed in this study. Based on the increased heart weights at 125 ppm, the no-observed-adverse-effect-level (NOAEL) for repeated inhalation exposure was 25 ppm.

2125 SINGLE-PASS BUBBLE AEROSOL GENERATOR FOR INHALATION STUDIES.

R. Jaeger[1], G. Mainelis[3], K. DeVoe[2] and M. Yao[3]. *[1]CH Technologies, Westwood, NJ, [2]BGI, Walham, MA and [3]Department Env Sciences, Rutgers University, New Brunswick, NJ.*

Inhalation exposure studies of pathogens require stable and reliable aerosol generators. As fragile organisms are aerosolized, their structural and biological integrity must be preserved. Air jet as well as ultrasonic nebulizers induce significant stress and reduced *via*bility due to forceful agitation, high shear force and repeated "recycling" of the carrier media. Recently, an aerosol generator that produces particles from a bubbling liquid was reported. This technique promised low shear force but still recycled the fluid. We describe an improvement in particle generation from a bubbling liquid by eliminating fluid reuse. In this device, a thin film of liquid is pumped onto the upper surface of an encapsulated porous stainless steel disk. Porosity can be set from 0.2 to 100 microns. Air is forced through the disk from below and breaks the liquid film into bubbles that grow and subsequently burst, releasing aerosol particles to the air. The particles are gently stirred and entrained by eight or more streams of dry air parallel to the surface. These radial streams are above the disk. Particles not entrained collect in the glass vessel and play no further role. We aerosolized P. fluorescens bacteria and polystyrene latex particles (1 micron) and determined that the particle concentration increased as the airflow increased from 2 to 5 L/min. Drying airflow from 10 to 30 L/min increased aerosol output. Distance above the porous disk had little effect. Constant generation rate over one hour demonstrated stability. Since organisms go through the aerosolization process only once, no significant decrease in bacterial *via*bility is expected during prolonged generation. As such, this instrument is useful in inhalation studies where extended delivery of stable and undamaged biological aerosols is required.

2126 COLLECTION, VALIDATION AND GENERATION OF BITUMEN FUMES FOR CHRONIC INHALATION STUDIES IN RATS.

G. Pohlmann, A. Preiss and W. Koch. *Aerosoltechnologie, Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany.* Sponsor: H. Muhle.

The objective of the study was to develop a laboratory generated exposure atmosphere to be used for chronic inhalation toxicity studies in rats that resembles, as closely as possible, personal exposures seen in workers during road paving operations. To achieve this, bitumen fume condensate (BFC) samples were collected from hot bitumen storage tanks and compared analytically with atmospheric workplace samples collected by different sampling devices and strategies. Two different laboratories analyzed personal and static samples taken at road paving worksites by different methods. Parameters determined were: Total Particulate Matter, Benzene Soluble Matter, Semi-Volatiles and Total Organic Matter, Boiling Point Distribution (BPD), Polycyclic Aromatic Hydrocarbons and UV Fluorescence (UVF). Different sampling methods were used to allow comparison of the standard German sampling method with the most commonly used industrial methods. A collecting procedure was developed that allowed sampling from hot bitumen storage tanks in an operational asphalt mixing plant. The sampling procedure has been optimized to collect material that matches the workplace samples as closely as possible. The validation procedure has been performed using a range of parameters that could be analyzed in both the workplace samples and the BFC collected from the tanks. BPD, UVF and content of individual PAHs were selected as parameters. For example the BPD of the collected sample did not differ more than 17 degree C from the average boiling point distribution of the work place samples, in the range from 5 to 95%. UVF of the BFC nearly exactly matched the average fluorescence of the workplace samples (105%). As a result, approximately 16 liters of BFC have been collected. A laboratory set-up for the re-generation of the airborne fume at three different exposure concentrations but with a similar composition was developed and has been running successfully for several months. The study was sponsored by the Bitumen industry assocaitions, ARBIT, Germany and Eurobitume, EU.

2127 INHALATION TOXICITY OF THE FLAVORING AGENT, DIACETYL (2, 3-BUTANEDIONE), IN THE UPPER RESPIRATORY TRACT OF RATS.

A. F. Hubbs, L. A. Battelli, R. R. Mercer, M. Kashon, S. Friend, D. Schwegler-Berry and W. T. Goldsmith. *HELD, NIOSH, Morgantown, WV.* Sponsor: D. Porter.

Diacetyl (2, 3-butanedione) is a diketone found naturally in foods such as butter and "generally recognized as safe" for use in low concentrations as a food additive. Diacetyl imparts the odor and flavor of butter to foods and also has industrial applications. Recently, an increased prevalence of fixed airways obstruction was reported in workers at a microwave popcorn plant and the lung disease correlated

with diacetyl exposure. In a previous study, inhalation of diacetyl-containing artificial butter flavoring caused necrosis of the nasal, bronchial, and bronchiolar epithelium in rats. We have now investigated the hypothesis that inhalation of diacetyl produces epithelial injury. Therefore, male Sprague-Dawley rats were exposed in a whole-body inhalation chamber for 6 hours to 0, 99.3 ± 0.07, 198.4 ± 0.10, or 294.6 ± 0.20 ppm diacetyl and euthanized the next day. Four levels of nose, three levels of trachea, and two lung sections were examined by light microscopy. In addition, the nose was examined by transmission electron microscopy (TEM) and the trachea was examined by TEM and scanning electron microscopy (SEM). At 198.4 ppm or higher, diacetyl inhalation resulted in significant necrosis of nasal epithelium with associated neutrophilic inflammation. At 294.6 ppm, diacetyl inhalation also caused significant necrosis of tracheal epithelium with associated neutrophilic inflammation. By SEM, diacetyl-induced tracheal changes included multifocal denuding of basement membrane with cell swelling, loss of microvilli, and loss of ciliated cells in the remaining epithelium. By TEM, tracheal changes included epithelial necrosis, denuded basement membrane, and elongation of epithelial cells near foci of exposed basement membrane. Diacetyl did not produce significant changes in the lung under these exposure conditions. These findings suggest that acute exposure to diacetyl alone is sufficient to cause upper respiratory tract epithelial necrosis in rats at concentrations of 198.4 ppm or higher.

2128 THE EVALUATION OF TWO ANESTHETICS FOR USE IN A BRONCHODILATOR SCREEN MODEL.

A. Sivillo, R. Moutvic, M. J. Brooker and I. M. Grossi. *Toxicology, Battelle, Columbus, OH.*

The goal of this study was to develop a model for screening bronchodilator drugs using propofol and isoflurane as alternative anesthetics to morphine-chloralose. The first phase involved comparing 2 models: one using propofol and the other using isoflurane anesthesia, when performing methacholine (Mch) challenge experiments (using escalating graded-doses of Mch) in 3 canines. In establishing a bronchoconstrictive model, it was found that with both anesthetic models, the plateau dose-response (seen through dynamic lung resistance measurements) was not consistent even though the peak response was very stable. Shifting to the second phase of the study, the propofol-anesthetized model was chosen over the isoflurane-anesthetized model as it resulted in a lower hypotensive response to the Mch challenge. The second phase used 6 canines and implemented measuring the peak response only, performing a second challenge (directly following the first challenge) to evaluate short-term repeatability of the model, and repeating these experiments using morphine-chloralose anesthesia as a standard for comparison. Following the morphine-chloralose experiments, animals were euthanized due to the known long-term neurological side effects resulting after chloralose anesthesia. Results demonstrated that the propofol model did not produce consistent results to repeat Mch challenge whereas the morphine-chloralose model was repeatable. In conclusion, it is suggested that the morphine-chloralose anesthetized bronchoconstrictive dog model may have potential as a screen for bronchodilator drugs.

2129 VALIDATION OF AN ISO-KINETIC DILUTOR FOR USE WITH AN ANDERSON CASCADE IMPACTOR.

J. Frye, R. Moutvic, M. J. Brooker and I. M. Grossi. *Toxicology, Battelle, Columbus, OH.*

The Anderson Cascade Impactor (ACI) is one of the most widely used and accepted devices employed by the Pharmaceutical industry for characterizing particle size distribution (PSD) of aerosols. For animal based pre-clinical toxicology studies, use of an ACI for PSD measurements is impractical due to the inlet flow rate required for operation (28.3 L/min). It is necessary to match impactor inlet flow rates to the aerosol available at the animal model-breathing zone. To accommodate the different inlet flows an iso-kinetic diluter was constructed for use with the ACI. The diluter allows the ACI to operate at its designed flow yet draw a low flow sample from the breathing zone in the exposure apparatus. The dilutor sample inlet tube cross-sectional face velocity was maintained equal to the ACI inlet cross-sectional face velocity, accommodating mixing sample aerosol with the dilution air in an iso-kinetic stream. Dilution air was added from a filtered compressed air source. Flow rates of the ACI and the dilution air were controlled and measured with calibrated mass flow meters. Two sample flow rates, representing small (0.5 L/min) and large (3.0 L/min) animal species were selected for validation. Jet nebulizers and solutions containing Certified Particle Size Standards (micro-spheres) were used to produce nearly mono-disperse aerosols in a holding plenum. The aerosol was dried in process before collection with the ACI. For each flow rate, three impactor collection samples were obtained at each of three particle sizes. Impactor stage masses were measured gravimetrically and the results analyzed for MMAD and GSD for each sample. Review and comparison of the results indicate good correlation between particle size samples collected at lower flow rates with the diluter and the nominal particle size used in aerosol generation. This diluter allows the use of the ACI to collect particle size samples from pre-clinical animal toxicology models at sample collection flow rates similar to production rates without significant alteration of PSD

2130 ACUTE RESPIRATORY RESPONSES OF THE MOUSE TO CHLORINE.

W. S. Wilkie[1], D. J. Shusterman[2] and J. B. Morris[1]. *[1]Toxicology Program, University of Connecticut, Storrs, CT and [2]Occupational and Environmental Medicine, University of California at San Francisco, San Francisco, CA.*

In human subjects, fifteen minute exposure to 0.5 - 1.0 ppm chlorine gas causes a nasal obstructive response in the absence of a marked sensation of irritation. For comparative purposes, the current investigation was designed to assess the respiratory responses of the mouse to this irritant gas. Towards this end, respiratory physiological responses were measured in female C57Bl/6J mice exposed to 0.6 to 3.0 ppm chlorine. Chlorine was a potent sensory irritant, with an RD50 of 2.3 ppm. Chlorine exposure produced airway obstruction as indicated by a concentration dependent increase in specific airways resistance (sRaw) during exposure, which persisted for fifteen minutes post exposure. At 0.6 ppm, chlorine produced mild sensory irritation (<20% change in breathing frequency) and significant obstruction (65% increase in sRaw). Pretreatment with atropine was without effect on the obstructive response suggesting the lack of involvement of cholinergic pathways. Pretreatment with the sensory nerve toxin, capsaicin, dramatically reduced both the sensory irritation and obstructive responses, strongly suggesting the involvement of sensory nerves. Studies were also performed using the surgically isolated upper respiratory tract of the anesthetized mouse. Chlorine was efficiently scrubbed from the airstream (>95%) in that site and produced an obstructive response that was of sufficient magnitude to account for the entire response observed in the intact animal. In summary, chlorine gas produces an immediate nasal obstructive response in the mouse that appears to be similar to that in the human.

2131 COMPARISON OF DOSE AND TOXICITY AFTER ADMINSTRATION OF A FLUOROALKYLETHYL PHOSPHATE SURFACTANT BY DERMAL AND INHALATION ROUTES IN RATS.

C. Finlay, D. P. Kelly, N. E. Everds, J. F. Hansen and J. C. Stadler. *DuPont Haskell Laboratory, Newark, DE.*

Fluorosurfactants are used as specialty wetting and leveling additives in cleaning and coatings formulations. Previously, this fluoroalkylethyl phosphate surfactant was evaluated for subchronic, developmental, and reproductive toxicity in gavage studies; the liver was the most sensitive target organ. Since the intended uses of this surfactant would predict that dermal or respiratory tract exposures are likely, studies were conducted to assess internal exposure and toxicity by these routes. In the 28-day study, the test substance was applied to the skin of male rats each day for 6 hours at doses of 0, 10, 100, and 1000 mg/kg/day (35% ai). There were no adverse effects on mortality, clinical signs, body weights, food parameters, anatomic pathology, or hematology. Aspartate- and alanine-aminotransferases and sorbitol dehydrogenase activities were increased at 1000 mg/kg/day. The NOEL for dermal exposure was 100 mg/kg/day. In the inhalation study, male rats were exposed nose-only to 0, 0.2, 2, or 20 mg/m3 of aerosol for 6 hrs/day, 5 days/wk for 2 wks. There were no effects on body weights, clinical signs, or clinical pathology parameters. The only histological effects were mild inflammation in the larynx and lung at 2 and 20 mg/m3. The NOEL for inhalation was 0.2 mg/m3. Internal exposures by the dermal, inhalation, and oral routes were compared by fluorine blood analysis (Wickbold Torch Combustion Method). Minimal levels of fluorine were detected at the two lowest doses administered dermally. Fluorine levels were apparent in all rats exposed by inhalation, however, the levels were equal to or less than levels in rats exposed orally. Based on the fluorine dosage results in the above studies and the findings in the respiratory tract after inhalation exposure, inhalation appears to be a more sensitive route of exposure than the dermal or oral routes for this fluorosurfactant.

2132 DEVELOPMENT OF A METHOD FOR THE SIMULTANEOUS ANALYSIS OF VINYL ACETATE AND ACETALDEHYDE CONCENTRATIONS IN THE NASOPHARYNGEAL CAVITY AND EXHALED BREATH OF HUMAN VOLUNTEERS.

R. E. Schwartz[1], J. J. Soelberg[2], R. A. Corley[2], K. K. Weitz[2], L. Bloemen[3], M. S. Bogdanffy[4] and K. D. Thrall[2]. *[1]Otolaryngology, Richland, WA, [2]Battelle, Pacific Northwest Laboratories, Richland, WA, [3]Dow Benelux, Terneuzen, Netherlands and [4]DuPont, Newark, DE.*

Vinyl acetate is a volatile monomer used in the plastics, coatings, and polymer industries. Inhaled vinyl acetate is absorbed by the nasal mucosa and rapidly hydrolyzed to acetaldehyde and acetic acid. A PBPK model has been developed to describe the uptake of vinyl acetate in the rat and human nasal cavity based on

parameters obtained from studies in rats. Thus, human nasal dosimetry data would be useful to verify the PBPK model predictions for humans. This study focused on the development of a real-time method to simultaneously measure vinyl acetate and acetaldehyde concentrations in the nasopharyngeal region of human volunteers on a breath-by-breath basis. A small-diameter air-sampling probe was inserted roughly 9 cm into one nostril such that the tip of the probe was in the nasopharyngeal cavity. The probe was threaded through a port on a facemask containing two one-way non-rebreathing valves and connected to a Teflon transfer line that fed directly into an ion-trap mass spectrometer to provide data sampling every 0.8 sec. A second transfer line attached to the exhale port on the facemask to permit the concentration of exhaled vinyl acetate to be measured using a second mass spectrometer. The 6 volunteers inhaled 13-C-labeled vinyl acetate at 1, 5, or 10 ppm for approximately 30 min under resting and exercise conditions while 13-C-vinyl acetate and 13-C-acetaldehyde were continually monitored in both the exhaled breath and in the nasopharyngeal cavity. A plethysmograph was used to match the airway concentrations to inhalation/exhalation oscillations. Preliminary results show little intra- or inter-variability in volunteers, thus indicating the feasibility of the method to differentiate the clearance process of vinyl acetate within a select region of the respiratory tract. (Supported by the Acetyls Sector Group, CEFIC).

2133 THE USE OF FLUORESCENTLY LABELED NANOPARTICLES TO DETERMINE THE EFFECT OF PARTICLE SIZE ON TRANSLOCATION FROM THE LUNG.

J. M. Carter[1], J. M. Kennedy[1], G. Oberdorster[3] and E. D. Clark[2]. *[1]Central Product Safety, Procter & Gamble, Cincinnati, OH, [2]The Health and Environmental Safety Alliance, Cincinnati, OH and [3]University of Rochester, Rochester, NY.*

Nanoparticles or ultrafine particles, are particles generally considered to be less than 100nm in size. These particles have been implicated in morbidity and other adverse reactions associated with PM2.5. Due to their small size, these particles may translocate from the lung to remote sites such as the heart, liver, brain or other extrapulmonary organs after inhalation exposure. Additionally, it has been suggested that the translocation of these particles may be responsible for adverse effects from ultrafine particles. We have initiated studies to determine whether nanoparticles translocate to extrapulmonary organs after inhalation of particles in rats. Briefly, Fisher 344 female rats were exposed *via* an aspiration model to fluorescently labeled polystyrene ranging in size from 20 nm to 400 nm. Animals were sacrificed 1, 3, 7 and 14 days after exposure to nanoparticles. Lung, heart, brain, spleen, kidney and liver tissues were removed and preserved for histological examination. Blood was also collected for examination of nanoparticles. Tissue was examined *via* confocal microscopy for presence of fluorescent nanoparticles. *In vitro* experiments using EpiAirway tissue constructs were also performed to evaluate membrane/cell/particle interactions. Histological evaluations were performed on these tissues 2, 8, and 24 hours post exposure. *In vivo* experiments indicate a correlation between size and the ability of these particles to migrate from the lung after inhalation exposure. Additionally, *in vitro* data yielded similar findings indicating particle size is a factor in predicting translocation potential of nanosized particles.

2134 THE EFFECT OF DOSAGE ERRORS AND STEP SELECTION METHOD ON THE PERFORMANCE OF THE UP-AND DOWN METHOD.

D. R. Sommerville. *US Army Edgewood CB Center, Aberdeen Proving Ground, MD.* Sponsor: S. Thomson.

The up-and-down method is a common approach for estimating the median effective dose/dosage (ED50) of a quantal response curve. It has been demonstrated in the literature that the method provides an accurate ED50 estimate while using a minimum number of individual trials. A major aspect of the method is the use of equally spaced log(dose) levels, typically chosen to be roughly equal to the standard de*via*tion (σr) of the response distribution of the test items. With some applications (e.g. inhalation toxicology), it is not possible to maintain equally spaced dosage levels. Irregular spacing will occur due to random errors from dosage administration (σA) and measurement (σM). The former is from the difficulty in precisely generating the target dosage, while the latter is the error in measuring what dosage was actually produced. A series of Monte-Carlo simulations of 6 to 10 items per trial series were performed to investigate the effect of σA and σM on the performance of the up-and-down method for small samples. Two separate sets of simulations were conducted: one where the dosage for the next step was based on the measured dosage from the previous trial (Method M) and the other from the previous target dosage (Method T). Two other factors were also investigated: the nominal step size and the location of the initial trial dosage relative to the true ED50. The method's efficacy was judged by how many individual trials were needed in a test series before a reliable/useable dataset was obtained. An example of a non-useable dataset is a series composed of either all responses or all non-responses. It was found that the method of step selection has a major effect on the efficacy and accuracy of the up-and-down method. Method T is superior to Method M with respect to both efficacy and accuracy. The negative influence of σA and σM on efficacy is greater for Method M than for Method T. In addition, σM has 2.3 times the effect (compared to σA) on increasing the mean square error (MSE) of the difference between the estimated and actual ED50 for both methods (M and T) of step selection.

2135 CHEMESTHESIS IN 15-MINUTE EXPOSURES TO OCCUPATIONALLY-PERTINENT CONCENTRATIONS OF GLUTARALDEHYDE VAPOR.

W. S. Cain, R. Schmidt and A. A. Jalowayski. *Otolaryngology, UCSD, La Jolla, CA.* Sponsor: J. Cometto-Muñiz.

In previous research, we found that human females could detect feel (chemesthesis) from brief exposures to concentrations of glutaraldehyde vapor in the range of hundreds of parts per billion (v/v). Threshold for feel in the eye (25-sec exposures) equaled 394 ppb and for feel in the nose (single-sniffs) equaled 472 ppb. Because chemesthesis shows the property of temporal integration for many materials, detection of glutaraldehyde might occur at lower concentrations in longer exposures. This study focused on levels pertinent to occupational exposure limits. Healthy females (n=50) between 18 and 35 years had four 15-min exposures to glutaraldehyde and five exposures to either blank air or VOC control in the course of a 7-hr day, as follows: once each to 35, 50, 75, and 100 ppb glutaraldehyde, three times to blank air, and twice to 10 ppm n-heptane. Exposures took place in a chamber, typically with two subjects present simultaneously. Analytical measurement (OSHA 64 method) confirmed the levels of glutaraldehyde. Subjects rated their confidence in the presence of stimulus-induced chemesthesis minute by minute. The outcome showed greater activity in the nose than in the throat or eyes. The activity increased progressively during exposure, but manifested itself as a reduction in the confidence of no chemesthesis rather than in the confidence of its presence. Even at the highest concentration, subjects failed to reach a 50% threshold for positive detection of chemesthesis by the end of exposure.

2136 ASSESSMENT OF HUMAN SENSORY AND RESPIRATORY RESPONSES TO CONSUMER PRODUCTS.

University. Vedula[5], L. Fell[2], S. Selim[4], R. Rogers[3], P. Dalton[6] and T. G. Osimitz[1]. *[1]Science Strategies, Charlottesville, VA, [2]ToxLink, LLC, Racine, WI, [3]Toxcon, Edmonton, AB, Canada, [4]selim and Associates Toxicology, Camelia, CA, [5]S.C. Johnson, Racine, WI and [6]Monell Institute for the Chemical Senses, Philidelphia, PA.*

The prediction of human responses to consumer products is an important element of product stewardship. Conventional animal toxicology studies often provide little information on the subjective responses (shortness of breath, coughing, nasal congestion) that consumers sometimes report. To address this gap, a series of physiologic tests and survey instruments were used to assess such responses in 371 adults (including mild and mild to moderate persistent asthmatics) exposed to consumer product formulations in simulated residential exposure rooms (16 ft x 16 ft x8 ft). An Institutional Review Board approved the protocol and informed consent package prior to conducting the study. Objective measures were chosen for pulmonary function, nasal congestion, and ocular irritation. Subjective Symptoms were evaluated by a self-administered survey for 20 different responses reflecting upper respiratory, lower respiratory, and non-specific symptoms. Experimental substances included a Test Substance (water-based air sanitizer - 6.1% triethylene glycol), a Reference Substance (water-based air freshener), and a Control Substance (distilled water). The products were sprayed in the exposure chamber according to label directions. The full set of assessments was performed immediately prior to exposure, after 5 min of exposure (mean TWA concentration of TEG = 4.33 ±1.75 mg/m3), and after 30 min of exposure during a period from 60-90 min (mean TWA concentration of TEG = 0.098 ± 0.023 mg/m3). Sporadic changes in subjective and objective measures occurred throughout the study in across all groups. Overall, no clinically significant symptoms, or increase in symptoms from baseline, were observed regardless of exposure to Test, Reference, or Control Substances. In addition, the response of asthmatics was not clinically significantly different than that of non-asthmatics exposed to any of the substances tested.

2137 COMBINED ARTERIAL BLOOD GAS ANALYSIS AND PLETHYSMOGRAPHY TO EVALUATE PULMONARY FUNCTION IN THE RAT.

K. D. Lake-Bruse, K. J. Smith and G. J. Schaefer. *Safety Pharmacology, WIL Research Laboratories, Inc., Ashland, OH.*

The purpose of this study was to evaluate the sensitivity and feasibility of combining plethysmography with arterial blood gas analysis to provide a more complete evaluation of pulmonary function without increasing the number of animals on

study. Restraint acclimated rats were implanted with arterial vascular access ports 7 days prior to pulmonary assessment. Animals (8 males/group) were dosed with saline or pentobarbital (PB; 10, 25, and 45 mg/kg, i.p.). Pulmonary function data consisting of pulmonary frequency (F), tidal volume (TV) and minute volume (MV) were collected at baseline, and at 0-6 hours and 24 hours post dosing. Pulmonary arterial O2 (PaO2) and CO2 (PaCO2) levels were determined in blood samples collected at pretest, and at 0.25, 1, 2 and 24 hours post-dosing. PB elicited a time and dose-dependent anesthetic effect with statistically significant changes in F, TV, MV, PaCO2 and PaO2 compared to saline control. Overall, peak decreases in F, TV, and MV occurred at 0.5 and 1 hr following the 25 and 45 mg/kg doses, respectively. F was decreased 15-37% for 1 hr and 22-44% for 2 hrs following the 25 and 45 mg/kg doses, respectively. TV was decreased 26-31% for up to 30 min and 30 38% for up to 2 hrs following the 25 and 45 mg/kg doses, respectively. MV was decreased 16% at 15 min, 21-56% for up to 1 hr, and 19-62% for up to 3 hrs following the 10, 25 and 45 mg/kg doses, respectively. From the blood gas analysis, the PaCO2 parameter was more statistically sensitive than PaO2. PaCO2 was increased 21% at 1 hour following the 25 mg/kg dose of PB, and increased 19%, 43% and 58% at 0.25, 1, and 2 hrs, respectively, following the 45 mg/kg dose of PB when compared to saline control. The 45 mg/kg dose of PB decreased overall (11 13%) the PaO2 for up to 24 hrs post dosing. In conclusion, combining plethysmography and arterial blood gas analysis within the same animal is a feasible approach that provides a more complete pulmonary function evaluation in rats.

2138 MECHANISMS OF ORGANOPHOSPHATE INSECTICIDE-INDUCED AIRWAY HYPERREACTIVITY.

P. Lein[1] and A. D. Fryer[2]. *[1]CROET, Oregon Health & Science University, Portland, OR and [2]Physiology and Pharmacology, Oregon Health & Science University, Portland, OR.*

It has been suggested that insecticide exposure is a causal factor for the increased incidence of asthma in the United States and other industrialized nations. Mechanism(s) by which insecticides trigger asthma have yet to be established. Airway tone is controlled by the parasympathetic nerves in the lung. Dysfunction of autoinhibitory M2 muscarinic receptors on parasympathetic nerves is associated with airway hyperreactivity, a characteristic of asthma. One widely used class of insecticides, the organophosphates, has been shown to decrease M2 receptor expression and function in the central nervous system. Thus, exposure to the organophosphate insecticides may contribute to airway hyperreactivity *via* inhibition of neuronal M2 receptors in the lung. To test this hypothesis, airway hyperreactivity was measured in guinea pigs 24 hr after subcutaneous injection with varying concentrations of chlorpyrifos, diazinon, parathion and the non-organophosphate insecticide permethrin. Electrical stimulation of the vagus nerves caused frequency-dependent bronchoconstriction that was significantly potentiated in animals treated with the organophosphates, but not with permethrin. Neuronal M2 receptor function was tested using the M2 agonist pilocarpine, which inhibits vagally-induced bronchoconstriction in control animals. In animals treated with organophosphate insecticide, pilocarpine dose-response curves were shifted significantly to the right, demonstrating decreased responsiveness of neuronal M2 receptors. In contrast, M3 muscarinic receptor function on airway smooth muscle was not altered by any of the organophosphates. Chlorpyrifos and diazinon caused airway hyperreactivity at doses that did not significantly inhibit acetylcholinesterase. These data demonstrate that organophosphate insecticides can inhibit neuronal M2 receptor function resulting in increased acetylcholine release from the parasympathetic nerves and airway hyperreactivity.

2139 CONTROLLED VENTILATION INHALATION EXPOSURE AND LUNG SCINTIGRAPHY APPLIED TO A PHARMACOKINETIC AND TISSUE DISTRIBUTION STUDY.

R. Moutvic[1], D. B. Cearlock[2], R. L. Beihn[3], A. Zutshi[4] and M. J. Brooker[1]. *[1]Toxicology, Battelle, Columbus, OH, [2]Zivena, Columbus, OH, [3]Scintiprox, Inc., Indianapolis, IN and [4]Pfizer, Inc., Kalamazoo, MI.*

The study was designed to evaluate the pharmacokinetic profile and tissue distribution in the dog of a 14C labeled doxirubicin (C14D) administered intravenously and *via* inhalation. A combination of controlled-ventilation inhalation exposure and scintigraphic evaluation of lung deposition were utilized for the inhalation leg. The deep lung was the target for deposition of inhaled aerosols. Exposures to C14D *via* inhalation were performed at tidal volumes that were 75% of vital capacity and included a 4 second breath hold. Inhalation exposures were controlled by a computer program that manipulated pressure within a Spangler box (iron lung). The test subjects were anesthetized and entubated. The C14D solution was co-labeled with 99mTc for scintigraphic imaging and C14D aerosols were administered to 12 dogs at a deposited dose of 1.65 mg. Scintigraphic images were collected immediately post exposure and C14D deposition in the lung was determined quantitatively. A second set of 12 dogs was exposed to 1.90 mg of C14D *via* bolus intravenous infusion. Serial blood samples were collected for pharmacokinetic analysis. Animals were serially necropsied and tissues were harvested to evaluate time dependent tissue distribution of C14D through out the body. Results indicated inhalation and intravenous PK profiles with similar kinetic distributions and elimination half lives. Systemic bioavailability *via* inhalation was 65%. Tissue distribution analysis indicated that the inhalation exposure group had substantially lower systemic exposure indicated by lower C14D levels in all tissues except the lung at all time points evaluated.

Author Index

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Key Word Index