National Center for Toxicological Research
Research Accomplishments and Plans

FY 2003 - 2004

Jefferson Laboratories of the FDA

Leaders in Health Science Research for the FDA

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Preface

The National Center for Toxicological Research (NCTR) plays a critical role in the U.S. Food and Drug Administration's (FDA) and Department of Health and Human Services' (DHHS) mission to promote and protect public health. The Center, a component of the Jefferson Laboratories of the FDA, is located in Jefferson, Arkansas, approximately 30 miles south of Little Rock.

The NCTR conducts FDA mission-critical, peer-reviewed critical path (translational) research that is targeted to develop a scientifically sound basis for regulatory decisions and reduce risks associated with FDA-regulated products. This research is aimed at evaluating the biological effects of potentially toxic chemicals or microorganisms, defining the complex mechanisms that govern their toxicity, understanding critical biological events in the expression of toxicity, and developing methods to improve assessment of human exposure, susceptibility and risk. Customized bioassessment of chemicals of vital interest to the FDA involves the coordination of expertise in the areas of biochemical and molecular markers of carcinogenicity, quantitative risk assessment, transgenics (mimicking responses in animal models by insertion of toxicologically relevant genes into a test animal or tissue culture), neurotoxicology, microbiology, chemistry, and genetic or reproductive/developmental toxicology.

Using its existing strengths in methods development, statistics, analytical chemistry and spectroscopy, NCTR is developing and standardizing new technologies, such as genomics, proteomics, metabonomics and nanotechnology to apply toward traditional toxicological endpoints. In addition NCTR is using toxicoinformatics (data collection, interpretation, and storage of information about gene and protein expression) to manage and integrate data from these new technologies with traditional toxicological data to provide a basis for predictive toxicology. Application of these new tools in animal surrogates will provide us with mechanistic biomarkers that will have more relevance for extrapolation of risk to humans; provide a better understanding of the present models used to assess risk in humans; and direct the development of more useful surrogate models that will increase our understanding of toxic responses in humans.

A significant contribution to our research accomplishments is the benefit gained by sharing knowledge through collaborations with scientific staff in all disciplines in other FDA Centers as well as in other government agencies, academia, and industry. As a critical resource for enhancing the science base of the FDA, the center director and scientists foster scientific forums with NCTR stakeholders, namely the product centers and the Office of Regulatory Affairs (ORA) as well as the scientific community. NCTR also receives guidance and advice on the relevance and quality of its research programs from an extramural Science Advisory Board.

A major emphasis for NCTR is to conduct translational and applied research on compounds nominated by the FDA for evaluation by the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP). In addition to these collaborations with the other FDA centers and NTP, NCTR partners with ten other government agencies, over twenty-five universities and medical centers (including local, national and foreign), other research facilities around the world, and several industries to investigate predictive toxicology issues.

The NCTR views its public health role as a key element in the development and modification of toxicology safety standards through the application of innovative scientific research. New health concerns, such as bovine spongiform encephalopathy (BSE), AIDS, pediatric initiatives, obesity, skin cancer, antibiotic resistance, counter terrorism and emerging foodborne pathogens, in addition to traditional concerns, are challenging the conventional ways in which the regulatory agencies (both national and international) set safety standards designed to protect public health. An example of the NCTR contribution is our participation in national and international consortia that are developing standards for using emerging genomic technologies and standards for interpreting the data derived from these technologies. In addition, NCTR is the editor and publisher of an on-line scientific journal entitled *Regulatory Research Perspectives* (http://www.fda.gov/nctr/) which highlights some of the latest research topics in the regulatory arena.

I am proud to present this report that summarizes NCTR research accomplishments for fiscal year 2003 and plans for fiscal year 2004.

Daniel A. Casciano, Ph.D.
Director, NCTR

NCTR Washington Operations

Science Advisory Board

Function

The NCTR Science Advisory Board (SAB) advises the Director in establishing, implementing and evaluating the research programs that assist the Commissioner of the Food and Drug Administration (FDA) in fulfilling regulatory responsibilities. This external body of recognized scientific experts is a key component of the review and planning process, and helps to ensure that the research programs at NCTR are scientifically sound and pertinent to the FDA.

FY 2003 Accomplishments

A full meeting of the SAB was held June 19-20, 2003. The Board was given an overview of the research being conducted at the NCTR, including (1) Basic Research, i.e. hypothesis-driven investigations aimed at scientific discovery and knowledge generation; (2) Translational Research, i.e. hypothesis-driven investigations aimed at interpretation and/or revision of basic scientific concepts to address emerging public health needs; and (3) Applied Research, i.e. investigations aimed at developing and applying standards to public health needs nationally and globally via harmonization.

The Board was informed that over the last year and a half, the NCTR had developed five new Centers of Excellence, including (1) the Functional Genomics Center, (2) the Structural Genomics Center, (3) the Toxicoinformatics Center, (4) the Phototoxicology Center, and (5) the Hepatotoxicity Center. A synopsis of the scientific activities ongoing in each of the Centers and NCTR Divisions was also provided. The Divisions include: the Division of Biometry and Risk Assessment, the Division of Genetic and Reproductive Toxicology, the Division of Biochemical Toxicology, the Division of Molecular Epidemiology, the Division of Neurotoxicology, the Division of Chemistry, and the Division of Microbiology.

The Board received a report from the Site Visit Team (SVT) that reviewed the Division of Biometry and Risk Assessment on May 5-6, 2003, which they voted to accept. This was followed by an initial response to that report from the Division Director. In addition, the Board heard a follow-up response to the SVT report on the Division of Chemistry, whose site visit took place on February 12-13, 2002.

The Board was provided with an update on the activities of the Pharmaceutical Safety Working Group (PSWG), including a history, an industry perspective, and the interface between the PSWG and the CDER Pharmacology and Toxicology Committee. In addition, an update on progress made by two specific biomarker working groups, i.e. the Cardiotoxicity Working Group and the Vascular Injury Working Group was provided.

The site visits reports and the minutes of the SAB meetings can be accessed at http://www.fda.gov/nctr/science/committees/committees.htm.

Science Advisory Board Membership Roster

NAME/TITLE	AFFILIATION	TERM ENDS	EXPERTISE
Dr. Daniel Acosta, Jr.* Dean, College of Pharmacy	University of Cincinnati	6/30/07	Pharmacology and Toxicology
Dr. Nancy Ann Gillette Sr. Vice President Sierra Biomedical	Charles River Laboratories	6/30/07	Veterinary Medicine and Pathology
Dr. Jerry Kaplan Associate Dean for Research	University of Utah School of Medicine	6/30/04	Molecular Biology
Dr. John Groopman	Bloomberg School of Public Health Department of Environmental Health Sciences	6/30/06	Toxicology
Dr. Pat R. Levitt	Vanderbilt University, John F. Kennedy Center for Research and Human Development	6/30/06	Neurobiology
Dr. E. Albert Reece Vice Chancellor and Dean	University of Arkansas College of Medicine	6/30/06	Physician
Dr. Alberto Luis Rivera-Rentas	School of Environmental Affairs, Ana G. Mendez University System	6/30/06	Neurobiology Electrophysiology
Dr. Paul J. Catalano Associate Professor of Biostatistics	Harvard School of Public Health	6/30/06	Biostatistics
Dr. Kenneth R. Tindall Senior Vice President	North Carolina Biotechnology Center	6/30/04	Biomedical Science, Genetics

*Chair

FDA Coordination Activities—Safety Testing

Function

The NCTR Office of Washington Operations serves as the Agency coordinator for activities of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and represents the Agency on Economic Cooperation and Development (OECD) matters related to the Test Guidelines Program of OECD.

The ICCVAM coordinates and advises on interagency issues on development, validation, and regulatory acceptance of new, improved and alternative test methods, and the national and international harmonization of such methods. Congress recently enacted the ICCVAM Authorization Act (Public Law 106-545, December 19, 2000) "to establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness." As a result, ICCVAM, which was initially assembled as an *ad hoc* committee and had evolved to a standing committee, became a permanent committee.

ICCVAM's charge includes:

- Promote the scientific validation and regulatory acceptance of new, improved, and alternative test methods.
- Coordinate the review/evaluation of new/revised alternative test methods of interagency interest.
- Facilitate and provide guidance on test method development, the validation process, validation criteria, regulatory acceptance criteria, and submission requirements.
- Provide recommendations to Federal agencies on the validation status of test methods and their regulatory suitability.
- Facilitate interagency regulatory acceptance and promote international harmonization and adoption of scientifically validated test methods.
- Facilitate awareness of and training for accepted test methods (end-users, regulators).

The Scientific Advisory Committee for the Validation of Alternative Methods (SACATM), established and chartered December 18, 2001, provides scientific and administrative advice to ICCVAM and its operational center, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The SACATM held its first meeting in Crystal City, VA on December 5, 2002, and their second meeting in Research Triangle Park, NC on August 12-13, 2003.

Information about ICCVAM and NICEATM can be found at http://iccvam.niehs.nih.gov. The SACATM charter, related *Federal Register* notices, and future meeting announcements can be found on the ICCVAM/NICEATM website at: http://iccvam.niehs.nih.gov/about/sacatm.htm.

FY 2003 Accomplishments

- Dr. Leonard Schechtman, Chair of ICCVAM, and Dr. William Stokes, Director of NICEATM, were formally named as the U.S. liaisons to the European Commission's European Center for the Validation of Alternative Methods (ECVAM) Scientific Advisory Committee (ESAC).

- ICCVAM has adopted a new process by which "test method nominations" and "test method submissions" to ICCVAM are considered and prioritized for review and evaluation. This process allows for any new, revised, or alternative test method to be nominated for evaluation by any organization or individual.

- ICCVAM revised the "ICCVAM Guidelines for Nomination and Submission of New, Revised, and Alternative Test Methods" (NIH Publication No. 03-4508), September 2003. This document provides improved guidance on the information needed by ICCVAM to evaluate the validation status of new or revised test methods at any stage of development. It also includes a framework for organizing the information supporting the validity of a test method.

- ICCVAM has developed a process for establishing performance standards for validated and accepted test methods. The purpose of performance standards is to communicate the basis by which new test methods have been determined to have sufficient accuracy and reliability for specific testing purposes. Performance standards can be used to evaluate the reliability and accuracy of other test methods that are based on similar scientific principles and measure or predict the same biological or toxic effect.

- A new internationally harmonized test guideline (TG 429) on skin sensitization using the mouse Local Lymph Node Assay (LLNA) was officially adopted at the OECD National Coordinators meeting in April 2002. The LLNA reduces and refines animal use compared to the traditional guinea pig test methods for which it substitutes. An advantage over the traditional test is that it also provides dose-response information. The validity of the new guideline was supported by the report of the independent scientific peer review evaluation of the LLNA coordinated by ICCVAM/NICEATM.

- In collaboration with the European Commission's European Center for the Validation of Alternative Methods (ECVAM), ICCVAM/NICEATM designed and initiated a multi-laboratory international study to evaluate the usefulness of cytotoxicity data from the BALB/c 3T3 Neutral Red Uptake (NRU) and the Normal Human Keratinocyte (NHK) NRU assays for estimating the acute oral toxicity potential of chemicals. Completion of the validation study is scheduled in 2004.

- ICCVAM and NICEATM are collaborating with ECVAM to conduct a validation study to evaluate three *in vitro* test methods for assessing dermal irritation. NICEATM has requested data from stakeholders for chemicals that can be considered as potential reference chemicals for the validation study.

- ICCVAM representatives participated in and/or co-sponsored, co-organized, co-chaired three workshops convened at ECVAM (Ispra, Italy) in 2002 and 2003. These included:

  — An ECVAM Status Seminar (June 4-6, 2002), which reviewed the progress made during the first ten years of the ECVAM, and provided perspectives on future collaborative opportunities between ICCVAM and ECVAM. The proceedings were published in ATLA, Vol 30, Suppl 2, 2002.

  — An ECVAM Workshop on Strategies to Replace *In Vivo* Acute Systemic Toxicity Testing (September 15-18, 2003), the purpose of which was to establish the state-of-the-art in the field and to develop a strategy toward the replacement of *in vivo* testing for acute systemic toxicity.

  — A Workshop on Validation Principles and Approaches for Toxicogenomics-Based Test Systems (December 11-12, 2003), the purpose of which was to consider the validation and regulatory acceptance aspects of this technology as potential alternative predictive testing and screening methods that could reduce, refine, and replace animals. This workshop addressed the specific considerations necessary for adequate validation of toxicogenomics-based test methods, recognizing that new approaches will be necessary to standardize and evaluate the scientific validity of test methods based on toxicogenomics and that the entire validation process will be different and more complex than that for classical alternatives since both the predictive test system and the applied new technology will need to be validated. Inasmuch as data are already being generated using this technology, it was considered both timely and important to address this issue now with the aim of establishing the foundation that will facilitate future regulatory acceptance of scientifically valid toxicogenomics-based test methods. Some areas considered included differences in platforms, impact of changes of arrays and sets of genes, quality assurance and GLP compliance, degree of acceptable variability, assessment of intra- and inter- laboratory reproducibility, data evaluation and analysis procedures, reference materials and databases. Additionally, the possibility of transferring validation processes and principles for toxicogenomics to other new technologies (e.g. proteomics, metabonomics) was also discussed.

- In March 2003, the ICCVAM and ECVAM made joint presentations to an OECD GLP Working Group on the need for further international guidance on the application of Good Laboratory Practices (GLPs) to *in vitro* toxicological testing. With the increasing use of non-animal testing procedures, such guidance will facilitate the acceptable use of new test methods and generation of data in accordance with the requirements of GLPs. The full OECD GLP Working Group endorsed and initiated planning to develop this additional guidance in September 2003.

- ICCVAM representatives served on the Organizing Committee for the "International Conference on Validation and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment" held in Stockholm, Sweden, March 6-8, 2002. Several ICCVAM representatives also served as invited

discussion leaders and rapporteurs. ICCVAM publications were used as key discussion documents. As a result of this conference, participants drafted an OECD Guidance Document (No. 34) entitled, "The Development, Validation and Regulatory Acceptance of New and Updated Test Methods in Hazard Assessment." This document is currently being circulated to OECD member countries for comments which are to be submitted January/February 2004.

- Test method evaluations, recommendations, and related efforts included those for the following:

  — ICCVAM recommended the revised Up-and-Down Procedure (UDP) as a replacement for the conventional LD_{50} test for regulatory hazard classification and labeling purposes.

  — ICCVAM, in partnership with the EPA and the International Life Sciences Institute (ILSI), held a training workshop on Acute Toxicity Testing Methods on February 19-21, 2002, at the NIH Natcher Conference Center in Bethesda, Maryland. The Workshop provided practical information and case studies to facilitate understanding and the implementation of the UDP and other *in vivo* and *in vitro* alternative methods for acute toxicity.

  — All U.S. Federal regulatory agencies that require acute oral toxicity testing have now accepted and adopted the revised UDP, which is expected to reduce the use of animals for this purpose by 60-70%.

  — ICCVAM recommended that *in vitro* toxicity test methods should be considered as one way to estimate the starting dose for acute oral toxicity studies, thereby reducing the number of animals required by up to 40%.

  — ICCVAM completed an expedited review and published a comprehensive technical report for three alternative *in vitro* test methods – EpiDerm ™ (EPI-200), EPISKIN ™, and the Rat Skin Transcutaneous Electrical Resistance (TER) assay –for assessing skin corrosivity. ICCVAM recommended that these test methods be used as screening assays for corrosive chemicals.

  — Skin Corrosivity and Irritation Test Methods:
    - ICCVAM completed an expedited review and published a comprehensive technical report for three alternative *in vitro* test methods – EpiDerm ™ (EPI-200), EPISKIN ™, and the Rat Skin Transcutaneous Electrical Resistance (TER) assay –for assessing skin corrosivity. ICCVAM recommended that these test methods be used as screening assays for corrosive chemicals. Chemicals that are predicted to cause skin corrosion by these test methods do not have to be tested in animals.
    - Corrositex®, an *in vitro* test method for assessing the dermal corrosivity potential of chemicals, was recommended by ICCVAM and accepted by U.S. Federal regulatory agencies in 2000. A generic, international test

guideline for similar *in vitro* membrane barrier test systems for skin corrosion was developed by ICCVAM and its Dermal Corrosivity and Irritation Working Group (DCIWG) and proposed to the Organisation for Economic Cooperation and Development (OECD) Test Guidelines Program in March 2003.

- In response to a request from the EPA, ICCVAM and NICEATM developed proposed performance standards for three types of *in vitro* dermal corrosivity test methods (human skin model systems, membrane barrier test systems, skin TER assays) previously reviewed by ICCVAM. Final ICCVAM performance standards will be published as addendums to previously published ICVAM reports on these test methods and will be forwarded to Federal agencies for their consideration in early 2004.

— *In Vitro* Endocrine Disruptor Screening Methods:

- NICEATM/ICCVAM completed a comprehensive evaluation of four *in vitro* test methods under consideration by the EPA for identifying potential endocrine disrupting chemicals. Comprehensive review documents include over 4,000 test results for more than 1,000 chemicals evaluated in estrogen and androgen receptor binding and transcriptional activation assays.
- NICEATM and ICCVAM convened an international Expert Panel Meeting to evaluate the validation status of the four types of *in vitro* binding and transcriptional assays. Based on the Panel's report, ICCVAM recommended reference chemicals and minimum procedural standards to facilitate standardization and scientific validation of these assays. Final documents were published in 2003.

Center for Hepatotoxicology

The mission of the Center for Hepatotoxicology is to provide expertise in liver toxicology to the FDA. The focus of this group is two-fold and reflects the expertise of its members in the mechanistic analysis of toxic responses of the liver and in liver carcinogenesis.

The vision for the Center is to develop and apply an integrated biochemical, transcriptomic, proteomic and metabonomic approach to questions related to liver toxicology. Both cross-species and cross-cell type within the liver analyses will be performed. Biomarker profiles of liver toxicity will be generated for more effective assessment for risk of acute toxicity and of liver cancer development for application to individualization of therapy based on efficacy and safety profiles.

FY 2002 Accomplishments

In FY 2003, the Center for Hepatotoxicology was newly developed. A study on the effect of chemical carcinogens on gene expression in human hepatocytes was completed.

FY 2003 Plans

Staff will develop biomarkers for liver injury and disease in mouse, rat, and human samples. Biomarker development will be accomplished through an integrated "omic" analysis of biofluids and tissue from liver injury and liver disease to establish microarray, NMR- and MS-based metabonomic, and Seldi and 2D-based proteomic signatures. The integration of signaling mechanisms for cell proliferation, apoptosis, and differentiation will provide the framework in which to assess these biomarkers. Our primary focus will be on acetaminophen, PPARa and PPARg agonists.

Research Projects

Title	Project Number	Strategic Research Goal
PI: Dragan, Yvonne		
◆ **Training in Hepatocyte Perfusion and Hepatic Cell Isolation**	**P00610**	**Predictive Toxicology**

Objective(s):

Train member(s) of the Hepatotoxicology Center in primary liver cell isolation and culture. The long-term goals will be to obtain signature gene and protein expression patterns of each cell type for comparison to toxin-induced changes. Training must be provided to give confidence in the integrity of liver cells following perfusion, separation, and culture of the liver cells.

PI: Harris, Angela		
◆ **Modulation of Gene Expression in Chemical Carcinogenesis: Analysis of Aflatoxin B_1-Induced Gene Expression in Human Hepatocytes**	**E0704701**	**Concept-Driven**

Objective(s):

1) Verify aflatoxin B1 effects on steady-state mRNA levels of eight genes previously identified by differential hybridization of a gene filter array to be aflatoxin B1 (AFB_1)-responsive in human hepatocytes. Use Northern blot, RT-PCR and/or RNA protection assay to establish AFB_1 time and dose-response curves for maximal gene expression and also determine the minimum dose at which gene expression can be detected;
2) Identify additional AFB_1-induced genes using differential display PCR (DD-PCR) and differential hybridization of a high-density filter array utilizing mRNA from human hepatocytes treated with low, moderate, and cytotoxic levels of AFB_1.
3) Evaluate selected genes as described for objective #1;
4) Distinguish genes involved in toxicological response to AFB_1 exposure from those that contribute to the carcinogenic response by comparing the gene expression profile of human hepatocytes treated with the hepatotoxic non-carcinogenic chemical, acetaminophen; and
5) Compare gene expression of selected genes in human hepatocytes treated with known rat liver chemical carcinogens, including 2-acetylaminofluorene, dimethylnitrosamine, and methapyrilene.

◆ **Development and Characterization of Conditionally Immortalized Human Primary Hepatocyte Cell Lines from Female and Male Donors**	**E0714101**	**Concept-Driven**

Objective(s):

Develop *in vitro* model systems for the study of mechanisms of toxicity in humans from different genders and/or ethnic populations.

Publications

Harris, A.J., Comparison of Basal Gene Expression in Cultured Primary Rat Hepatocytes and Freshly Isolated Rat Hepatocytes, in press*, Toxicology Mechanisms and Methods,* 2003.

Harris, A.J., Shaddock, J., Delongchamp, R., Dragan, Y., Casciano, D., Effect of Substratum on Hepatic Gene Expression, in press*, Methods in Toxicology,* 2003.

Harris, A.J., Dial, S., Casciano, D., Comparison of Basal Gene Expression Profiles and Effects of Hepatocarcinogens on Gene Expression in Cultured Primary Human Hepatocytes and HepG2 Cells, in press, *Mutation Research*, 2003.

Yim, S., Ward, J., Dragan, Y., Yamada, A., Scacheri, P., Kimura, S., Gonzales, F., Microarray Analysis using Laser Capture Microdissection of Microscopic Hepatocellular Precancerous Lesions and Frozen Hepatocellular Carcinomas Reveals Unique and Consistent Gene Expression Profiles, in press, *Toxicologic Pathology,* 31: 295-303, 2003.

Biochemical Toxicology

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Marta Pogribna setting up cell culture for DNA methylation studies.

Executive Summary

Introduction

The Division of Biochemical Toxicology conducts fundamental and applied research specifically designed to define the biological mechanisms of action underlying the toxicity of products either regulated by or of interest to the Food and Drug Administration (FDA). This research centers on assessing the toxicities and carcinogenic risk associated with specific chemicals and gene-nutrient interactions, and the introduction of new techniques to assess toxicities and carcinogenic risk. The risk assessment research is firmly rooted in mechanistic studies focused on the understanding of toxicological endpoints, an approach that allows greater confidence in the subsequent carcinogenic risk assessments. Research within the Division capitalizes on scientific knowledge in the areas of biochemistry, organic chemistry, cellular and molecular biology, immunology, nutritional biochemistry, and pharmacology. It is supported by sound technical skills, the availability of state-of-the-art equipment, and internal and external collaborations and funding.

FY 2003 Accomplishments

A major emphasis within the Division is to conduct research on compounds nominated by the FDA for evaluation by the National Institute of Environmental Health Sciences, National Toxicology Program (NIEHS/NTP). This focus reflects the fact that the NCTR has superb animal facilities supported by a multi-disciplinary staff of scientists with strong mechanistic research experience; as such, the Center has the capability to conduct subchronic and chronic toxicological assessments in a rigorous manner to address the FDA's needs. While acknowledging the limitations of animal bioassays, these studies currently serve as the benchmark by which toxicological assessments are made by federal agencies, including the FDA. In addition to providing basic information on toxicological endpoints, such as cancer, these experiments form the basis for mechanistic studies to ascertain if the response detected in the experimental model is pertinent to humans.

During FY 2003, in response to an NTP nomination by the Center for Veterinary Medicine (CVM), division investigators completed an NTP study to determine carcinogenicity of malachite green, a therapeutic agent used in aquaculture. These studies indicated that

Project Number Codes:
E–Ongoing P–Preliminary S–Support

leucomalachite green, a metabolite of malachite green, is a mouse liver carcinogen. Division investigators had previously elucidated the mechanism by which riddelliine, a pyrrolizidine alkaloid found in herbal teas, is activated to a genotoxic carcinogen. During the current year, they expanded their studies to include monocrotaline, retrorsine, heliotrine, lasiocarpine, and clivorine, and further demonstrated that the dietary supplements comfrey and coltsfoot contain pyrrolizidine alkaloids that can be activated to genotoxins. At the request of the Center for Food Safety and Applied Nutrition (CFSAN), experiments were initiated on acrylamide, a carcinogen found in fried foods. These investigations have emphasized dose-response relationships and the development of biomarkers for assessing exposure.

An area of particular concern to the FDA, in particular CFSAN, is the potential toxicity of cosmetic ingredients due to their interaction with light. To address this concern, the NCTR, in collaboration with the NIEHS/NTP, constructed a phototoxicity facility that is located within the Division. Studies at the NCTR Center for Phototoxicity have focused on the co-carcinogenic effects of simulated solar light and topically-applied α- and β-hydroxy acids, *Aloe vera*, and retinyl palmitate. The aims of other experiments have been to quantify the chemicals used in tattoo inks, investigate the photostability and safety of pigments used for tattooing, including permanent make-up, and develop a transgenic mouse model to investigate the mechanisms for the induction of cutaneous malignant melanoma and cutaneous ocular melanoma.

As part of the NTP effort, the division investigators studied a series of endocrine-active compounds, including genistein, ethinyl estradiol, nonylphenol, and vinclozolin. These studies have involved both short term and long term assessments that are unique in terms of the number of endpoints evaluated and the inclusion of chronic toxicity assessments using varied exposure windows.

Anti-retroviral drugs are being used to prevent the mother-to-child transmission of human immunodeficiency virus type 1, the virus responsible for acquired immunodeficiency syndrome. While effective in preventing viral transmission, the long-term consequences of perinatal exposure to these drugs are presently unknown. Division investigators have conducted a series of investigations to examine the genotoxic consequences of the anti-retroviral reverse transcriptase inhibitors zidovudine, lamivudine, stavudine, didanosine, and zalcitabine in neonatal mice. During FY2003, these studies were expanded to assess the effects of perinatal exposure of zidovudine and lamivudine in combination with the non-nucleoside reverse transcriptase inhibitor nevirapine and the protease inhibitor nelfinavir.

Tamoxifen is an adjunct chemotherapeutic agent for the treatment of breast cancer and a chemoprotective agent for breast cancer prevention. Despite being beneficial in regard to breast cancer, tamoxifen is known to increase the risk of endometrial cancer in women. Division investigators have conducted experiments to elucidate the mechanisms for tumor induction, with emphasis on characterizing the DNA adducts formed from this drug. As part of this effort, mass spectral methods were developed with sufficient sensitivity to detect and quantify tamoxifen DNA adducts and these methods were applied to endometrial and breast samples obtained from women receiving the drug.

A strong emphasis within the Division has been in the area of nutritional folic acid deficiency and tumor progression. As part of this program, Division investigators evaluated the progressive changes in global DNA hypomethylation and promoter region hypermethylation in target and non-target tissues for carcinogenesis.

FY 2004 Plans

In FY 2004 the Division's NTP efforts will include completion of final reports on the carcinogenicity of α- and β-hydroxy acids. Final reports for the multigeneration studies on genistein, nonylphenol, and ethinyl estradiol will also be prepared. Photocarcinogenicity and mechanistic studies will continue on topically-applied *Aloe vera* and retinyl palmitate, and the toxicity of *Aloe vera* will be assessed following oral exposure. Studies will continue to assess the effects of perinatal exposure of zidovudine and lamivudine in combination with nevirapine and nelfinavir. Experiments will continue on acrylamide, including assessing its carcinogenicity following perinatal and chronic exposures.

Investigators associated with the NCTR Center for Phototoxicity will continue to study the interaction of light with tattoo pigments and develop transgenic mouse models for photocarcinogenesis, with emphasis on the induction of cutaneous and ocular melanoma. They will also initiate studies of furcoumarins found in lemon and lime oil and Padimate O, a component of suntan preparations.

Results from the studies with endocrine-active compounds have indicated that soy-containing diets may protect against adrenal and renal toxicities. Gene and protein expression will be used to elucidate the mechanisms behind the effect upon adrenal gland. In addition, experiments will be initiated to investigate the protective effects of soy-containing diets against the renal toxicities of nonylphenol and di(2-ethylhexyl)phathalate.

Experiments with tamoxifen will be expanded to investigate the role of estrogen receptors in the formation of tamoxifen-DNA adducts. The investigations with pyrrolizidine alkaloids will be expanded to investigate if these compounds are present in a variety of dietary supplements. Finally, studies will be initiated to determine if global and locus-specific DNA hypomethylation could be a common mechanism in genotoxic and non-genotoxic hepatocarcinogenesis.

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Research Projects

Title	Project Number	Strategic Research Goal

PI: Beland, Frederick

- **Effect of Ethanol on the Tumorigenicity of Urethane (Ethyl Carbamate) in $B_6C_3F_1$ Mice** — **E0212001** — **Agent Driven Research**

  Objective(s):

  Determine the effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in $B_6C_3F_1$ mice.

  Status: Completed on 5/19/2003

- **Perinatal Carcinogenicity of Drug Combinations used to Prevent Mother-to-Child Transmission of HIV** — **E0214111** — **Agent Driven Research**

  Objective(s):

  Determine the carcinogenicity, genotoxicity and metabolism of antiretroviral drug combinations administered to mice transplacentally, perinatally, or neonatally.

  Status: Started/Ongoing

- **Genotoxicity and Carcinogenicity of Acrylamide and its Metabolite, Glycidamide, in Rodents - Two-Year Chronic Carcinogenicity Study** — **E0215001** — **Agent Driven Research**

  Objective(s):

  Compare the carcinogenicity of acrylamide and its metabolite glycidamide in $B_6C_3F_1$ mice and F344 rats treated chronically for two years.

  Status: Project Under Review

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **DNA Adducts of Tamoxifen** — **E0701101** — **Agent Driven Research**

  Objective(s):

  The nonsteroidal antiestrogen tamoxifen, which is currently being used in clinical trials as a chemoprotective agent against breast cancer, has been associated with the induction of certain malignancies. In order to determine if tamoxifen is acting through a genotoxic mechanism, this project will characterize DNA adducts from suspected tamoxifen metabolites, and develop methods for their detection and quantitation. Additional objectives include assessing the mutagenicity of tamoxifen and its metabolites in relation to DNA adduct formation in tissues of the Big Blue rat, determining the extent adduct formation in women treated with tamoxifen, and investigating the extent of DNA adduct formation from tamoxifen analogues including toremifene and GW5638.

  Status: Started/Ongoing

- **Genotoxicity ad Carcinogenicity of Acrylamide And Its Metabolite, Glycidamide, in Rodents: Neonatal Mouse Bioassay** — **E0718501** — **Agent Driven Research**

  Objective(s):

  Compare the extent of DNA adduct formation, induction of micronuclei, and carcinogenicity of acrylamide and its metabolite glycidamide in $B_6C_3F_1$ mice treated neonatally.

  Status: Project Under Review

- ***Salmonella* Mutagenicity Testing for Regulatory Needs** — **S00179** — **Concept Driven Research**

  Objective(s):

  Use the Ames *Salmonella* mutagenicity test system to determine the mutagenicity of compounds of regulatory interest to the Agency.

  Status: Started/Ongoing

- ***In Vivo* DNA Adduct Standards** — **S00198** — **Concept Driven Research**

  Objective(s):

  Develop methods to detect, characterize, and quantify DNA adducts *in vivo*.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Boudreau, Mary

- **Effects of *Aloe Vera* Components on Cell Proliferation and DNA Adduct Formation in SKH-1 Mice Following Simulated Solar Light Exposure** — **E0214001** — **Agent Driven Research**

  Objective(s):

  1) Determine the dose-response and acute kinetics of topical exposure to *Aloe vera* plant components on the structure of SKH-1 mouse skin in the absence of simulated solar light exposure;
  2) Determine the effects of topical exposure of *Aloe vera* plant components on the amount of simulated solar light required to induce skin edema in the SKH-1 mouse;
  3) Determine the subchronic effects of repeated co-exposure to *Aloe vera* plant components and simulated solar light on skin cell edema, proliferation, and DNA damage in the SKH-1 mouse;
  4) Determine the tumor-promoting activities of *Aloe vera* plant components following simulated solar light tumor initiation; and
  5) Determine the influence of *Aloe vera* components on simulated solar light-induced tumor formations in mice.

  Status: Started/Ongoing

- **Bioassays in the F-344 Rat and the $B_6C_3F_1$ Mouse Administered *Aloe Vera* Plant constituents in the Drinking Water** — **E0214201** — **Agent Driven Research**

  Objective(s):

  The use of *Aloe vera* is not limited to over-the-counter dermal therapeutics and cosmetics. *Aloe vera* is also taken internally. *Aloe vera* for internal consumption is also widely used as a prophylaxis and treatment for a variety of unrelated systemic conditions. In view of the complexities inherent in aloe pharmacology and the inconsistencies reported in literature, the objective of these studies is to conduct bioassays in rats and mice using standardized preparations of *Aloe vera* to explore the limits of safety for the *Aloe vera* leaf constituents present in commercial products.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Chou, Ming

- **A Collaborative Research Proposal to Assess Cancer Risk Posed by Intermittent Exposure to Aflatoxin B_1 in Rats** — **E0688801** — **Agent Driven Research**

  Objective(s):

  1) Test the hypothesis that a chemically-induced tumor incidence is a function of the accumulated lifetime exposure, and is predictable from the average daily dose for various dosing regimens, such as continuous and intermittent dosing; and
  2) Study correlations between the chemically-induced tumor incidence and various biomarkers of the initiation and the promotion stage of carcinogenesis for continuous and intermittent dosing.

  Status: Started/Ongoing

- **Effects of Dietary Restriction on the Post-Initiation Stages in Aflatoxin B_1-Induced Carcinogenesis on Male F-344 Rats Fed Methyl-Deficient Diets** — **E0695201** — **Concept Driven Research**

  Objective(s):

  Study the interactions of dietary restriction (DR) and methyl deficiency (MD) on the alterations of hepatic oxidative DNA damages, DNA methylation, cell proliferation, oncogene and tumor suppressor gene mutation, preneoplastic foci formation and tumor incidence during the post-initiation stages of AFB_1-induced carcinogenesis in male F344 rats. The results of these studies will: 1) test the hypothesis that DR may be an antagonist to the promotional effect of MD in the AFB_1-induced carcinogenesis; and 2) evaluate the correlations between the effects of DR and MD on the formation of AFB_1-induced preneoplastic foci and tumors and various biomarkers during the post-initiation stages of carcinogenesis.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

◆ **A Study of Genotoxic Mechanisms of Carcinogenic Pyrrolizidine Alkaloids and Pyrrolizidine Alkaloid N-Oxides** — **E0710401** — **Predictive Toxicology**

Objective(s):

1) Characterize the structures of the eight DHP-derived DNA adducts;
2) Study metabolism of retronecine-based pyrrolizidine alkaloids, heliotridine-based pyrrolizidine alkaloids, otonecine-based pyrrolizidine alkaloids, and pyrrolizidine alkaloid N-oxides by liver microsomes of F344 rats, $B_6C_3F_1$ mice, and humans of both sexes, and compare metabolism profiles;
3) Study the DNA adduct formation *in vitro* (from liver microsomal metabolism of the pyrrolizidine alkaloids described above in the presence of calf thymus DNA) and *in vivo*, and determine whether or not the same set of DHP-derived DNA adducts is formed in all cases;
4) Determine whether or not the levels of DHP-derived DNA adducts from different types of necine-based pyrrolizidine alkaloids formed in target tissues (liver) are significantly higher than those in non-target tissues;
5) Determine whether or not pyrrolizidine alkaloid N-oxides can be metabolized by rat and mouse liver microsomes to the parent pyrrolizidine alkaloids and whether or not DHP-derived DNA adducts are formed in significant amounts both *in vivo* and *in vitro;*
6) Determine whether or not some dietary supplements sold in the United States contain genotoxic pyrrolizidine alkaloids;
7) Determine the effect of liver carboxyesterases on DHP-derived DNA adduct formation from rat and human liver microsomal metabolism in the presence of calf thymus DNA;
8) Determine the effect of liver carboxyesterase inhibitors on DHP-derived DNA adduct formation from rat and human liver microsomal metabolism in the presence of calf thymus DNA; and
9) Determine the effect of Chinese herbs, such as liquorice, and their active components, such as glycyrrhizin and glycyrrhetinic acid, on inhibition of DHP-derived DNA adduct formation *in vivo* and *in vitro*.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Culp, Sandra

- **Two-Year Bioassay in Mice Administered Malachite Green or Leucomalachite Green in the Diet** — **E0212701** — **Agent Driven Research**

  Objective(s):

  Determine the risk associated with exposure to malachite green or leucomalachite green.

  Status: Started/Ongoing

- **Two-year Bioassay in Rats Administered Malachite Green or Leucomalachite Green in the Diet** — **E0212801** — **Agent Driven Research**

  Objective(s):

  Determine the risk associated with exposure to malachite green or leucomalachite green and establish if the demethylated derivatives of malachite or leucomalachite green found in tissues of treated rodents lead to the reactive species that bind to DNA.

  Status: Started/Ongoing

PI: Delclos, Kenneth

- **Range Finding Study for the Evaluation of the Toxicity of Genistein Administered in the Feed to CD (Sprague-Dawley) Rats** — **E0212201** — **Agent Driven Research**

  Objective(s):

  Determine the doses of genistein to be used in a multigeneration bioassay for establishing the effects of this naturally occurring isoflavone on development of reproductive organs, reproduction, cancer of the reproductive organs, and neurological and immunological function.

  Status: Completed on 1/10/2003

- **Range Finding Study for the Evaluation of the Toxicity of Nonylphenol Administered in the Feed to CD (Sprague-Dawley) Rats** — **E0212501** — **Agent Driven Research**

  Objective(s):

  Determine the doses of nonylphenol for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.

  Status: Completed on 10/23/2003

Title	Project Number	Strategic Research Goal

- **Range Finding Study for the Evaluation of the Toxicity of Vinclozolin Administered in the Feed to CD (Sprague-Dawley) Rats** — **E0212601** — **Agent Driven Research**

  Objective(s):

  Determine the doses of vinclozolin for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.

  Status: Started/Ongoing

- **Range Finding Study for the Evaluation of the Effects of Ethinyl Estradiol Administered in the Feed to CD (Sprague-Dawley) Rats During Development** — **E0212901** — **Agent Driven Research**

  Objective(s):

  Determine the doses of ethinyl estradiol (EE2) for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.

  Status: Started/Ongoing

- **A Comparison of Weight Gain and Fertility in CD Rats Fed a Standard Diet (NIH-31) or a Soy- and Alfalfa-free, Casein-containing Diet (NIH-31C)** — **E0213001** — **Agent Driven Research**

  Objective(s):

  Evaluate effects of NIH-31C on fertility by comparing pregnancy rates and litter size and weight in CD rats treated according to the treatment regimen to be used in the F0 generation of the multigeneration.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Genistein: Evaluation of Reproductive Effects Over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages** — **E0213201** — **Agent Driven Research**

  Objective(s):

  1) Determine the effects of genistein, a naturally occuring isoflavone, on reproduction and on the development of reproductive and other selected hormone-sensitive organs when administered to CD rats over multiple generations;
  2) Determine if subtle effects observed in the dose range finding study are magnified through multiple generations;
  3) Evaluate the reversibility of any observed effects;
  4) Evaluate the chronic toxicity of genistein, particularly potential induction of cancer of the reproductive organs, following exposures that will include various life stages (*in utero* through early adulthood, *in utero* and continuous for two years after birth, *in utero* and lactational only, and postweaning only); and
  5) Assess plasma and tissue genistein levels and evaluate additional endpoints modulated by genistein. These additional endpoints may be mechanistically associated with compound-induced alterations observed in the dose range finding studies that are also anticipated in animals from the multigeneration studies.

  Status: Started/Ongoing

- **Para-Nonylphenol: Evaluation of Reproductive Effects over Multiple Generations** — **E0213501** — **Agent Driven Research**

  Objective(s):

  1) Determine the effects of p-nonylphenol, an intermediate in the production of surfactants and other industrial products, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations;
  2) Determine if subtle effects observed in the dose range finding study are magnified through multiple generations;
  3) Evaluate the reversibility of any observed effects; and
  4) Assess the effect of nonylphenol on aromatase activity in the central nervous system in male rats at birth and on testosterone production and steroid receptor expression at puberty and at PND 140.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Ethinyl Estradiol: Evaluation of Reproductive Effects over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages** — **E0213801** — **Agent Driven Research**

  Objective(s):

  1) Evaluate the effects of ethinyl estradiol, a potent synthetic estrogen widely used in prescription drugs, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats in the diet over multiple generations;
  2) Determine if subtle effects observed in the dose range finding study are magnified through multiple generations;
  3) Evaluate the reversibility of any observed effects;
  4) Evaluate the chronic toxicity of ethinyl estradiol, particularly the potential induction of cancer of the reproductive organs, following exposures that will include various life stages; and
  5) Assess pharamacokinetics of ethinylestradiol (F0 generation only) by measuring serum levels using high pressure liquid chromatography and atmospheric pressure chemical ionization tandem mass spectrometry following an oral gavage dose.

  Status: Started/Ongoing

- **Sexual Dimorphism in the Inflammatory Response to Biomaterials** — **E0696301** — **Agent Driven Research**

  Objective(s):

  Determine if a sex difference in the *in vitro* response of human monocytes and mouse peritoneal macrophages to various biomaterials can be demonstrated. Based on existing literature, we hypothesize that there will be a significant sex difference in the synthesis and release of inflammatory mediators that could influence the biocompatibility of the material.

  Status: Completed on 10/27/2003

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **The Effects of Dietary Genistein on the Growth of Chemically-Induced Mammary Tumors in Ovariectomized and Intact Rats** — **E0702701** — **Agent Driven Research**

  Objective(s):

  1) Determine whether or not, in the absence of endogenous ovarian estrogens, dietary genistein can promote or suppress the growth of neoplastic mammary tissue at various stages of the carcinogenic process; and

  2) Evaluate the expression and activity of CYP1B1 in NCTR CD (Sprague-Dawley) female rats fed a soy- and alfalfa-free diet (5K96) relative to the same rats fed NIH-31 chow and to commercially available Sprague-Dawley (SD) rats. Based on the previously observed sensitivity of NCTR CD rats fed 5K96 diet to DMBA-induced adrenal toxicity, we hypothesize that either the 5K96 diet up-regulates CYP1B1 activity in the rat adrenal or that the NCTR CD (SD) rat contains higher CYP1B1 activity than SD rats from common commercial suppliers (Charles River and Harlan).

  Status: Started/Ongoing

- **Effects of Endocrine-active Agents on Bone** — **E0710601** — **Agent Driven Research**

  Objective(s):

  Determine if the administration of the endocrine-active agents genistein and ethinyl estradiol (EE2) will alter bone growth and remodeling and if the direction and extent of the effect depends on the window of exposure to the compounds.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Protective Effects of Soy-Containing Diets Against the Renal Toxicity of P-nonylphenol (NP) and Di(2-ethylhexyl)phthalate (DEHP)** — **E0714201** — **Concept Driven Research**

  Objective(s):

  1) Determine that the cystic kidney disease previously shown to be induced by nonylphenol in developing NCTR CD rats fed a soy-free diet is decreased in incidence and/or severity in rats fed soy-containing diets and to determine if the degree of protection is related to the amount of soy in the diet;
  2) Evaluate the renal toxicity of dietary DEHP in developing rats maintained on a soy-free diet;
  3) Evaluate potential early markers of renal cystogenesis in nonylphenol - and DEHP-treated rats and their modulation by soy-containing diets;
  4) Evaluate the roles of modulation of antioxidant defenses and cyclooxygenase activities in the protective effect of soy against nonylphenol - and, if demonstrated, DEHP-induced renal toxicity; and
  5) If renal toxicity of DEHP and a protective effect of soy against that toxicity are demonstrated, the effect of diet on the hepatic and testicular toxicity of DEHP will be assessed by measurement of the peroxisomal enzyme palmitoyl coA-oxidase in liver and histopathological evaluation of the tests.

  Status: Project Under Review

- **Optimization of Procedures for 1) Laser Capture Microdissection of Rat Kidney for Gene and Protein Expression Studies; and 2) Measurement of Renal Cyclooxygenases, Antioxidant Enzymes and Isoprostanes** — **P00619** — **Method Driven Research**

  Objective(s):

  1) Determine optimal parameters for laser capture microdissection to collect distinct renal cell populations for analysis of mRNA and proteins;
  2) Optimize conditions for the measurement of cox-1, cox-2, glutathione peroxidase, superoxide dismutase and quinone reductase; and
  3) Evaluate the feasibility of utilizing commercial ELISA kits for the determinations of prostaglandin and isoprostane levels in renal cortex and medulla.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Doerge, Daniel

- **ADDEND: Evaluation of Lactational Transfer of Genistein in CD (Sprague-Dawley) Rats** — **E0213231** — **Method Driven Research**

  Objective(s):

  Measure transfer of genistein from the lactating dam to nursing pups by measuring genistein in serum and milk using high pressure liquid chromatography (LC) and electrospray (ES) tandem mass spectrometry (LC-ES/MS/MS) following consumption of genistein-fortified soy-free basal diet.

  Status: Started/Ongoing

- **ADDEND: Evaluation of Serum Nonylphenol in CD (Sprague-Dawley) Rats Exposed to Dietary Nonylphenol** — **E0213531** — **Agent Driven Research**

  Objective(s):

  Assess serum and tissue nonylphenol levels (F2 generation only) by high pressure liquid chromatography and atmospheric pressure chemical ionization mass spectrometry (LC-APCI/MS).

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Genotoxicity, Mutagenicity and Exposure Biomarkers of Acrylamide and Its Metabolite, Glycidamide, in Rodents** — **E0214601** — **Agent Driven Research**

  Objective(s):

  1) Synthesize chemically and characterize spectroscopically the major glycidamide-DNA adducts;
  2) Develop and validate LC-ES/MS/MS assays to quantify the major glycidamide-DNA adducts;
  3) Determine glycidamide-DNA adduct levels in rodent tissues following short-term exposures of rodents to acrylamide and to glycidamide;
  4) Determine toxicokinetics and compare bioavailability of acrylamide and glycidamide following exposure by intravenous, oral gavage, and dietary administration;
  5) Correlate the levels and kinetics of glycidamide-DNA adduct in target tissues and circulating lymphocytes with acrylamide- and glycidamide-hemoglobin adducts in rodent exposure studies for future use in monitoring human exposure through occupation, smoking, and the diet; and
  6) Determine *in vivo* mutagenesis of acrylamide and glycidamide using transgenic mice (Big Blue).

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Toxic Hazards from Anti-Thyroid Chemicals** — **E0692001** — **Concept Driven Research**

Objective(s):

1) Determine inhibition mechanisms for environmental goitrogens using purified thyroid peroxidase and lactoperoxidase;
2) Determine the mechanism for covalent binding suicide substrates to purified peroxidases using electrospray-mass spectrometry to analyze intact adducted proteins and/or proteolytic fragments;
3) Determine mechanism of goitrogen uptake into isolated thyroid cells in primary culture and subsequent inhibition of iodination/coupling reactions involved in thyroid hormone synthesis;
4) Determine the structure-activity relationship for uptake of goitrogens into the thyroid and inhibition of thyroid hormone synthesis rats;
5) Correlate *in vivo* thyroid hormone status endpoints from the NTP endocrine disruption rat bioassay with the *in vitro* activity of rat microsomal thyroid peroxidase to better define the anti-thyroid mechanism for genistein;
6) Determine whether administration of genistein to rats results in an increase in the amounts of circulating auto-antibodies to TPO; and
7) Develop analytical methodology to quantify genistein/daidzein and the respective sulfate and glucuronide conjugates in blood and mammary tissue.

Status: Completed on 10/29/2003

- **Development of Methods for Analysis and Confirmation of β-Agonists** — **E0694501** — **Method Driven Research**

Objective(s):

1) Develop determinative and confirmatory procedures using Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry (LC-APCI/MS) for multiresidue screening β-agonists in livestock tissues;
2) Develop synthetic procedures to produce authentic β-agonist standards for use in regulatory screening. These methods will provide the flexibility to adapt to the potential for "designer drug" modifications by clandestine laboratories; and
3) Explore the use of packed column supercritical fluid chromatography (SFC) coupled to APCI/MS as a more efficient technique for chromatographic separation in the screening of large numbers of β-agonists in livestock tissues.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **ADDEND: Development of Methods for Analysis and Confirmation of β-Agonists** — **E0694511** — **Method Driven Research**

  Objective(s):

  Generate incurred ractopamine residues in livestock species that may be targets of off-label ractopamine use. Tissues and excreta from dosed animals will be used to validate immunochemical and confirmatory assays of ractopamine. The USDA will provide tissues from an incurred residue feeding study and NCTR will expand our multiresidue LC/MS method to include an additional compound, ractopamine, that was recently approved for commercial use by CVM.

  Status: Started/Ongoing

- **Measurement of Oxidative DNA Damage in Normal and Hepatitis C-Infected Human Liver** — **E0706401** — **Method Driven Research**

  Objective(s):

  1) Develop simple synthetic methods to produce stable labeled analogs of 8-oxo-dG, etheno-dA, etheno-dC, and M1-dG;
  2) Develop an automated on-line sample preparation method to maximize detection sensitivity for 8-oxo-dG, etheno-dA, etheno-dC, and M1-dG, in a single sample analysis, using liquid chromatogrpahy and tandem mass spectrometry;
  3) Apply methodology to the analysis of hepatic DNA from humans and animals; and
  4) Determine feasibility for application to clinical trials of therapeutic agents and toxicity/carcinogenicity testing in experimental animals.

  Status: Completed on 10/27/2003

- **Effect of Soy-Containing Diets on Ammonium Perchlorate-Induced Thyroid Toxicity in Sprague-Dawley Rats** — **E0716301** — **Agent Driven Research**

  Objective(s):

  Determine the effect of dietary soy and genistein, the principal soy isoflavone, on the dose-response characteristics for perchlorate-induced thyroid toxicity in male Sprague-Dawley rats.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

◆ **Human Studies of Isoflavone Safety and Efficacy** — **S00607** — **Method Driven Research**

Objective(s):

Bioanalytical analysis of soy isoflavones (and metabolites) in support of clinical trials at the University of Miami and Wayne State University.

Status: Started/Ongoing

PI: Fu, Peter

◆ **Effect of Topically Applied Skin Creams Containing Retinyl Palmitate on the Photocarcinogenicity of Simulated Solar Light in SKH-1 Mice** — **E0214301** — **Predictive Toxicology**

Objective(s):

Study the effects of topically applied skin cream containing retinyl palmitate on the photocarcinogenicity of simulated solar light in SKH-1 mice.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal
◆ **The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells**	**E0687901**	**Predictive Toxicology**

Objective(s):

1) Determine if the neonatal mouse bioassay can be employed to evaluate the tumorigenic potential of therapeutic drugs;
2) Examine concurrently as positive controls the genotoxic carcinogens: 4-aminobiphenyl, benzo(a)pyrene, 6-nitrochrysene, and aflatoxin B_1;
3) Study the metabolism and DNA adduct formation of benzodiazepine and antihistamine drugs by mouse and human liver microsomes to determine which if any cytochrome P450 is responsible for metabolic activation in mice and humans;
4) Transgenic human lymphoblastoid cell lines expressing appropriate CYP isozymes will also be employed to study the mutations and DNA binding of the subject drugs;
5) Determine whether or not the $B_6C_3F_1$1 neonatal mouse tumorigenicity bioassay is sensitive to chemical carcinogens that exert their tumorigenic activity by a secondary mechanism;
6) Assess the carcinogenicity of methylphenidate hydrochloride (ritalin), 4-hydroxy-2-nonenal, malondialdehyde, crotonaldehyde, and acrolein;
7) Assess the carcinogenicity of phenolphthalein and Tacrin;
8) Determine the tumorigenicity, mutagenicity, and DNA adduct formation of anti-HIV nucleosides in the $B_6C_3F_1$ neonatal mouse bioassay; and
9) Assess the tumorigenicity and estimate DNA adduct formation of a set of known or suspected human carcinogens in the $B_6C_3F_1$neonatal mouse.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal
◆ **A Study of the Secondary Mechanisms of Carcinogenesis: Lipid Peroxidiation and Endogenous DNA Adduct Formation from Chloral Hydrate, Benzodiazepines, Antihistamines, and Other Chemicals**	**E0700401**	**Predictive Toxicology**

Objective(s):

The specific aims outlined below are critical for the development of methodologies to study secondary mechanisms of carcinogenesis, including lipid peroxidation and endogenous DNA adduct formation, for determination of the mechanisms by which chemicals, such as FDA-regulated drugs including benzodiazepines and antihistamines, may induce cancer, and for the continued development of the neonatal mouse bioassay as a regulatory alternative tumorigenicity bioassay:

1) Develop analytical methodologies for analysis of lipid peroxidation products and endogenous DNA adducts;
2) Determine whether or not the drugs of FDA interest, including benzodiazepines and antihistamines studied in E687901, and other chemicals induce lipid peroxidation and endogenous DNA adduct formation *in vitro*;
3) Determine the inhibitory effect of lipid- and water-soluble antioxidants on drug-induced lipid peroxidation and endogenous DNA adduct formation *in vitro*;
4) Determine whether or not the malondialdehyde-modified MG-1 DNA adduct and/or other endogenous DNA adducts can be used as biomarkers of lipid peroxidation; and
5) Determine the mutagenicity of the benzodiazepine and antihistamine drugs in *Salmonella typhimurium* TA 104 and determine whether or not mutagenicity in *Salmonella typhimurium* TA104 can be used as a biomarker of lipid peroxidation induced by chemicals that generate free radicals upon metabolism.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Howard, Paul

- **The Role of Fumonisin B_1 in *Fusarium* sp. Tumorigenicity in Rats** — **E0211101** — **Agent Driven Research**

  Objective(s):

  1) Determine the effect of fumonisin B_1 on signal transduction pathways in cultured human esophageal epithelial tissues;
  2) Determine if DNA damage occurs *in vivo* in F344 rats when fed in the diet cultures of *Fusarium graminearum, Fusarium subglutinans, Fusarium moniliforme* or a combination of the three fungi, using ^{32}P-postlabeling technique;
  3) Determine the pharmacokinetics of fumonisin B_1 in $B_6C_3F_1$ mice and F344 rats under conditions similar to those used in the chronic bioassay, and in non-human primates; and
  4) Use the Rhesus monkey as a model to determine if fumonisin B_1 crosses the placenta.

  Status: Started/Ongoing

- **Comparative Toxicity of Fumonisin Derivatives in Female $B_6C_3F_1$ Mice** — **E0212401** — **Agent Driven Research**

  Objective(s):

  The primary objective of the study is to compare the toxicity of several fumonisin derivatives in female $B_6C_3F_1$mice.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **The Effects of Chemoexfoliation using α- and β-hydroxy Acids on Cell Proliferation and DNA Adduct Formation in SKH-1 Mice Exposed to Simulated Solar Light** — **E0213101** — **Agent Driven Research**

  Objective(s):

  The NIEHS/FDA Phototoxicity Center is designed to address the effects of compounds on the induction of skin cancer in mice using light sources that are relevant to humans. Input into the design of the facility has been obtained from experts in phototoxicity and photocarcinogenicity. These experts will continue to provide critical advice on the design of the experimental protocols. As a result, a facility will be developed that will meet the rigors of scientific scrutiny, and will generate data for human health risks from the effects of compounds on light-induced skin cancer. The facility is also designed for expansion to allow simultaneous examination of the toxicity or cocarcinogenicity of compounds in the presence of either simulated sunlight or fluorescent UVB light. The mechanistic studies in this proposal will provide the data necessary to design and interpret properly the future α-hydroxy acid and simulated solar light cocarcinogenicity studies.

  Status: Started/Ongoing

- **Effect of Topically Applied Skin Creams Containing Glycolic and Salicylic Acid on the Photocarcinogenicity of Simulated Solar Light in SKH-1 Mice** — **E0213701** — **Agent Driven Research**

  Objective(s):

  Determine if the application of creams containing α- and β-hydroxy acids to the skin of male and female SKH-1 hairless mice alters the tumor incidence induced by simulated solar light in the mouse skin.

  Status: Started/Ongoing

- **The Use of DNA Microarray Technology to Quantify the Effects of 8-methoxypsoralen (8-MOP) and UVA Light Treatment on SKH-1 Mouse Skin** — **E0213901** — **Agent Driven Research**

  Objective(s):

  Determine the effects of PUVA treatment on gene expression in the skin of SKH-1 mice. Success of this project will lead to a more extensive protocol in collaboration with NIEHS.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **DNA Adduct Formation by Nicotine Metabolites** — **E0692501** — **Concept Driven Research**

  Objective(s):

  1) Determine the structural identity of the nicotine delta 1',2'- and delta 1',5'-iminiumion DNA adducts, and modify existing ^{32}P-post labelling techniques to detect the adduct; and
  2) Quantify the presence of these adducts *in vitro* and *in vivo* in mice.

  Status: Started/Ongoing

- **Purification of Ceramide Synthase** — **E0705901** — **Concept Driven Research**

  Objective(s):

  1) Isolate rat ceramide synthase;
  2) Identify the gene coding for rat ceramide synthase;
  3) Develop antibodies to rat ceramide synthase; and
  4) Use the antibodies to study tissue specific expression of ceramide synthase.

  Status: Started/Ongoing

- **Methodology for Safety Testing of Pigments Used for Tattooing, Including Permanent Make-up** — **E0710501** — **Method Driven Research**

  Objective(s):

  1) Determine the chemicals in tattoo pigments and their metabolism *in vitro*;
  2) Develop methodology for tattooing SKH-1 hairless mice in a quantitative and reproducible manner;
  3) Determine the extent of inflammation induced by the implanted pigment, and determine the time of recovery following tattooing;
  4) Determine the acute toxicity of several tattoo inks and permanent make-up inks in SKH-1 hairless mice in the presence and absence of simulated solar light;
  5) Determine if tattoo pigments are photococarcinogenic in the SKH-1 hairless mouse using simulated solar light; and
  6) Determine if visual or infrared frequency imaging technology can be used for the detection of skin tumors in SKH-1 mice.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Development of a Research Plan and Research Protocol for Furocoumarins in Lemon and Lime Oil** — **P00624** — **Agent Driven Research**

  Objective(s):

  1) Conduct a literature search and summarize the occurrence, use, pharmacokinetics, toxicity, mutagenicity, and carcinogenicity of lemon and lime oil furocoumarins; and
  2) Develop a draft research protocol for submission to the NCTR Protocol Review and IAG Toxicology Study Selection and Review Committee.

  Status: Started/Ongoing

- **Historical Database of Skin Tumor Formation in SKH-1 Mice** — **S00213** — **Knowledge Bases**

  Objective(s):

  1) Enter into the NCTR MultiGen system the historical data (Argus Research Laboratories) of the tumor incidence in SKH-1 mice treated with simulated solar light (SSL) (no test compounds). Argus Research Laboratories will provide the weekly individual animal observation records for as many studies as deemed reasonable by Argus Research Laboratories. NCTR will be responsible for entering the data into the NCTR MultiGen database;
  2) NCTR will generate tumor incidence reports summarizing the occurrence of tumors in the animal groups from the animal tumor data that were obtained from Argus Research Laboratories. The data will then be analyzed for various parameters pertinent to these types of studies (e.g. time-to-first-tumor, mean time-to-first-tumor, tumors/mouse). The data, summary data, and statistical analyses will be shared with Argus Research Laboratories;
  3) NCTR will share with Argus Research Laboratories the incidence data on the occurrence of tumors in controled SKH-1 mice treated with SSL at NCTR. NCTR will also share the development of any statistical methods for analyzing the tumor incidence data with SKH-1 mice; and
  4) Both parties agree to share information concerning future development of animal rack and caging systems; devices for holding the SSL lamps; control of SSL lamps; devices for housing fluorescent light fixtures; devices, software, or protocol for monitoring the irradiance from SSL or fluorescent lights; and database systems for collecting animal information.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: James Gaylor, Sandra

- **Nutritional Modulation of Apoptosis and Chemosensitivity: A Novel Anticancer Strategy** — **E0700301** — **Concept Driven Research**

  Objective(s):

  1) In Nitroso methylurea (NMU)-initiated mammary epithelial cells, to determine whether nutritional manipulation of the cell cycle combined with low-dose chemotherapy will permanently eliminate p53-independent and p53-dependent preneoplastic and neoplastic cells; and
  2) Determine the mechanisms of cell death induced by nutritional manipulation and low-dose chemotherapy by examining molecular endpoints associated with p53-dependent and independent pathways of apoptosis.

  Status: Started/Ongoing

- **Genes, Micronutrients, and Homeobox-Related Malformations** — **E0707201** — **Predictive Toxicology**

  Objective(s):

  A more specific understanding of the genetic and environmental factors that contribute to the etiology of birth defects is a necessary prerequisite for the design of effective preventive strategies to reduce infant and maternal risk. This project has the potential to significantly advance current knowledge of specific etiological factors involved in maternal and infant risk, to aid in the design of nutritional intervention strategies, and to provide a basis for future mechanistic studies of human malformation. NCTR's main objective is investigation of maternal risk factor for neural tube defects and congenital heart defects.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Pogribna, Marta

- **Folic Acid Metabolism in Children with Down Syndrome** — **E0708501** — **Concept Driven Research**

Objective(s):

Determine whether supplementation with the nutrients folic acid and betaine will increase plasma levels of methionine, S-adenosylmethionine (SAM) and S-adenosylhomocysteine SAH, which have shown to be low in children with Down Syndrome (DS). The experiments will focus on the biochemical lesions in one-carbon metabolism stemming from trisomy 21 gene overdose and the potential to normalize metabolic imbalance with targeted nutritional intervention. With better understanding of the metabolic and molecular aberrations of CBS gene overdose in DS, the potential to ameliorate or prevent these progressive disease processes with nutritional intervention could become a reality. In the proposed study, baseline levels of homocysteine, methionine, cystathionine, cysteine, glutathione, cysteinyl-glycine, SAM, SAH, and adenosine in plasma of DS children will be determined at baseline and after 3 months supplementation with folic acid and betaine. This will define the metabolic abnormalities in one-carbon metabolism caused by the presence of an extra copy of chromosome 21 and is an important first step in determining whether there is a potential for nutritional intervention to correct the metabolic imbalance. The long-term goal for this study is to determine whether nutritional intervention in children with DS at 2-10 years of age will have a positive effect on their growth, immunologic function, and cognitive development. Adults with DS have already reached a plateau of growth and development and therefore the likelihood that nutritional intervention will affect their growth and development is minimal.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Pogribny, Igor

- **Mechanisms and Consequences of DNA Damage and Methylation Dysregulation during Rat Hepatocarcinogenesis** — **E0712801** — **Concept Driven Research**

Objective(s):

1) Confirm that the presence of uracil and abasic sites in preneoplastic DNA from folate/methyl deficient rats creates nonproductive high affinity binding sites for the DNA methyltransferase that compromise normal DNA methylation at the replication fork resulting in genome-wide hypomethylation;
2) Determine a) whether the double-stranded loss of cytosine methylation is maintained in folate/methyl deficient rats after nutritional repletion of methyl donors or whether the original methylation pattern and chromatin structure can be reestablished; and b) whether the increase in dnmt1 expression is stimulated by global loss of methyl groups and whether dmnt1 expression is decreased by methyl repletion;
3) Determine the temporal relationship between the appearance of DNA lesions and site-specific methylation within the CpG island of the p16 promoter region in p16 gene expression with alterations in local chromatin structure and DNA methyltransferase mRNA levels and activity; and
4) Use microarray slides printed with the rat cDNA library in collaboration with Dr. James Fuscoe, of the Center for Functional Genomics, as a tool to screen for methylation-related down-regulation of candidate genes in hepatic preneoplastic foci, preneoplastic nodules, and tumor tissue from folate/methyl deficient rats.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Roberts, Dean

- **Antigenic Biomarkers of Estrogen Catechol Metabolism for Use in Epidemiological Studies** — **E0705701** — **Predictive Toxicology**

  Objective(s):

  1) Prepare immunogenic conjugates for immunization of rabbits and antigenic conjugates for the characterization of antisera and for affinity purification of antibodies;
  2) Develop IA/LC/MS methods to detect the antigenic biomarkers in urine and/or serum; and
  3) Initiate studies to validate the use of the antibody reagents and IA/LC/MS methods developed in aim 1 and 2 using human urine and serum samples collected in an ongoing study of reproductive events, carcinogen metabolism, and interindividual variability.

  Status: Completed on 12/13/2002

- **Padimate O: Development of a Research Plan and Research Protocol** — **P00626** — **Agent Driven Research**

  Objective(s):

  1) Conduct a literature search and summarize the occurrence, use, stability, vehicle effects, pharmacokinetics and toxicity of padimate O; and
  2) Develop a draft research protocol for submission to the NCTR Protocol Review and IAG Toxicology Study Selection and Review Committee.

  Status: Started/Ongoing

PI: Tolleson, William

- **Molecular Basis of Tumor Promotion and Increased Somatic Growth in Yellow Avy/a Mice: Mitogenic Effects of Agouti Protein *In Vitro*** — **E0701201** — **Concept Driven Research**

  Objective(s):

  Determine whether or not the agouti protein stimulates mitogenesis *in vitro*.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **The Role of Human Metabolism in Endocrine Disruption** — **E0702301** — **Method Driven Research**

  Objective(s):

  Humans may be exposed to compounds in the diet or in the environment that disrupt endogenous endocrine responses in various tissues. We propose to utilize cell biological approaches to determine the role of human cytochromes P-450, UDP-glucuronosyltransferases, and sulfotransferases in the antiestrogens. The relative abilities of the various human enzyme systems expressed by individual cell lines to alter the extent of green fluorescent protein synthesis will indicate those human enzyme activities that activate or deactivate endocrine disrupting agents.

  Status: Started/Ongoing

- **Photoinduction of Cutaneous Malignant Melanoma in TP-*ras*/ink4A ($^{+/-}$) Transgenic Mice** — **E0708901** — **Predictive Toxicology**

  Objective(s):

  1) Characterize photochemical DNA damage in the skin of TP-*ras*/ink-4a mice exposed to UVA+UVB radiation;
  2) Determine whether cutaneous malignant melanoma can be induced in neonatal TP-*ras* ($^{+}$) ink4a ($^{+/-}$) transgenic mice using UVA+UVB radiation;
  3) Identify photochemically-induced mutations within the ink4a/p16/CDKN2A and p53 foci in tumor tissues; and
  4) Determine whether UVA+UVB exposure at an early age creates a greater risk for developing cutaneous melanoma in TP-*ras* ($^{+}$)ink4a($^{+/-}$) mice compared with chronic UVA+UVB exposure of older animals.

  Status: Started/Ongoing

PI: Wolff, George

- **Caloric Restriction and Gene Expression in Agouti Mice** — **E0260301** — **Concept Driven Research**

  Objective(s):

  The total amount of fat and calories we consume in our diet is highly correlated with the occurrence of cancer in North America and other highly developed nations. The studies proposed will help us learn how calories modify the development of cancer in mice and the mechanism underlying cancer development in humans.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Tumor Promotion and Neurochemical Changes in Mice During Chronic Feeding of the Antidepressant Fluoxetine** — **E0688201** — **Agent Driven Research**

  Objective(s):

  1) Determine if chronic feeding of fluoxetine (Prozac) results in promotion of mouse mammary carcinomas; and
  2) Determine if chronic feeding of fluoxetine: a) produces changes in the concentrations of serotonin and its metabolite, 5-hydroxyindoleacetic acid, in different regions of the mouse brain; and b) induces changes in serotonergic receptor and uptake sites in different regions of the mouse brain.

  Status: Started/Ongoing

- **Cellular and Molecular Responses to Chronic Iron Overload in Animal Models** — **E0691201** — **Agent Driven Research**

  Objective(s):

  1) Determine the health effects of chronic iron overload in mice and rats;
  2) Determine neurochemical changes after chronic iron overload in mice and rats; and
  3) Develop an animal model for identifying the cellular and molecular mechanisms underlying the hepatic and pancreatic effects of chronic iron overload which are characteristic of the human disease idiopathic hemochromatosis and possible neurochemical mechanisms which associate effects of iron with neurological disorders, e.g., Parkinson and Alzheimer diseases.

  Status: Started/Ongoing

Publications

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Project Number Codes:
E–Ongoing P–Preliminary S–Support

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Project Number Codes:
E–Ongoing P–Preliminary S–Support

Xia, Q., Chou, M.W., Kadlubar, F.F., Chan, P.C. and Fu, P.P., Human Liver Microsomal Metabolism and DNA Adduct Formation of the Tumorigenic Pyrrolizidine Alkaloid, Riddelline, *Chem. Res. Toxicol.*, 16:66-73. Accepted: 10/30/2002 (E0710401)

Concept Papers

Title	Project Number	Strategic Research Goal

PI: Beland, Frederick

- **4-Aminobiphenyl, p53 Mutations, and the Induction of Bladder Cancer** — **E0719401** — **Concept Driven Research**

  Objective(s):

  Status: Approved Concept Paper

PI: Howard, Paul

- **Induction of Melanoma in HGF/SF Transgenic Mice using Simulated Solar Light and Role of Sunscreens in Protecting Against Melanoma** — **E0716401** — **Agent Driven Research**

  Objective(s):

  1) Construct a solar simulator with variable spectral output and irradiance greater than 1 SED/min;
  2) Establish a breeding colony for the HGF/SF transgenic mice;
  3) Determine the effect of sunscreens on the induction of melanoma in neonatal HGF/SF mice using simulated solar light; and
  4) Determine if the rate of dose of simulated solar light affects the outcome of the melanoma development in the HGF/SF transgenic melanoma model.

  Status: Approved Concept Paper

Title	Project Number	Strategic Research Goal

PI: Pogribny, Igor

- **Global and Locus-Specific DNA Hypomethylation: A Common Mechanism Involved in Genotoxic and Non-genotoxic Rat Hepatocarcinogenesis** — **E0718101** — **Predictive Toxicology**

  Objective(s):

  1) Determine if the temporal alteration in genomic methylation profile in preneoplastic liver tissue and non-target tissues observed in the folate/methyl deficient model of rat endogenous hepatocarcinogenesis also occurs in other carcinogenesis model;
  2) Determine genes that are steadily up-regulated in target tissue during the promotion stage of carcinogenesis; and
  3) Evaluate whether the global and locus-specific DNA hypomethylation along with aberrant expression of related genes is specific only to target tissues and may be used for early detection of chemicals with carcinogenic potential.

  Status: Approved Concept Paper

Biometry and Risk Assessment

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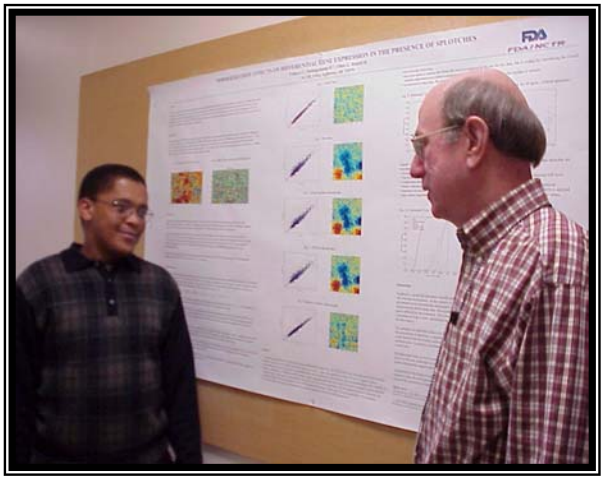

Drs. Molefe and Kodell discuss statistical methods for cDNA microarray data.

Executive Summary

Introduction

Risk assessment is a process for determining the extent of health hazard as a function of the conditions of exposure to toxic substances. The Division of Biometry and Risk Assessment conducts research to develop new and improved methods for assessing human health risks associated with exposure to chemicals and biological organisms. The Division is comprised of four mathematical statisticians, two research biologists, two computational chemists, two information technology specialists, one postdoctoral fellow and one program support specialist. Division scientists conduct both individual research within the Division and collaborative research with scientists from other NCTR Divisions, other FDA Centers, other government agencies, and academic institutions.

In 2002 the Center for Toxicoinformatics was established within the Division. The mission of the Center is to conduct research in bioinformatics and chemoinformatics, and to develop and coordinate informatics capabilities in support of NCTR's toxicological research in genomics, proteomics and metabonomics.

The main functions of the Division of Biometry and Risk Assessment are to:

- Develop statistical testing methods and predictive systems for identifying potential health hazards associated with toxic substances;
- Develop biometrical methods for estimating risks associated with toxic substances to enable setting exposure levels that correctly reflect underlying uncertainties;
- Develop mathematical models for better representation of internal exposure levels and of biological mechanisms in order to reduce uncertainty in estimates of risk;
- Provide analytical expertise to NCTR scientists on the design, conduct and analysis of research studies to evaluate the toxicity of regulated products;
- Assist other FDA Centers in conducting risk assessments for the regulation of specific products and in investigating generic risk assessment issues;
- Participate in interagency risk assessment activities to maintain knowledge of the state of the art, and to promote the improvement and unification of risk assessment practices across agencies; and
- Coordinate research and support in toxicoinformatics at NCTR relative to data arising from new technologies in genomics, proteomics and metabonomics.

FY 2003 Accomplishments

During FY 2003, scientists in the Division engaged in research addressing a variety of problems in biometry and risk assessment relevant to science-based regulation. Research projects included the following:

- Developing models and tests for assessing the risk of skin cancer due to interactions of cosmetics with sunlight;
- Developing improved statistical estimators and tests for assessing tumorigenicity in long-term rodent bioassays;
- Building an organ-specific database of carcinogenic and noncarcinogenic chemicals for SAR analysis;
- Developing novel computer-based classification systems to predict the toxicity of untested chemicals;
- Using a mixture-of-genotypes model for classifying enzyme variants in the development of biomarkers of disease risk;
- Developing quantitative methods for assessing the cumulative risk from exposure to mixtures of chemicals;
- Using two- and three-parameter dose-response models to assess and incorporate model uncertainty in microbial risk assessment;
- Enhancing pharmacokinetic simulation software to simultaneously model a parent chemical and several metabolites;
- Developing sensitive sub-population models for the spread of infection and disease caused by foodborne pathogens;
- Developing statistical designs and analytical techniques for functional genomic studies that measure changes in gene expression using cDNA microarrays;
- Developing network algorithms to model microarray gene expression data; and
- Developing an integrated system of databases, libraries and analytical tools for toxicoinformatics.

FY 2004 Plans

For FY 2004, scientists in the division will conduct research related to the design, analysis and interpretation of genomics studies; research to develop improved methods of data mining and class prediction; research on improved analytical methods for both long-term and short-term tumorigenicity studies; research on the spread and assessment of microbiological pathogens; and research on physiologically based pharmacokinetic (PBPK) models.

Planned research activities, identified by project number, include:

- Continuing to develop statistical adjustments to account for the simultaneous testing of multiple genes for differential expression among comparison groups (E07112.01);
- Developing network architecture to identify precursor genes, co-expressed genes and target genes for constructing genetic profiles of risk (E07159.01); and
- Developing robust statistical designs for functional genomics studies aimed at hazard identification (E07184.01)

- Continuing to develop a computer-based system that integrates databases, libraries and analytical tools for managing and analyzing "omics" data (S00617);
- Continuing to develop computer-based systems to predict the risk of organ-specific toxicity using multiple inputs based on chemical structures and spectra (E07083.01);
- Developing a novel prediction method, decision forest, for classification of subjects into risk categories based on genomic and proteomic data (E07169.01);
- Continuing to refine statistical tests for distinguishing tumor frequency risks from tumor latency risks in multiple-tumor photococarcinogenicity studies (E07061.01);
- Developing improved survival-adjusted statistical tests (E07171.01) and estimators (E07172.01) for hazard identification in long-term tumorigenicity bioassays;
- Continuing to develop statistical procedures for incorporating dose-response-model uncertainty into microbial risk assessment (E07045.01);
- Conducting animal experiments on host susceptibility and strain virulence to predict the spread of microbial pathogens through a population (E07082.01); and
- Continuing to develop a Windows-based program to implement a multi-component (parent chemical & 3 metabolites) PBPK model that accommodates postnatal growth in laboratory animals and humans (E07130.01).

In addition to the above highlighted projects, research will continue on all other active projects. Center-wide consultation on statistical, pharmacokinetic and toxicoinformatic problems will continue, as will the provision of oversight to on-site contract activities associated with statistical analyses and experimental support.

Public Health Significance

Human health risk estimates influence the regulation of exposure to toxic substances, thereby affecting both the health of the U.S. population and the health of the U.S. economy. The nature of the research carried out in the Division of Biometry and Risk Assessment is diverse, with projects characterized by development of mathematical and statistical theory and methods for risk assessment; biological experimentation and pharmacokinetic modeling with specific agents; and development of computational systems for predicting toxicity through knowledge discovery in databases. The ultimate goal of the research carried out in the Division is to improve the regulation of natural or synthetic toxic substances occurring in foods, drugs, cosmetics, biologics, medical devices and animal drugs. Continued significance to the FDA is fostered through interactions with individuals and committees at other FDA Centers involved in evaluations of risk for the regulation of specific products. Participation by Division scientists on interagency risk-assessment committees ensures relevance of the Division's research not only to FDA's regulatory needs, but also to broad public health issues.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Chen, James

- **Analysis of Multiple Tumor Sites** — **E0700901** — **Method Driven Research**

  Objective(s):

  1) Develop analytical and numerical techniques for computing the experiment-wise error rate in testing of multiple tumor sites;
  2) Evaluate and compare the experiment-wise error rate and power of various methods of p-value adjustment and recommend an optimal method for test of site-specific effects;
  3) Evaluate the experiment-wise error rate and power of global statistics for an overall test of carcinogenicity; and
  4) Recommend optimal procedures, which control the experiment-wise error rate and still maintain the power, for the analysis of multiple tumor sites.

  Status: Completed on 10/17/2002

- **Cumulative Risk Assessment for Chemical Mixtures** — **E0708701** — **Predictive Toxicology**

  Objective(s):

  Develop and apply the relative potency factors approach for estimating the risk from combined exposures to a set of chemicals having a common mode of action.

  Status: Started/Ongoing

- **Experimental Design and Analysis of GeneArray Expression Data** — **E0711201** — **Method Driven Research**

  Objective(s):

  Develop statistical and computational procedures for the design, analysis, and interpretation of gene expression data from microarray experiments.

  Status: Started/Ongoing

- **Research Scientists Council** — **S00188** — **Center Support (Research)**

  Objective(s):

  An advisory committee to the Director and Associate Director for Research on issues pertaining to the conduct of scientific research at NCTR.

  Status: Completed on 9/17/2003

Project Number code:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Delongchamp, Robert

- **Mortality Among Atomic Bomb Survivors Who Were Exposed In Utero** — **E0702901** — **Agent Driven Research**

  Objective(s):

  1) Estimate the dose-response relationship between noncancer mortality and radiation exposure;
  2) Assess the effect of gestational age at exposure on mortality; and
  3) Appraise the role of severe mental retardation in mortality.

  Status: Completed on 12/16/2003

- **A Mixture Model Approach to Classifying CYPIA2 Variants that Adjusts for their Current Smoking Status** — **E0703701** — **Method Driven Research**

  Objective(s):

  1) Examine statistical methods for parametric density estimation based upon a mixture of normal distributions; and
  2) Apply the method to a data set where hepatic cytochrome P4501A2 activity appears to be induced by smoking cigarettes.

  Status: Completed on 9/8/2003

- **An Investigation of the Effects of Adjusting Intensities from cDNA Arrays on the Assessment of Differential Gene Expressions** — **E0709601** — **Method Driven Research**

  Objective(s):

  1) Evaluate the advantages/disadvantages of using either the mean or median for normalizing array data in the presence of nuisances;
  2) Determine an optimal size of subsets for normalizing data in the presence of nuisances that merit their use; and
  3) Assess the bias induced by nuisances and the extent to which normalization procedures are able to remove them.

  Status: Started/Ongoing

Project Number code:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Kodell, Ralph

- **Attribution of Tumor Lethality in the Absence of Cause-of-Death Information** — **E0689601** — **Method Driven Research**

  Objective(s):

  1) Develop a nonparametric procedure for estimating distributions of time-to-onset of and time-to-death from occult tumors in the absence of cause-of-death information;
  2) Develop a method for entering the number of fatal tumors in an experiment that lacks cause-of-death data, in order to modify the International Agency for Cancer Research (IARC) cause-of-death test;
  3) Develop a procedure for estimating the lag time between onset of and death from an occult tumor, when cause-of-death data are unavailable; and
  4) Illustrate the new procedures using data from the Project for Caloric Restriction (PCR) studies.

  Status: Completed on 10/28/2003

- **Dose-Response Modeling for Microbial Risk Assessment** — **E0704501** — **Predictive Toxicology**

  Objective(s):

  1) Evaluate existing dose-response models for microbial risk assessment;
  2) Develop improved models for estimating probabilities of infection and disease;
  3) Develop methods for incorporating model uncertainty into microbial risk assessment.

  Status: Started/Ongoing

Project Number code:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Statistical Analysis of Tumor Multiplicity Data** — **E0706101** — **Predictive Toxicology**

  Objective(s):

  1) Investigate the model of Kokoska, et al. for analyzing tumor multiplicity data from single-induction experiments, using the negative binomial distribution for the number of induced tumors and the Weibull distribution for the time to observation of such tumors;
  2) Develop likelihood-ratio approach, adapted from the model of Kokoska, et. al. for testing between-group differences with respect to the expected number of induced tumors as well as the distribution of time to observation;
  3) Develop tests for dose-related trend with respect to the expected number of induced tumors and the distribution of time to observation;
  4) Extend the model to situations involving multiple or continuous dosing, and situations in which there is a background of spontaneous tumors;
  5) Conduct a Monte Carlo simulation study to compare the new methodology to conventional analytical approaches, and to evaluate its robustness and identifiability; and
  6) Develop user-friendly software for easy implementation of the proposed analytical procedures.

  Status: Started/Ongoing

- **Interagency Agreement on Developing and Evaluating Risk Assessment Models for Key Waterborne and Foodborne Pathogens and Chemicals** — **P00422** — **Predictive Toxicology**

  Objective(s):

  Developing and evaluating risk assessment models and chemical risk assessments for food and water. This is a proposal for a new interagency agreement between NCTR and EPA's National Center for Environmental Assessment.

  Status: Started/Ongoing

- **Modification and Application of Quantitative Risk Assessment Techniques to FDA-Regulated Products** — **S00174** — **Method Driven Research**

  Objective(s):

  In response to requests from scientists and regulators at CDRH, CDER, CFSAN, and CVM, using available toxicological data, conduct cancer and noncancer risk assessments of FDA-regulated products to assist in establishing "safest" conditions of exposure to toxic substances.

  Status: Started/Ongoing

Project Number code:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Application of Biometrical Procedures for NTP Projects** — **S00175** — **Concept Driven Research**

  Objective(s):

  In response to requests from NCTR scientists, modify and/or apply statistical techniques to the design, conduct, analysis, and interpretation of NTP studies to identify and assess the cancer and noncancer risks of potentially toxic substances.

  Status: Started/Ongoing

PI: Moon, Hojin

- **Development of Improved Survival-Adjusted Tests for Animal Carcinogenicity/Tumorigenicity Data** — **E0717101** — **Method Driven Research**

  Objective(s):

  1) Develop new statistical methods for investigating the carcinogenic potential of drugs and other chemical substances; and
  2) Develop a statistical testing methodology for a dose-related trend in tumor incidence rates of an occult tumor.

  Status: Started/Ongoing

- **Estimation of Lag Time Between Onset of and Death from an Occult Tumor via Attribution of Tumor Lethality** — **E0717201** — **Method Driven Research**

  Objective(s):

  Develop new statistical methods for estimating the elapsed time between onset of and death from an occult tumor when cause-of-death (COD) for each animal or context of observation for each tumor is not available.

  Status: Started/Ongoing

Project Number code:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Tong, Weida

- **Development of a Novel Class Prediction Method, Decision Forest, for Analysis of Genomic and Proteomic Data** — **E0716901** — **Method Driven Research**

  Objective(s):

  1) Develop the two-class Decision Forest method. The method will be developed on several publicly available gene expression and SELDI-TOF data sets, and the results will be compared with others that derived from traditional classification techniques; and
  2) The multi-class Decision Forest method will be also developed in this protocol. The method will be demonstrated on a gene expression data set to classify the pediatric acute lymphoblastic leukemia (ALL) subtypes.

  Status: Project Under Review

- **ODASI (Omics Data Analysis Solutions Initiative) Committee** — **S00632** — **Predictive Toxicology**

  Objective(s):

  The committee will review concept papers to identify the needs for bioinformatics support, interact with PIs to provide suggestions about scope and procedure of data management and analysis required for the protocol, and recommend appropriate experts to assist with the project for experiment design, data analysis and pattern recognition.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Turturro, Angelo

- **Development of a Model for the Transmission Kinetics of Infection by Cryptosporidium Parvum with Acquisition of Data on Key Parameters** — **E0708201** — **Concept Driven Research**

  Objective(s):

  1) Standardize the virulence of doses of Cryptosporidium parvum used in this and subsequent studies;
  2) Investigate the suitability of the Brown-Norway rat as a model for Cryptosporidium parvum infectivity in humans, or the C57Bl/6 mouse chemically-suppressed with dexamethasone if BN is unsuitable;
  3) Compare Cryptosporidium parvum infectivity for model animals with age and pregnancy, which may influence immunocompetence;
  4) Compare Cryptosporidium parvum infectivity for model animals with treatment with chemicals which induce immunosuppression other than by dexamethasone;
  5) Compare Crypotosporidium parvum infectivity in animals with immunosuppression models similar to the effects of AIDS;
  6) Compare Cryptosporidium parvum infectivity in animals with physiological stress and nutritional immunosuppression models; and
  7) Use these data in pathogen virulence and host susceptibility in a model for the transmission dynamics of Cryptosporidium parvum in human outbreaks.

  Status: Started/Ongoing

PI: Young, John

- **Computational Predictive System for Rodent Organ-Specific Carcinogenicity** — **E0708301** — **Predictive Toxicology**

  Objective(s):

  Develop an expert system to predict rodent carcinogenicity using modern SAR technology and statistical approaches.

  Status: Started/Ongoing

- **Bio-Preg to Windows 2000 Upgrade** — **E0713001** — **Method Driven Research**

  Objective(s):

  Upgrade Bio-Preg to a Windows-based program that will be called Win-Preg.

  Status: Started/Ongoing

Project Number code:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal
◆ **Species Comparison Utilizing a PBPK Model**	**P00393**	**Predictive Toxicology**

Objective(s):

Pharmacokinetic data from the literature will be excerpted and adapted to be simulated via a PBPK model. Initially the literature data will be limited to dexamethasone, cocaine, and methylmercury. Species comparisons will be made utilizing this single pharmacokinetic model.

Status: Completed on 9/29/2003

Project Number code:
E–Ongoing P–Preliminary S–Support

Publications

Bowyer, J.F., Harris, A.J., Delongchamp, R.R., Jakab, R.L., Miller, D.B., Little, R. and O'Callaghan, J.P., Selective Changes in Gene Expression in Cortical Regions Sensitive to Amphetamine Neurotoxicity, *NeuroToxicology*. Accepted: 8/7/2003 (E0707301)

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Chen, J.J., Statistical Analysis for Developmental and Reproductive Toxicologists, *Developmental and Reproductive Toxicology, A Practical Approach.* Accepted: 10/15/2002 (S00116)

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Hashemi, R.R., Young, J.F., Grid Based Analysis Approach for Learning from Sparse Data, *1st ACIS International Conf. on Software Engineering Research & Applications (2003).* Accepted: 6/25/2003 (E0708301)

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Hashemi, R.R., Young, J.F., The Prediction of Methylmercury Elimination Half-Life in Humans using Animal Data: A Neural Network/Rough Set Analysis, *Journal Toxicology and Environmental Health.* Accepted: 5/23/2003 (E0708301)

Hsueh, H., Chen, J.J. and Kodell, R.L., Comparison of Methods for Estimating the Number of True Null Hypotheses in Multiplicity Testing, *J. of Biopharmaceutical Statistic.* Accepted: 3/24/2003 (E0711201)

Malling, H., Delongchamp, R.R. and Valentine, C.R., Three Origins of Am3 Revertants in Transgenic Cell Culture, *Environmental and Molecular Mutagenesis.* Accepted: 7/23/2003 (E0700201)

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Yu, S., Keenan, S.M., Welsh, W.J. and Tong, W., Influence of the Structural Diversity of Data Sets on the Statistical Quality of Three- Dimensional Quantitative Structure-Activity Relationship (3D-QSAR) Models: Predicting the Estogenic Activity of Xenoestrogens, *Chemical Research Toxicology*, 15(10):1229-1234. Accepted: 12/1/2002 (E0708301)

Zeise, L., Hattis, D., Andersen, M.E., Bailer, A., Clewell, H., Conolly, R.B., Crump, K.S., Kodell, R.L. and Krewski, D., Improving Risk Assessment: Research Opportunities in Dose Response Modeling to Improve Risk Assessment, *Human and Ecological Risk Assessment*, 8(6):1421-1444. Accepted: 10/1/2002 (S00032)

Concept Papers

Title	Project Number	Strategic Research Goal

PI: Delongchamp, Robert

- **Statistical Design and Analysis of Functional Genomic Studies that Estimate Changes in Gene Expression using cDNA Arrays** — **E0718401** — **Quantitative Risk Assessment**

  Objective(s):

  1) Compare the efficiency of experiment designs that are capable of adjusting estimated treatment effects for nuisance sources of variation, which are referred to as day, dye, and array effects; and
  2) Develop methods that estimate the number of true null hypotheses among a set of hypotheses tests.

  Status: Approved Concept Paper

PI: Tsai, Chenan

- **Network Algorithms to Model Gene Expression Data** — **E0715901** — **Concept Driven Research**

  Objective(s):

  Develop neuro-fuzzy and Bayesian network models to describe relationships between genes or gene clusters.

  Status: Approved Concept Paper

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Chemistry

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Chemist Beth Brown performs a dose certification HPLC analysis in support of an FDA-nominated NTP study.

Executive Summary

Introduction

The Division of Chemistry contributes to new NCTR/FDA-directed research initiatives in mass spectrometry (MS)-based proteomics and nuclear magnetic resonance (NMR)-based metabonomics programs. These new facilities are now fully operational and complement the program Centers in Structural and Functional Genomics, Toxicogenomics, Hepatotoxicology, and other research divisions to elucidate mechanisms of toxicity. The Division also has programs on MS-based investigations in counter-terrorism, environmental chemistry, biomarkers, and sensor technology for rapid screening. Computational chemistry and artificial intelligence are also used in predictive toxicology and pattern recognition of bioterror agents. The Division continues to provide expertise in analytical chemistry and spectroscopy to the National Toxicology Programs (NTP) and other research programs Center-wide.

FY 2003 Accomplishments

The Mass Spectrometry Proteomics Laboratory was established in 2002 and collaborations have been initiated within NCTR (Center for Hepatotoxicology, Divisions of Chemistry, Microbiology, and Neurotoxicology) and externally. Projects have ranged from the identification of differentially expressed proteins to large-scale proteome mapping. Proteomics of rat liver mitochondria was chosen as a first project because of the critical importance of this organelle in cell function (E7183). We identified more than 1500 unique proteins, which will enable us to examine changes in protein expression and mitochondrial function in disease states or following drug treatments. MS approaches (nanoLC/MS/MS and de novo sequencing) were also used to identify proteins induced in *Mycobacterium vanbaalenii* PYR-1 that were exposed to pyrene, a toxic polycyclic aromatic hydrocarbon (PAH) (E7118). These studies will aid us to elucidate the mechanisms by which bacteria decontaminate environmental toxins.

The Mass Spectrometry Counterterrorism Program has used matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) MS and a pyrolysis metastable atom bombardment (MAB)-TOF MS to produce unique mass spectra of pathogenic *Vibrio* and *Salmonella* spp. A computational method was invented to improve the quality of MALDI spectral methods of these biological pathogens and to correctly identify bioterror agents (E07146). With this approach, bioterror agents or hoax materials, such as talc or flour, may be rapidly characterized. In

collaboration with the Microbiology Division and the Arkansas Regional Laboratory's (ARL) Microbiology staff, we are developing a method for microbial isolation that dramatically reduces analysis times of contaminated food to only a few hours.

In computational chemistry, the collaboration with the University of Arkansas for Medical Sciences (UAMS) continues on the brain tumor diagnostic method to achieve tissue characterization (E7131) and develop an early, noninvasive breast tumor detection method. Models have been developed using NMR spectral information and internal molecular structural connectivity information for predictive toxicology. A patent was submitted for the three-dimensional quantitative spectrometric data-activity relationship (3D-QSDAR) method (E7126). QSDAR modeling was used to predict the toxic equivalence factors (TEFs) for a small set of chlorinated dioxin molecules (E7077), that were assumed as nontoxic, and half of them predicted toxic with our model. FDA/ORA experimentally validated these predictions to the same level of magnitude, indicating the impressive accuracy of the QSDAR approach and the alarming toxicity of these substances. Rapid methods such as 3D-QSDAR may rapidly estimate toxicity of molecules and minimize the use of experimental animals.

A NMR-based Metabonomics Research Program has been established to aid the FDA in nonclinical and clinical drug safety assessment and drug efficacy issues. Metabonomics is a quantitative spectral "fingerprint" measurement of the dynamic endogenous metabolite response of living systems to pathophysiological stimuli or genetic modification. We are investigating metabolic changes in biofluids caused by drugs, analgesics and herbal products that may produce beneficial or deleterious health effects. Our program includes investigations on acetaminophen, tamoxifen, glitazone (with CDER), TCDD, doxorubicin (with CDER), cancer and liver diseases. Collaborations with UAMS scientists have been initiated to investigate the biological effects of ephedrine, ethanol, baby diets, cardiovascular disease and aging on health using the metabonomics approach.

In environmental chemistry research, collaboration with the Division of Molecular Epidemiology was established to assess the safety of hair dyes (E6978), which have been reported to increase the risk of developing bladder cancer with frequent usage. MS methods were developed to detect the bladder carcinogen 4-aminobiphenyl (4-ABP) in hair dyes. Some batches of para-phenylenediamine (PPD), a key reagent used in hair color development, were contaminated with 4-ABP and may be a source of 4-ABP contamination in hair dyes. We provided this analytical method to the cosmetic industry so that they may monitor the purity of PPD and other aromatic amine constituents used in hair dyes to assure the safety of hair coloring products. Investigations on the stability of biologically active constituents of herbal products containing St. John's Wort were carried out and showed some key chemicals degrade under simulated gastric conditions, or in acidic fruit drinks, and through exposure to light. Food Quality Indicator (FQI), a rapid, chemical sensor to assess food quality (E7080) has been evaluated by The Canadian Center for Fisheries Innovation (CCFI), St. Johns, Newfoundland, Canada. Their findings show that FQI is rapid, sensitive, rugged, and simple enough that multiple analysts can obtain results of equal quality. A Cooperative Research and Development Agreement (CRADA) has been developed with Litmus for a commercial outlet and partial support for extension of the FQI technology. An interagency agreement has been established with the Federal Aviation Administration (FAA) to develop rapid sensor detection methods to screen for explosives in counterterrorism (E7081).

The Chemistry National Toxicology Program (NTP) Analytical Team and Mass Spectrometry Branch continued analyses of test articles of numerous NCTR/National Institute of Environmental Health Sciences (NIEHS) Interagency Agreement (IAG) studies and collaborations Center-wide. Analytical support was provided to toxicological investigations on *Aloe barbadensis*, ethinyl estradiol, zidovudine, lamivudine, Ephedra sinica (Ma Huang), nelfinavir, nevirapine, retinol palmitate, retinoic acid, and 13-cis-retinoic acid. Analytical methods were developed or validated to separate Ephedra alkaloids, isomers of retinol palmitate and retinoic acid. Guidance was provided to National Institute of Health (NIH) on the alkaloid content of Ephedra sinica to be used in studies being conducted at NIEHS and NCTR.

FY 2004 Plans

Proteomics:

- Development of bioinformatic tools to aid in the analysis of proteomics data.
- Identify protein markers associated with the toxicity of acetaminophen and other hepatotoxins (with the Center for Hepatotoxicology).

Counterterrorism:

- Develop automated systems for rapid sample isolation and cell preparation, and for robotic spectral acquisition. Other foodborne pathogens to be profiled include Enterohemorhagic *E. coli* and *Listeria monocytogenes*.
- Continue refinement of pattern recognition/mass spectral methods for the rapid identification of biological pathogens and hoax materials.

NMR-Based Metabonomics:

- Acetaminophen, a hepatotoxin, will be used in a NMR-based metabonomics study (E7150) in collaboration with the Center for Hepatotoxicology, Functional Genomics, Proteomics, Biometry, CDER, and UAMS.

Computational Chemistry:

- Develop and validate a Monte-Carlo-based computational technique that can predict the tertiary structures of proteins and effects of genetic polymorphisms (in collaboration with the Division of Molecular Epidemiology, UALR, and CBER).

Environmental Chemistry:

- Determine if 4-ABP in hair dyes is bioavailable and poses a public health risk.

Public Health Significance

Proteomic and metabonomic technologies will advance our understanding of mechanisms of antibiotic resistance, signature proteins in disease states, biochemical modifications of proteins associated with cellular metabolism, and protein function following drug treatments or toxicity. These data can aid in the establishment of FDA policies and interpretation of data provided during an investigational new drug (IND) or new drug applications (NDA) submission to the FDA. The NCTR counterterrorism research provides a rapid means of analysis of bacteria that may aid in rapid identification of biohazards. Rapid analytical methods and MS-based monitoring studies provided a means to assure product quality and safety for the consumer.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Ang, Catharina

- **ADDEND: Development of Analytical Methodologies for Assessing Bioactive Herbal Ingredients in Functional Food Systems** — **E0705611** — **Method Driven Research**

  Objective(s):

  Expand the original protocol to include functional foods as additional substrates and to include active components of Echinacea and marker compounds as analytes. The scope of this protocol addendum covers the analytical methodology development aspect for St. John's Wort and Echinacea. Functional food items to be investigated may include tea, drink, soup, snack, cereal and candies.

  Status: Started/Ongoing

- **Analytical Methodology Development for Assessing Bioactive Herbal Ingredients in Functional Foods** — **E0716101** — **Method Driven Research**

  Objective(s):

  Develop qualitative and quantitative methods for determination of specific marker compounds, such as terpene trilactones (ginkgolides and biolobalide) and kava lactones in raw plant materials, dietary supplements and functional food products containing ginkgo, kava kava or their extracts. A minor objective of this proposed work is to include other minor compounds which do not meet the selection criteria but may be of safety concerns. These compounds include ginkgolic acids, ginkotoxin and urushiols in gingko products and unknown factors in kava.

  Status: Started/Ongoing

- **Influence of Hyperforin Concentration on Drug Interactions** — **P00436** — **Method Driven Research**

  Objective(s):

  Quantify hyperforin concentrations in plasma samples collected at University of Arkansas for Medical Sciences (UAMS) as a part of studies evaluating drug interactions between St. John's Wort and the conventional medicines.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Beger, Richard

- **Methods for Predicting Toxicological Properties of Molecules from Their NMR Chemical Shifts Through-Bond and Through-Space Distance Connectivity Patterns** — **E0712601** — **Predictive Toxicology**

  Objective(s):

  Produce models that use NMR data and infuse three-dimensional atom-to-atom through-bond connectivity and atom-to-atom through-space intramolecular distance information into a three-dimensional pattern that can be used by pattern recognition software to build a model of a biological or toxicological endpoint. The results of the 3D-QSDAR models will be compared to the results of QSDAR and QSAR models from protocols E0706801, E707701 and E0708301.

  Status: Started/Ongoing

- **ADDEND: Methods for Predicting Toxicological Properties of Molecules from Their NMR Chemical Shifts Through-bond and Through-space Distance Connectivity Patterns: Task Order 853 - Automated Analysis of NMR Chemical Shifts** — **E0712611** — **Predictive Toxicology**

  Objective(s):

  Produce and investigate models of biological activity that are produced based on the activity factors calculated from the minimum summed chemical shift deviations between a set of known compounds and an unknown compound to be predicted by the model. Task order #853 set up for programming to facilitate analysis of NMR chemical shifts; calculation of minimum shifts, pattern recognition on shift patterns.

  Status: Started/Ongoing

PI: Buzatu, Dan

- **Comparison of Principal Components Analysis (PCA) and Artificial Neural Networks (ANN) for Prediction of Qualitative and Quantitative Biological End Points from Spectromentric Data** — **E0707701** — **Predictive Toxicology**

  Objective(s):

  This study will introduce and evaluate a new ANN-based method for the correlation of spectrometric data to biological endpoints/activities. The evidence and methodology needed to expand the existing FDA-owned patent covering the use of spectrometric data for predicting biological endpoints will be provided.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **High Speed Parallel Distributed Neural Network Project** — **E0713101** — **Method Driven Research**

  Objective(s):

  Development of a high-speed computational platform for an Internet-based parallel-distributed artificial neural network. This is intended not only as an increase in the data-size handling capacity of the network, but equally important as a drastic enhancement of its efficiency. Improvements in both of these factors will enable the parallel neural network to become a powerful tool for elucidating important patterns in proteomic, genomic and other large size data-sets.

  Status: Started/Ongoing

- **The Development of Dynamic Mass Spectral/Pattern Recognition-Based Methods for the Rapid Identification of Bioterror Agents** — **E0714601** — **Method Driven Research**

  Objective(s):

  Develop the necessary computational capability to enable the rapid identification of pathogen/nonpathogen microorganisms, nonbiological hoax materials, and mixtures of all mentioned collected real world situations. An analysis will be done of the salient spectral features necessary for identifying these substances, and the effect of both instrumental and pattern definition techniques on the ability to use these features for rapid identification.

  Status: Started/Ongoing

- **Analysis of Proton MRS Data Using a Distributed Artificial Network** — **E0719501** — **Predictive Toxicology**

  Objective(s):

  Evaluate whether a self-optimizing, parallel distributed neural network can use the data from *in vivo* proton magnetic resonance spectroscopy (MRS) exams to provide additional information about a brain lesion. If so, this project will lead to improved brain tumor diagnoses from proton MR spectra.

  Status: Project Under Review

Title	Project Number	Strategic Research Goal

PI: Feuers, Ritchie

- **Methods for Support of a Functional Proteomics Facility at NCTR** — **E0713501** — **Method Driven Research**

  Objective(s):

  1) Establish and standardize for routine use procedures for whole cell and subcellular organellar isolation for a variety of tissues;
  2) Develop and standardize specific and sensitive markers of cell type and organellar purity and yield;
  3) Identify, adapt, develop and standardize appropriate 2-D protein separation techniques; and
  4) Integrate results of specific aims 1-3 to provide "front-end" components of a functional proteomics facility.

  Status: Started/Ongoing

PI: Siitonen, Paul

- **Analytical Method Validation and Characterization of Ephedra Alkaloids in *Ephedra sinica* Staph - NTP Test Article Materials** — **P00628** — **Agent Driven Research**

  Objective(s):

  NIEHS/CFSAN/NCTR have identified six ephedra alkaloids of toxicological significance to be included for evaluation of Test Articles prior to the NTP Study. Several potential appropriate methods of analysis for epehdra alkaloids have been published by other scientists. Selection, validation and/or modification of these methods is required by the Division of Chemistry personnel prior to characterization of the ephedra test article for the NTP study. The Division of Chemistry has received NTP experimental test articles for maintenance of custody throughout the experiment. Published methods of analysis will be applied to the NTP materials or surrogate material to determine the reproducibility and ruggedness prior to selection and application to chemical characterization analyses for the NTP experiment. Numerous reports of adverse health effects linking ephedra containing supplement use and athletic training or weight loss have appeared in recent years. Validated methods are needed to determine levels of ephedra alkaloids prior to initiation of the NTP experiment.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Turesky, Robert

- **Human Risk Assessment of Heterocyclic Aromatic Amines: Exposure, Development of Novel Biomarkers of Cytochrome P450 1A2 Activity and DNA Adduct Formation** — **E0709101** — **Agent Driven Research**

  Objective(s):

  1) Analyze HAAs by HPLC-MS in previously unreported grilled foods that are indigenous to southern cooking style, including Cajun-type foods;
  2) Establish sensitive biomarkers for interspecies extrapolation and human health risk by utilizing HPLC-MS methods to measure metabolites and excised DNA adduct of MeIQx and PhIP in human urine for cohort studies;
  3) Determine if specific metabolites of MeIQx and PhIP in human urine are catalyzed by P45 aA2, which is believed to be the major P450 involved in the toxication of these chemicals;
  4) Evaluate the effect of chemoprotective agents and dietary supplements on enzyme modulation, and its impact on HAA metabolism and DNA adduct formation in human hepatocytes for eventual chemoprotective studies *in vivo*; and
  5) Interspecies metabolism to assess the capacity of human and rat P45 1A2 orthologues in metabolic activation and detoxication of HAAs to assess human risk.

  Status: Started/Ongoing

- **Dietary Factors in the Etiology of Human Cancer, Biomonitoring of Heterocyclic Aromatic Amines – CRADA-Funded portion of E0709101** — **E0709102** — **Agent Driven Research**

  Objective(s):

  1) Determine the extent of heterocyclic aromatic amine exposure via urine analysis and determine whether HAA may contribute to human cancer development based upon the nested case-control studies;
  2) Develop analytical methods to measure the HAA metabolites and DNA adducts in urine; and
  3) Correlate HAA metabolite profiles with genotype and phenotype data associated with xenobiotic enzymes associated with cancer risk, such as cytochrome P450 1A2, N-acetyltransferases.

  Status: Project Under Review

Title	Project Number	Strategic Research Goal

◆ **Toxicological Effects of Ochratoxin A** — **E0709401** — **Predictive Toxicology**

Objective(s):

1) Establish chemical and biological markers of oxidative stress to proteins using biochemical and mass spectrometry techniques;
2) Establish markers of oxidative damage to DNA by measurement of abasic site formation and oxidized DNA lesions by affinity detection and LC-MS methods;
3) Investigate changes in gene expression and protein expression in liver and kidney as a function of OTA treatment; and
4) Correlate differences in these above endpoints with *in vivo* mutagenesis using the Big Blue Rat experimental model.

Status: Started/Ongoing

PI: Wilkes, Jon

◆ **Combining MAB/MS with Pattern Recognition to Sub-Type Bacteria** — **E0707901** — **Method Driven Research**

Objective(s):

This work is intended to demonstrate the validity of the combination of pyrolysis/metastable atom bombardment (MAB)/mass spectrometry (PyMAB/MS) with computerized pattern recognition (PattRec) for bacterial sub-typing. The work should produce a scientifically and technologically validated basis for commercial licensing of an NCTR-patented process: a method for assembling coherent spectral databases for use in rapid chemotaxonomy at the strain and sub-strain level.

Status: Started/Ongoing

◆ **Evaluation of Pyrolysis MAB/Tof MS and MALDI/Tof MS for Rapid Characterization of Presumptive Bio-Terror Agent Samples** — **E0714701** — **Method Driven Research**

Objective(s):

The suitability of mass spectral data obtained from both pyrolysis metastable atom bombardment MS and matrix-assisted laser desorption/ionization time-of-flight MS techniques will be evaluated for the purpose of rapidly characterizing presumptive bio-terror agent samples. This includes analysis of the salient spectral features necessary for identifying microorganisms from contaminated samples and differentiating tainted samples from hoax sample materials collected from the environment, as well as evaluating the effects of both instrumental and pattern definition techniques on the ability to use these features for rapid identification.

Status: Started/Ongoing

Publications

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Beger, R., Buzatu, D.A. and Wilkes, J.G., Using Simulated 2D 13C-13C NMR Spectral Data to Model a Diverse Set of Estrogens, *J. Molecular Design*, 2:435-453. Accepted: 5/1/2003 (E0712601)

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Project Number Codes:
E–Ongoing P–Preliminary S–Support

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Project Number Codes:
E–Ongoing P–Preliminary S–Support

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Concept Papers

Title	Project Number	Strategic Research Goal
PI: Beger, Richard		
◆ **Case-Control Study of NMR Metabonomic Signatures for Prostate, Breast and Colorectal Cancer** **Objective(s):** Demonstrate that there are unique NMR spectral signatures in urine and/or serum obtained from cancer patients compared to controls. **Status:** Approved Concept Paper	**E0717601**	**Predictive Toxicology**
PI: Edmondson, Rick		
◆ **Development of Automated High Sensitivity Nano LC/MS/MS Systems for Proteomics** **Objective(s):** **Status:** Approved Concept Paper	**E0718201**	**Method Driven Research**
◆ **Managing Sample Complexity and Dynamic Range During Proteome Analyses** **Objective(s):** Studies aimed at optmizing the analytical approaches adopted at NCTR regarding comprehensive proteome analysis. **Status:** Approved Concept Paper	**E0718301**	**Method Driven Research**

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Genetic and Reproductive Toxicology

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The genomics team from the Functional Genomics Center in the Division of Genetic and Reproductive Toxicology.

Executive Summary

Introduction

The Division of Genetic and Reproductive Toxicology (DGRT) conducts applied basic research to address specific high priority issues regarding genetic or reproductive/developmental toxicology. Division research is directed toward developing and validating new methods that can be used for the identification of potentially hazardous food additives, human and animal drugs, biological therapies and medical devices. In addition, in collaboration with other NCTR scientists, DGRT utilizes the methodologies that it develops to conduct research to understand the potential toxicity of specific high priority drugs, dietary supplements and/or other agents. Genistein and the AIDS therapeutic drugs zidovudine and lamivudine are currently undergoing extensive evaluations in cross-Division collaborative research efforts. New studies to understand the ability of acrylamide to cause cancer are being initiated.

Currently there are four basic focus areas in the Division research program. Genetic Toxicology research addresses the development of methods to assess the potential for chemicals to negatively impact human genetic material or the function of the genetic material. Reproductive/Developmental Toxicology focuses on methods to understand normal human development and how chemicals might alter normal development. In addition to these disciplinary research areas, the Division conducts research to understand the impact of dietary supplementation. This research primarily focuses on understanding the physiological and genetic consequences of dietary modulation. The Center for Functional Genomics has completed its initial optimization experiments and is now conducting microarray analysis for several research projects.

FY 2003 Accomplishments

In the Developmental/Reproductive Toxicology area, a project evaluating the predictability of animal data for human developmental toxicity was completed. Marginal dietary biotin deficiency was found to be teratogenic in a mouse model. Structural activity relationships were found to be useful as predictors of chemicals capable of causing endocrine disruption.

Improvements were completed to the newly developed ACB-PCR method that can be used to directly measure specific mutations in genes (oncogenes) that are directly involved in tumor induction. Such mutations can be detected when they occur in as few as 1 cell in 100,000 cells.

Protocols were developed to apply this methodology to ultraviolet light-induced skin cancer and also for colon cancer.

A large NCTR research effort to study dietary restriction is nearing completion. In studies completed in 2003, a small decrease in diet (10%) was demonstrated to have a number of positive effects for the overall health of the rats studied over much of their lifetime.

A chapter written by DGRT scientists describing the methods for the conduct and interpretation of data from the mouse lymphoma assay was included in the new book, Optimization in Drug Discovery: *In-vitro* Methods, to be published by The Humana Press.

DGRT scientists participated as members in two of the expert workgroups of the International Workshop for Genotoxicity Tests held in Plymouth, England, an effort to harmonize the protocols and interpretation of data from genotoxicity tests.

An international collaborative ILSI project evaluating the use of microarrays to distinguish between genotoxic and non-genotoxic chemicals in cell culture was completed.

During 2003, the NCTR Functional Genomic Center established its procedures for microarray analysis and also for the handling and interpretation of data. Sources of variation in microarray gene expression data were identified and a public access database for toxicoinformatic data was established. An experiment was completed describing the changes in the expression level of genes in rats as a function of time of day.

FY 2004 Plans

DGRT scientists will continue with the development of new approaches to directly measure mutations. They have developed a new cell line into which they inserted the gene for a fluorescent protein and another gene that controls its production. When a chemical causes a mutation in the controlling gene, the cell produces a fluorescent protein and becomes visible under UV light and can be quantified using cell-sorting instrumentation. Division scientists are currently trying to insert this gene into mice.

The new ACB-PCR technology will be applied to the study of skin cancer and colon cancer. A new project is being developed to study prostate cancer, and one of the current transgenic models (the Phi X mouse) will be evaluated for its utility in detecting mutations caused by UV light. DGRT scientist will participate in a new NTP study to evaluate acrylamide.

Work will continue on several ongoing projects including: (1) An Office of Women's Health project that is investigating whether genistein can decrease the induction of carcinogen caused mutations; (2) A collaborative project with UAMS investigating the influence of biotin on the developing embryo; (3) A project to investigate whether the drug azathioprine will increase the background level of mutations causing Lesch-Nyhan Syndrome and thus increase the incidence of this genetic disease in the offspring of individuals taking the drug; (4) The NTP project investigating the ability of the AIDS therapeutic drugs and malachite green to cause mutation in rodents; (5) A project to investigate whether the developing embryo and/or the neonate is

particularly sensitive to the induction of mutation following exposure to known carcinogens; and (6) A multi-organization collaborative project to establish a flow cytometric analysis of micronuclei in rats, nonhuman primates and humans.

The Functional Genomics Center will continue its validation and utilization of microarray technology. The ongoing validation includes a collaborative project with the National Institute of Standard Technology (NIST). Several research projects utilizing microarray technology are currently underway and additional projects will be initiated.

Public Health Significance

Genetic toxicology is the investigation of the ability of chemicals to alter the genetic material. The FDA requires that petitioners provide data evaluating the potential genetic toxicity of their products as a part of the product approval process. Because genetic damage is believed to be important in tumor development, this information is used as a part of the evaluation of suspected carcinogens. Regulatory decisions are based not only on the identification of potentially genotoxic substances, but also on an understanding of their mode of action. Research within the Division centers on the development and validation of new methods by which to assess genetic risk. While tissue culture approaches are used to detect potential genotoxicity and to generate hypotheses concerning the basic mechanisms of genotoxicity, the Division specializes in the development and validation of *in vivo* mammalian systems. An increased understanding of mutational mechanisms, combined with test systems with an increased ability to detect genetic damage, will provide the FDA with better information for decision making. As new assays are validated, Division scientists work with international scientists to assure harmonization of protocols and the development of guidelines.

Reproductive/Developmental Toxicology is important to the Agency because one of the difficult challenges facing the FDA is the identification and regulation of chemicals, food additives, and biological therapies that may produce birth defects. Such defects affect 7% of the population at birth, another 7% have low birth weights, and at least 25% of pregnancies end in spontaneous abortion. The Division specializes in research to understand how toxicants may induce birth defects such as neural tube defects. Current research addresses the role that folic acid may play in the normal closure of the neural tube. This research supports current thinking that diet may play a role in the development of normal offspring and that interactions between diet and toxicants may be important in producing certain birth defects.

The new genomic technologies are moving toward providing new tools for making better public health decisions. International research efforts are providing the scientific and medical community with an increased understanding of the genetic material and how it functions in both humans and rodents. Utilizing this information, new molecular technologies are being rapidly developed and can be used to evaluate structural and functional changes to the genetic material of both rodents and humans. The Division has developed the NCTR Functional Genomics Center. This capability is now available to NCTR and other investigators and will allow these technologies to be applied to fundamental risk assessment questions. While current technologies in the field of genetic and reproductive/developmental toxicology generally evaluate single endpoints, these new genomic technologies will provide the opportunity to detect alterations in a

number of different endpoints. This new approach to evaluating toxicity will also allow for the integration of information across the various types of adverse health outcomes. For instance, when this technology is fully developed, it will be possible to concurrently evaluate chemicals for their ability to cause cancer, to impact the nervous system, to cause birth defects and to modify the immune function.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Aidoo, Anane

- **The Frequency and Types of Spontaneous Mutations Found in the *Hprt* and *lacI* Genes of Lymphocytes from Transgenic Big Blue Rats** — **E0697501** — **Predictive Toxicology**

  Objective(s):

  1) Determine the frequency of spontaneous mutation at the *Hprt* and *lacI* loci in pre-weanling, young (four-month-old) and old (18-month-old) Big Blue rats; and
  2) Determine the types of mutations present in the mutants from Objective 1

  Status: Completed on 7/21/2003

- **ADDEND: The Frequency and Types of Spontaneous Mutations Found in the *Hprt* and *lacI* Genes of Lymphocytes from Transgenic Big Blue Rats** — **E0697511** — **Predictive Toxicology**

  Objective(s):

  The approved master project (E0697501) has not yet begun, and no animals have been allocated for it as yet. We have recently developed a method for expanding mutant rat lymphocyte clones from the approximately 100,000 cells per colony that are scored as mutants in our 96-well assay dishes to cultures containing several million cells. It is important to alter the experimental procedure of E0697501 to take advantage of the new technology. Taking advantage of this technology, however, necessitates switching most of the animals used in the project from female to male.

  Status: Completed on 7/21/2003

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **The Development of a Genotypic Selection Assay and Analysis of the Age-Specific Patterns of Mutant Accumulation** — **E0706301** — **Predictive Toxicology**

  Objective(s):

  1) Develop a genotypic selection assay (GSA) allowing a direct measurement of mutant frequencies and molecular analysis of mutation in any non-polymorphic endogenous sequence and in any tissue;
  2) Determine the spontaneous mutant frequencies (MFs) and age-associated accumulation rates (ARs) in highly (Exon 3) and poorly (Exon 4) mutable regions of *Hprt* coding sequence in the *Hprt* lymphocyte mutation assay; and
  3) Compare the *in vivo* persistence of elevated Mfs in *Hprt* exons 3 and 4 induced after exposure to ENU.

  Status: Started/Ongoing

- **ADDEND: Evaluation of the Effects of Dietary Antioxidant Intake on Behavior, DNA Damage and Expression of Free Radical Scavenging Enzymes During Physical Exercise in Male and Female Fischer 344 Rats Treated with 2-Amino-1-Methyl-6-Phynelimidazo[4,5-F]Pyridine (Phip)** — **E0706311** — **Predictive Toxicology**

  Objective(s):

  This addendum will enable the use of both treated and untreated animals. We also intend to include measurement of mitochondrial DNA mutations as an additional end point to the nuclear DNA mutations already described in the main protocol. This aspect of the study will make it possible to compare *in vivo* mutations occurring in both nuclear and mitochondrial DNA, as mutations in both systems contribute to human disease burden.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Evaluation of the Effects of Daidzein and Genistein (Hormone Replacement Agents) on the Genotoxic and Carcinogenic Activity of the Model Mammary Carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) in Ovariectomized Transgenic Big Blue Rats** — **E0707001** — **Predictive Toxicology**

  Objective(s):

  Determine whether daidzein and genistein or estradiol supplementation, singly or in combination to ovariectomized rats would alter in mammary tisssues:

  1) DNA adducts produced by DMBA;
  2) Frequency and types of mutations produced by DMBA; and
  3) Tumor formation by DMBA and types of p53 and H-*ras* mutations in tumors.

  Status: Started/Ongoing

- **ADDEND: Evaluation of the Effects of Daidzein and Genistein (Hormone Replacement Agents) on the Genotoxic and Carcinogenic Activity of the Model Mammary Carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) in Ovariectomized Transgenic Big Blue Rats** — **E0707011** — **Predictive Toxicology**

  Objective(s):

  In Group 1A under experimental design, rats on the estrogen control diet plus DMBA were inadvertently omitted. In order to include this treatment group in the study, we are requesting an additional 20 Big Blue rats. These rats and the other 288 rats specified in the main protocol will be pair-housed in cages, with one animal ear-clipped to facilitate the monitoring of body weight and food intake.

  Status: Started/Ongoing

- **ADDEND: An Efficient Regulatory Method for Evaluating Chromosomal Damage: Analysis of Micronucleus in Different Rat Strains by Flow Cytometry** — **E0714011** — **Method Driven Research**

  Objective(s):

  Addendum requesting 50 additional animals for the use of both normal (intact spleen) and splenectomized Sprague-Dawley rats.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Akerman, Gregory

- **Effect of p53 Genotype on Gene Expression Profiles in Mice Exposed to the Model Mutagen, N-ethyl-N'-nitrosourea (ENU)** — **E0712901** — **Concept Driven Research**

  Objective(s):

  1) Determine the effect of mutation in the p53 tumor suppressor gene on gene expression profiles in young and aged mice; and
  2) Determine the effect of mutation in p53 tumor suppressor gene on gene expression profiles in young and aged mice exposed to the model mutagen, N-ethyl-N-nitrosourea.

  Status: Started/Ongoing

PI: Bishop, Michelle

- **Fluorescent-Based Detection of Oxidative DNA Damage in Cells Treated *In Vitro* Using Flow Cytometry and Fluorescence Microscopy** — **P00441** — **Method Driven Research**

  Objective(s):

  1) Develop a sensitive and reliable method for the detection of 8OHdG in cells by flow cytometry and fluorescence microscopy;
  2) Optimize conditions for the assay; and
  3) Apply the methods developed to evaluate free radical mechanism of drug-or chemical-induced DNA damage in cells.

  Status: Started/Ongoing

PI: Branham, William

- **Development of a Statistically Robust 3D-QSAR Model to Predict *In Vitro* Rat Uterine Estrogen Receptor Binding Activity** — **E0290001** — **Knowledge Bases**

  Objective(s):

  The primary purpose of this Cooperative Research and Development Agreement (CRADA) is the development and validation of a statistically robust model for prediction of isolated rat uterine estrogen receptor relative binding affinity (RBA) that could be used as part of a prioritization scheme to identify chemicals for further *in vitro*/*in vivo* screening tests.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

◆ **Development of a Statistically Robust Rat Androgen Receptor (AR) 3D-QSAR Model for Predicting Relative Binding Affinity (RBA) of Untested Chemicals** — **E0290101** — **Concept Driven Research**

Objective(s):

Develop and validate a statisticaly robust 3D-QSAR model to predict *in vitro* rat androgen receptor (AR) relative binding. Provide an alternative and/or supplemental method to prioritize chemicals for entry into Tier 1 screening under the Environmenatl Protection Agency's (EPA) Screening and Testing Program for endrocrine disruptors.

Status: Started/Ongoing

PI: Chen, Tao

◆ **Comparison of Mutation Induction and Types of Mutations in the cII Gene of Big Blue Mice Treated with Carcinogens as Neonates and Adults** — **E0709001** — **Predictive Toxicology**

Objective(s):

1) Determine the mutant frequencies in the cII gene of lambda/lacI transgenic mice treated with ethylnitrosourea, a direct-acting carcinogen, and the modifying role of age, sex and target organ;
2) Compare the mutant frequencies in the cII gene of livers from the transgenic mice exposed as neonates and adults to different doses of aflatoxin B_1, a human hepatocarcinogen that requires a metabolic activation;
3) Determine the effect of exposure of neonatal and adult Big Blue mice to 17 b-estradiol, a human hormone carcinogen, on subsequent spontaneous and carcinogen-induced mutations in the cII gene of the target organs; and
4) Determine the types of cII mutations in the mutants from Objectives 1, 2, and 3.

Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **DNA Adduct Formation, Mutations and Patterns of Gene Expression in Big Blue Rats Treated with the Botanical Carcinogens Riddelliine, Aristolochic Acid (AA) and Comfrey** — **E0710001** — **Predictive Toxicology**

  Objective(s):

  1) Treat Big Blue rats subchronically with riddelliine, AA, and comfrey using procedures appropriate for tumor induction;
  2) Analyze DNA adduct formation in the target tissues for carcinogenesis and in spleen lymphocytes;
  3) Determine the cII mutant frequencies and the types of cII mutations in the target tissues of treated rats;
  4) Determine global gene expression patterns in the target and surrogate tissues of treated rats; and
  5) Correlate gene expression patterns with DNA adduct formation and mutation induction in treated rats.

  Status: Started/Ongoing

- **Further Evaluation of the Types of Genetic Events Detected by the Mouse Lymphoma Assay (MLA) and the Role of the Assay in Mechanistically-Based Risk Assessment** — **E0711701** — **Predictive Toxicology**

  Objective(s):

  1) Determine if the L5178Y/*Tk*$^{+/-}$ Mouse Lymphoma Assay adequately detects both aneuploidy and mitotic recombination;
  2) Determine if the L5178Y mouse lymphoma cells have active recombinase functions which lead to a large proportion of mutants that result from recombinase-mediated rearrangements; and
  3) Determine what is/are the fundamental genetic mechanism(s) causing the small and large colony thymidine kinase mutant phenotypes.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Dobrovolsky, Vasily

- **Validation of the Mouse Targeted $tk^{+/-}$ *In Vivo* System for Use in Mutagenicity Studies** — **E0701801** — **Predictive Toxicology**

  Objective(s):

  1) Expand a colony of transgenic $tk^{+/-}$ mice using breeding of $Tk^{+/-}$ founders and C57Bl/6 mice, and transfer the $tk^{+/-}$ genotype to a C57Bl/6 background;
  2) Determine spontaneous mutant frequencies at the tk and *Hprt* loci of splenic T-lymphocytes for mice of different ages;
  3) Induce mutations in $tk^{+/-}$ transgenic mice using treatment with the point mutagen ENU and the clastogens BLM and y-radiation, and to measure the kinetics of mutant induction at the tk and *Hprt* loci;
  4) Breed transgenic $tk^{+/-}$ parents in an attempt to derive $tk^{-/-}$ knockout mice, and study the biological signifcance of the tk gene in mice;
  5) Determine how the $tk^{-/-}$ genotype may effect mutant frequencies at the *Hprt* locus.

  Status: Started/Ongoing

- **ADDEND: Validation of the Mouse Targeted *$Tk^{+/-}$ In Vivo* System for Use in Mutagenicity Studies** — **E0701811** — **Predictive Toxicology**

  Objective(s):

  1) Establish routine microbiological surveillance of the colony as indicated in the addendum. This surveillance will be conducted on sentinel animals removed from the colony on an approximately monthly basis, and will consist of tests for the microorganisms listed in the addendum; and
  2) Propose to extend the range of mutagens tested in *$Tk^{+/-}$* mice through a collaborative arrangement with Dr. Vernon E. Walker of the Wadsworth Center, NY Dept. of Health, Albany, NY, and Dr. Rogene Henderson of the Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **ADDEND: Validation of the Mouse Targeted *Tk*$^{+/-}$ *In Vivo* System for Use in Mutagenicity Studies** — **E0701821** — **Predictive Toxicology**

  Objective(s):

  Proposing to breed *Tk*$^{+/-}$ mice with Pms2+/- mice in order to derive *Tk*$^{+/-}$ mice that can be used for evaluating LOH mutation and that are also deficient in the Pms2 gene product. Will be using animals bred under the parent protocol E0701801 and another protocol (E0704101). Also extending proposed completion date of master project and associated addenda to 4/30/2004.

  Status: Started/Ongoing

- **Evaluation of the *Tk*$^{-/-}$ Knockout Mouse as a Model of Systemic Lupus Erythematosus** — **E0706901** — **Predictive Toxicology**

  Objective(s):

  Investigate whether the *Tk*$^{-/-}$ genotype in mice is lupus prone. Particular emphasis will be given to documentation of the putative immune-complex mechanism of the renal disease, and in-depth evaluation of the immune system in *Tk* KO mice, seeking comparison with published characteristics of SLE in mice and humans.

  Status: Started/Ongoing

- **Transgenic Mouse Model for Detecting *In Vivo* Mutation Using a Green Fluorescent Protein Reporter** — **E0713801** — **Predictive Toxicology**

  Objective(s):

  1) Produce two lines of transgenic mice expressing the tetracycline-repressor protein;
  2) Investigate the efficiency of *in vivo* repression of green flourescent protein (GFP) in various tissues of different lines of the double-transgenic mice; and
  3) Determine the frequency of spontaneous and y-ray-induced TetR mutation in lymphocytes of double-transgenic mice using flow cytometry.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Duffy, Peter

- **Effect of Different Levels of Caloric Restriction (CR) on Physiological, Metabolic, Biochemical, Immunological, Molecular and Body Composition Variables in Rats** — **E0692401** — **Concept Driven Research**

Objective(s):

1) Determine how various levels and durations of CR affect physiological function, enzymes related to intermediary and drug metabolism, hormonal regulation, blood chemistry, etc;
2) Determine the relationship between body fat (BF), fat-free mass (FFM), total body water (TBW) and total body electrical conductivity (TOBEC) as a function of strain, age, mass and nutritional status in rats;
3) Validate and automate the use of a new noninvasive electromagnetic scanning device to measure BF, FFM and TBW and to compare the results to a conventional chemical fat extraction technique;
4) Determine if CR alters the relative quantity and disposition of various types of lipids such as cholesterol, phospholipids, free fatty acid, etc. in various tissues, as well as in urine, feces and blood serum;
5) Develop control data related to CR that can be used by CFSAN to evaluate the toxicity and efficacy of low calorie foods, food additives, and food substitutes;
6) Determine temporal and environmental factors that modulate the effects of CR;
7) Develop experimental methods for utilizing a low-level of CR to increase the survival rate and to decrease variablity in the chronic bioassay; to provide the concomitant control data for comparison; and
8) Develop control data for a reference purified diet that has been formulated to conform to long-term nutrient requirements of rodent animal models typically utilized in toxicology and nutrition studies.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **ADDEND: Evaluation of Cellular Responses in Rats Cell Proliferation Study by Flow Cytometric Cell Cycle Analysis** — **E0692421** — **Concept Driven Research**

  Objective(s):

  Flow cytometric cell cycle analysis for cell proliferation studies was missing in the original master protocol of the CFSAN-NCTR collaborative study E0692401. Therefore, this addendum proposes to efficiently utilize the available tissues from E0692401 to perform flow cytometric cell cycle analysis to obtain or accumulate data on cell cycle effects by dietary restriction. Will utilize bone marrow, kidney, spleen, and thymus tissues that were not previously designed for study in E0692401 for further evaluation using flow cytometric cell cycle analysis to study cell proliferation activities of tissues obtained from rats that received various levels of dietary restriction. No additional animals needed.

  Status: Started/Ongoing

PI: Fuscoe, James

- **Development of Glass-Slide-Based Oligonucleotide Microarrays for Mouse and Human Genes** — **E0711601** — **Method Driven Research**

  Objective(s):

  Develop, print, and establish the methodology for using a "mouse chip" containing approximately 5000 genes; a "rat chip" containing approximately 4000 genes; and a "human chip" containing approximately 8300 genes. Once the methodology is established, these chips will be available for a wide variety of research projects within the NCTR.

  Status: Started/Ongoing

- **Assessment of the Global Gene Expression Changes During the Life Cycle of Rats** — **E0712201** — **Concept Driven Research**

  Objective(s):

  1) Use the NCTR rat microarray chip to quantitate the relative expression of approximately 4000 genes in the liver of rats at the following ages: 2 wks, 5 wks, 6 wks, 8 wks, 15 wks, 21 wks, 52 wks, 78 wks, and 104 wks. These data will serve as a baseline measurement of gene expression that will be available for future studies on drug metabolism, toxicity, and susceptibility; and
  2) Verify the relative expression levels by quantitative PCR or Northern analysis.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Evaluation of Performance Standards and Statistical Software for Regulatory Toxicogenomic Studies** — **E0716801** — **Predictive Toxicology**

  Objective(s):

  Supply the experimental and statistical analyses necessary to help develop a consensus within FDA as to what performance standards would be beneficial for assessing the quality of microarray data submitted to the FDA on sponsor-selected platforms. The experimental results and conclusions from this inter-center project will be shared with other consortial microarray standardization efforts and made publicly available through publication.

  Status: Started/Ongoing

- **General Support for Center for Functional Genomics** — **S00616** — **Predictive Toxicology**

  Objective(s):

  The Center for Functional Genomics (CFG) is a centralized facility to handle all aspects of microarray printing and processing. Its objectives are:

  1) Provide NCTR investigators with access to high quality microarray technology for the investigation of biological mechanisms of action underlying the toxicity of products regulated by the FDA, and related fundamental and applied research;
  2) Create a validated toxicogenomics database that will be a resource for the scientific and regulatory community;
  3) Be a focal point and scientific resource for issues in toxicogenomics; and
  4) Utilize advances in genomics to address issues critical to the FDA mission. In addition, the CFG will provide continual development of new and better approaches to microarray technologies, including larger gene collections, custom microarrays, validated gene expression databases, experimental design, and tools for handling and analyzing microarray data.

  Status: Started/Ongoing

PI: Hansen, Deborah

- **Developmental Toxicity of Synthetic Ephedrine and Botanical Ephedra in Rats** — **E0214701** — **Predictive Toxicology**

  Objective(s):

  Determine potential developmental toxicity of synthetic ephedrine and botanical ephedra in rats.

  Status: Project Under Review

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Physiological Effects of Synthetic Ephedrine and Botanical Ephedra in Rats and Rabbits** — **E0214901** — **Agent Driven Research**

  Objective(s):

  Determine potential physiological effects of synthetic ephedrine and botanical ephedra in combination with caffeine, in exercised and sedentary rats, and rabbits.

  Status: Project Under Review

- **Indices of Biotin Nutrition** — **E0703401** — **Concept Driven Research**

  Objective(s):

  Determine the human requirement for biotin in normal individuals and in individuals in certain circumstances in which biotin status may be impaired. Specific Aim#4 (which will be accomplished at NCTR) will determine whether biotin of similar severity to that observed in human pregnancy can cause significantly increased rates of fetal malformation in the mouse. In the pilot mouse study, marginal biotin deficiency in mouse dams that caused an increase in 3-HIA excretion similar to that seen in human pregnancy produced 10% incidence of cleft palate in the fetal mouse.

  Status: Completed on 9/18/2003

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal
◆ **Mechanism(s) of Folate-Responsive Dysgenesis**	**E0707401**	**Concept Driven Research**

Objective(s):

1) Determine if there is concordance between the expression of the folate receptor (FBPI) and the most proliferative cohorts of neural tube- and neural crest-cells during defined 12-hour windows on each day of gestation from GD 5 to GD 15;
2) Determine if the loss of these cohorts of cells during these windows of antifolate exposure gives rise to recognizable neural tube defects and neurocristopathies in the fetus at term;
3) Characterize the basal expression of FBPI isoforms and extent and mechansim of FBPI regulation in the placenta and various fetal tissues on GD 17 among cohorts of dams fed a folate-deficient or folate-replete diet;
4) Determine if sustained quenching of placental cytotrophoblast FBPI by antisense FBPI cDNA overexpression from GD 8 to GD 16 during maternal folate deficiency has an adverse impact on cytotrophoblastic proliferation leading to small placentas and global growth retardation of fetuses; and
5) Demonstrate that neural tube closure and neural crest cell function in the whole mouse embyro at GD 8.5 can be perturbed by down-regulating FBPI expression in neural tube cells through the introduction of antisense oligonucleotides to the 43-kDa trans-factor which is required for FBPI transcription.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal
◆ **Examination of Embryonic Gene Expression During Neural Tube Closure**	**E0710901**	**Concept Driven Research**

Objective(s):

1) Construct SAGE library of expressed genes from control untreated GD 8.0 and GD 8.25 CD-1 mouse embryos;
2) Construct SAGE library of expressed genes from GD 3.25 CD-1 mouse embryos treated with a teratogenic dose of valproic acid on GD 8.0;
3) Compare the libraries to determine which genes are up- or down-regulated by valproic acid treatment;
4) Use Northern blot techniques to determine if the mRNA transcripts for these genes are indeed increased or decreased in expression compared to control embryos;
5) Use Northern blot techniques to determine a time-course of altered gene expression for genes of interest;
6) Examine expression of some of these genes after treatment with teratogenic or non-teratogenic doses of valproic acid, valproate analogs or another developmental toxicant; and
7) Use in situ hybridization, laser capture microdissection and Northern techniques to determine if altered gene expression is specific for subsets of embryonic cells.

Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Mechanism of Biotin Deficiency-Induced Malformations** — **E0713301** — **Concept Driven Research**

Objective(s):

1) Determine if palatal tissue from biotin-deficient embryos is able to fuse *in vitro* in either biotin-sufficient or -deficient medium;
2) Determine if arachidonic acid increases palatal fusion and improved limb development and increases the length of the long bones *in vitro* from biotin deficient mouse embryos;
3) Determine if prostaglandin E2 increases palatal fusion and improved limb development and increases the length of the long bones *in vitro* from biotin deficient mouse embryos;
4) Determine if malonyl CoA increases palatal fusion and improves limb development and increases the length of the long bones *in vitro* from biotin deficient mouse embryos;
5) Determine fetal arachidonic acid content and synthesis *in vivo*; and
6) Determine if arachidonic acid is able to prevent biotin deficiency-induced orofacial clefting and limb hypoplasia *in vivo*.

Status: Started/Ongoing

PI: Hass, Bruce

- **Identification of Target Sites for UVB Irradiation in Gene A of ΦX174 Contained as a Transgene in Mouse Embryonic Cell PX-2** — **E0710101** — **Predictive Toxicology**

Objective(s):

1) Determine the dose-survival-response of PX02 cells to UVB/UVA light in order to determine UV doses that optimize mutation induction and cell survival;
2) Determine the induced mutant frequency in gene A of ΦX174 by a forward mutation assay using cultures of PX2 exposed to UVB; and
3) Sequence the UVB/UVA-induced mutants from treated and untreated cultures to identify specific target sequences.

Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

◆ **UV-Induced Mutations in Mouse Epidermis Using Gene A of ΦX174: Proof of Principle** — **E0718701** — **Method Driven Research**

Objective(s):

Establish that a UVB-induced dose-response in mutant frequency of mouse epidermis can be detected by the forward assay for ΦX174 analyzed by single bursts.

Status: Project Under Review

PI: Heflich, Robert

◆ **ADDEND: Micronucleus and Gene Mutation Analysis in Female Big Blue $B_6C_3F_1$ Mice Administered Malachite Green and Leucomalachite Green in the Diet (Addend to E0212821)** — **E0212841** — **Agent Driven Research**

Objective(s):

Assess the mutagenicity of malachite green and leucomalachite green in relation to DNA adduct formation in female Big Blue mice.

Status: Started/Ongoing

◆ **Effect of Azathioprine in Somatic Cell and Germline Hprt Mutant Frequencies in the Mouse** — **E0709901** — **Concept Driven Research**

Objective(s):

Test the hypothesis that *in vivo* selection by azathioprine affects both somatic cell and germline Hprt mutant frequencies using the mouse.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: MacGregor, James

- **An Efficient Regulatory Method for Evaluating Chromosomal Damage: Analysis of Micronucleus in Different Rat Strains by Flow Cytometry** — **E0714001** — **Method Driven Research**

Objective(s):

This collaborative project to evaluate a new method for monitoring chromosomal damage, involving NCTR, Health Canada, the National Institute of Health Sciences, Tokyo, CDER, CFSAN, CVM and Litron Laboratories, has established a flow cytometric method of scoring micronucleated cells that allows assessment of chromosomal damage *in vivo* using small samples of peripheral blood. The method is statistically superior to traditional microscopic scoring of bone marrow, and is applicable to rats, dogs, humans, and nonhuman primates. The kinetics of micronucleated cell appearance and disappearance is being determined in species of regulatory interest (rat, dog, non-human primate, human). It is expected that the new methodology, by allowing measurement in peripheral blood rather than bone marrow, will permit integration of studies of chromosomal damage into routine toxicological studies and will facilitate evaluation of chromosomal damage in human studies.

Status: Started/Ongoing

PI: Manjanatha, Mugimane

- **ADDEND: Micronucleus and Gene Mutation Analysis in F344 Big Blue Rats Administered Leucomalachite Green in the Diet for 4, 16, and 32 Weeks** — **E0212821** — **Agent Driven Research**

Objective(s):

Malachite and leucomalachite green are currently being tested for carcinogenicity under the NIEHS/NCTR IAG. Previous experiments indicate that both compounds form DNA adducts in rodents when administered in the diet. The objective of this project is to assess the mutagenicity of leucomalachite green in relation to DNA adduct formation in tissues of Big Blue rats.

Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

◆ **ADDEND: Micronucleus and Gene Mutation Analysis in F344 Big Blue Rats Administered Leucomalachite Green in the Diet for 4, 16, and 32 weeks. (Addendum 3 to E0212801)** — **E0212831** — **Agent Driven Research**

Objective(s):

Malachite and leucomalachite green are currently being tested for carcinogenicity under the NIEHS/NCTR IAG. Recent results from addendum E0212821, indicate a two-fold increase in lacl mutations in the livers of Big Blue rats fed leucomalachite green for 16 weeks. The objective of this addendum is to expand the analyses of the remaining rats on E0212821 (32-week dose groups) to include additional indicators of hepatic toxicity.

Status: Started/Ongoing

PI: Mckinzie, Page

◆ **Application of the MutEx/ACB-PCR Method of Genotypic Selection to the Detection of K-*ras* Mutations** — **E0706601** — **Predictive Toxicology**

Objective(s):

Establish assays that can provide mechanistic data for chemical risk assessment and aid in establishing the relevance of rodent models for predicting human risk. The proposed research approach is to apply a recently developed method, MutEx/ACB-PCR to the detection of human and rodent k-*ras* GGT → GAT and GGT → GTT mutations. The assays will then be used to study the chemical induction of these mutations.

Status: Completed on 9/23/2003

◆ **ACB-PCR Measurement of Azoxymethane-Induced Rat K-*ras* codon 12 GGT → GAT and GTT → GTT Mutations in Colonic Aberrant Crypt Foci Isolated Using Laser-Capture Microdissection** — **E0714901** — **Predictive Toxicology**

Objective(s):

Use newly established PCR-based methods to quantify the rat K-ras codon 12 GGT → GAT and GGT → GTT mutant fractions in rat colonic mucosa, aberrant crypt foci, and tumors at specified times after colon tumor initiation by azoxymethane treatment. Use this data in conjunction with K-*ras* mutant fraction data generated from studies of human colon to determine how rodent data can be extrapolated to human disease.

Status: Project Under Review

Title	Project Number	Strategic Research Goal

PI: Mittelstaedt, R.

- **ADDEND: Measurement of H-*ras* Codon 61 CAA to AAA Mutation in Mouse Liver DNAs Using the MutEx/ACB-PCR Genotypic Selection** — **E0704121** — **Predictive Toxicology**

  Objective(s):

  Quantify and identify lacI mutations in liver DNA of mice treated as neonates with 4-aminobiphenyl in order to establish mutation induction and specificity as an early event in hepatic tumorigensis.

  Status: Started/Ongoing

- **ADDEND: Measurement of H-*ras* Condon 61 CAA to AAA Mutation in Mouse Liver DNAs Using the MutE/ACB-PCR Genotypic Selection** — **E0704141** — **Predictive Toxicology**

  Objective(s):

  Project intended to serve as a recruiting tool for a UAMS INTOX graduate student. Results from these experiments will support experimentation being conducted with neonatal mice in E0704121.

  Status: Started/Ongoing

PI: Morris, Suzanne

- **P53 Transgenic Mouse Evaluations of Genistein: 28-Day and 36-Week Studies** — **E0213601** — **Predictive Toxicology**

  Objective(s):

  1) Determine the toxicity of genistein in the C57Bl6/J strain, and select doses for the 36-week studies;
  2) Identify the potential carcinogenicity of genistein in the p53 transgenic mouse model;
  3) Determine if the potential carcinogenicity of genistein relates to changes in the rates of cell death and cell proliferation; and
  4) Determine if exposure to genistein results in an increase in the mutant frequency in a reporter gene, (*Hprt*), in the splenic lymphocytes of the p53 mouse.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

◆ **ILSI/HESI Consortium on Application of Genomics and Proteomics to Mechanism-Based Risk Assessment** — **P00425** — **Knowledge Bases**

Objective(s):

The goal of the ILSI project is to 1) establish a database for genomics data; and 2) relate the changes in gene expression to *in vitro* genotoxicity measures that are utilized in hazard assessment. In this project, two cell strains will be exposed to known carcinogens, the mutant frequency at the Thymidine Kinase locus will be measured and the formation of specific DNA adducts will be quantified. RNA will be isolated and sent to CDER for gene expression analysis. The data generated from this project will be entered into the ILSI database for further analysis.

Status: Completed on 9/5/2003

PI: Parsons, Barbara

◆ **Measurement of H-*ras* Codon 61 CAA to AAA Mutation in Mouse Liver DNAs Using the MutEx/ACB-PCR Genotypic Selection** — **E0704101** — **Predictive Toxicology**

Objective(s):

1) Quantify somatic mutations in liver DNA of mice treated with 4-aminobiphenyl in order to establish and evaluate MutEx/ACB-PCR genotypic selection as an approach for human risk assessment; and
2) Determine whether or not the MutEx/ACB-PCR genotypic selection is sensitive enough to measure the spontaneous frequencies of H-ras codon 61 CAA to AAA mutation in three different mouse models: $B_6C_3F_1$, C57BL/6, and the Pms2 mismatch repair-deficient, transgenic mouse.

Status: Started/Ongoing

◆ **ADDEND: Measurement of H-*ras* Codon 61 CAA to AAA Mutation in Mouse Liver DNAs Using the MutEx/ACB-PCR Genotypic Selection** — **E0704111** — **Predictive Toxicology**

Objective(s):

Due to failure of a freezer, liver tissues being stored collected under the master protocol were thawed. The livers of the one-month post-treatment time-point of the newborn mouse assay were the ones destroyed. Additional animals and resources are being requested in order to repeat the one-month time-point of the $B_6C_3F_1$ newborn mouse assay.

Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Analysis of p53 Codon 270 CGT to TGT Mutation in Simulated Solar Light-Induced Skin Tumors and Exposed Mouse Skin** — **E0715201** — **Predictive Toxicology**

  Objective(s):

  1) Develop the ACB-PCR detection of mouse p53 codon 270 CGT→ TGT mutation;
  2) Measure the frequency of detection and levels of this mutation in mouse skin tumors;
  3) Measure the frequency of this mutation in skin tissue from tumor-bearing animals; and
  4) Measure the frequency of this mutation in skin exposed to decreasing levels of SSL.

  Status: Started/Ongoing

- **Measurement of Cancer-Associated Gene Mutation in Colon Tumor and Non-Tumor Tissue** — **E0716001** — **Predictive Toxicology**

  Objective(s):

  1) Determine K-*ras* codon 12 GGT → GAT and GGT → GTT mutant frequencies in colonic ACF, adenomas, and carcinomas; first by DNA sequencing and, if mutation is not detected, then by ACP-PCR;
  2) Determine K-*ras* codon 12 GGT → GAT and GGT → GTT mutant frequencies in tumor margin samples and tumor-distant, normal-appearing colonic epithelium from colon cancer patients; and
  3) Determine K-*ras* codon 12 GGT → GAT and GGT → GTT mutant frequencies in autopsy samples of colonic epithelium from colon-disease-free individuals.

  Status: Project Under Review

PI: Shaddock, Joseph

- **ADDEND: Lymphocyte *Hprt* Mutant Frequencies and Types of Mutations in PMS2 Mice Treated as Neonates With Solvent or With 4-aminobiphenyl** — **E0704131** — **Predictive Toxicology**

  Objective(s):

  Quantify and identify the *Hprt* mutations in spleen lymphocytes of $PMS2^{+/+}$, $PMS2^{+/-}$, and $PMS2^{-/-}$ mice treated as neonates with either dimethlysulfoxide (solvent control) or with 4-aminobiphenyl (4-ABP).

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Valentine, Carrie

- **Evaluation of the Potential of the Gene A Forward Mutational Assay of ΦX174 for Improving Sensitivity of Transgenic Mutation Assays** — **E0711501** — **Predictive Toxicology**

  Objective(s):

  1) Determine the appropriate experimental conditions to identify single bursts of mutations fixed *in vivo*;
  2) Develop a microplate scoring method that will identify *in vivo* bursts within numerous aliquots;
  3) Determine the spontaneous mutant frequency and ENU-induced mutant frequency by single burst analysis for mouse splenic lymphocytes; and
  4) Continue development of a frameshift assay for ΦX174 in gene J by our collaborator Dr. Bentley Fane.

  Status: Started/Ongoing

Publications

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Project Number Codes:
E–Ongoing P–Preliminary S–Support

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Thai, S., Allen, J.W., Deangelo, A.B., George, M.H. and Fuscoe, J., Altered Gene Expression in Mouse Livers After Dichloroacetic Acid Exposure, *Mutation Research*, 543:167-180. Accepted: 12/1/2002 (N/A)

Concept Papers

Title	Project Number	Strategic Research Goal

PI: Chen, Tao

- **DNA Microarray Analysis of Gene Expression Patterns in Mouse Bone Marrow and Hematopoietic Stem Cells at Different Time-Points after N-ethyl-N-nitrosourea (ENU) Treatment to Extrapolate the Mechanisms of Mutagenesis** — **E0720001** — **Predictive Toxicology**

  Objective(s):

  1) Obtain gene expression profiles at different time-points of the mutagenic process in the bone marrow and hematopoietic stem cells of ENU-treated mice; and
  2) Establish a model of functions, interactions and molecular regulatory networks of the genes for ENU-induced mutagenesis by comprehensive analysis of gene expression profiles.

  Status: Approved Concept Paper

PI: Desai, Varsha

- **Development of "Mitochip", a Glass-Based Oligonucleotide Microarray Containing Mitochondrial and Nuclear Genes Associated With Mitochondrial Function** — **E0718601** — **Method Driven Research**

  Objective(s):

  Develop a "MitoChip" containing genes associated with mitochondrial function such as oxidative phosphorylation, B-oxidation of fatty acid, tricarboxylic acid cycle, apoptosis as well as genes involved in the replication, transcription, translation of mitochondrial DNA, DNA repair and regulation of DNA copy number.

  Status: Approved Concept Paper

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Manjanatha, Mugimane

- **CONCEPT - 2-Amino-1methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) Induction of Mutagenic and Carcinogenic Effects in the Prostate of Rats and Modulation by Dietary Antioxidant** — **E0719701** — **Predictive Toxicology**

  Objective(s):

  1) Determine the PhIP-induced mutant frequencies and spectra of mutations in the lacI gene of the prostate stromal and epithelial gland in the presence and absence of antioxidants;
  2) Compare mutation induction in the prostate gland with mRNA levels of a rodent counterpart of the human clinical marker for prostate cancer in the presence and absence of antioxidants;
  3) Capture by laser capture microdissection p53 and K-*ras* mutated BPH cells using immunostaining and genotypic selection respectively, and determine types of p53 and K-*ras* mutations;
  4) Determine p53 and K-*ras* mutations in PhIP-induced prostate tumors by PCR;
  5) Compare *lacI* mutation profiles with p53 and K-*ras* mutations in initiated BPH cells and tumors in the presence and absence of antioxidants; and
  6) Determine the mRNA levels of testosterone and estrogen receptors as well as 5 alpha-reductase gene in the prostate in the presence and absence of antioxidants.

  Status: Approved Concept Paper

PI: Mei, Nan

- **CONCEPT - Development of a New Rat Model for Studying *In Vivo* Chemical Mutagenesis by Detecting Mutations in T-Cell Receptor (TCR) Genes** — **E0719601** — **Method Driven Research**

  Objective(s):

  1) Develop a new rat model for studying *in vivo* chemical mutagenesis by detecting mutations in T-cell receptor (TCR) genes;
  2) Determine TCR mutant frequencies in spleen lymphocytes of Fischer 344 rats treated with ethylnitrosourea; and
  3) Compare the mutant frequencies in the TCR genes and the cll gene in spleen lymphocytes of Big Blue transgenic rats treated with aristolochic acid to validate the TCR assay.

  Status: Approved Concept Paper

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Microbiology

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Senior Analyst, Don Paine, examines microscopically a sample for the presence of parasites.

Executive Summary

Introduction

The Division of Microbiology at the National Center for Toxicological Research (NCTR) serves a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of the Food and Drug Administration's (FDA's) responsibility in toxicology. The Division of Microbiology also responds to microbial surveillance and diagnostic needs for research projects within the NCTR and FDA. Projects are selected based on FDA priorities and programmatic expertise. The research program is divided into six focal areas: 1) Foodborne pathogens, food safety and methods development; 2) Antimicrobial resistance; 3) Gastrointestinal microbiology and host interactions; 4) Environmental biotechnology; 5) The use of microorganisms as models to predict the metabolic pathways by which drugs are metabolized in mammals; and 6) Microbiological surveillance and diagnostic support of research.

FY 2003 Accomplishments

The Division of Microbiology research scientists continue to provide valuable information to FDA for evaluating key regulatory issues in food safety and environmental biotechnology, with special emphasis on antimicrobial resistance in the food animal production environment.

Food Safety and Antimicrobial Resistance

Reports of antimicrobial-resistant bacteria from farms, animal carcasses and aquaculture facilities are raising concerns that antimicrobial use in food-producing animals may play a role in selecting for antibiotic resistance. The research and regulatory issues on antimicrobials used in food-producing animals are of great importance to the FDA. A number of collaborative research projects with other FDA Centers are being conducted in the Division of Microbiology.

In FY 2003, researchers in the Division of Microbiology collected litter, feed, and water samples from farms to isolate *Salmonella, Campylobacter*, and *Escherichia coli* to determine if they are fluoroquinolone-resistant. Molecular methods, such as ribotyping, pulsed field gel electrophoresis and polymerase chain reaction, were developed to screen for fluoroquinolone resistance genes in *Salmonella* spp., *Campylobacter* spp. and *E. coli* isolates from chicken and turkey farms. Molecular characterization of the fluoroquinolone-resistant strains was conducted. Romet (oxytetracycline and sulfadimethoxine-ormetoprim) is an antibiotic of choice to control

pathogenic bacteria in aquaculture. Intensive use of the antibiotic has resulted in the occurrence of antibiotic resistant bacteria. In addition, several tetracycline-resistant bacteria were isolated from samples obtained in aquaculture facilities. We characterized tetracycline resistance in the fish pathogen *Aeromonas* spp. by various molecular biology methods such as pulsed field gel electrophoresis (PFGE), polymerase chain reaction (PCR), southern hybridization and plasmid isolation. A multiplex PCR method was developed that will detect the presence of all known tetracycline-resistant genes in *Aeromonas* spp. We also developed and evaluated an oligonucleotide-microarray method to rapidly detect multiple antibiotic resistant genes from foodborne and clinical pathogens. Over 100 genes related to antimicrobials that are used in animal and poultry farming have been identified and the microarray antibiotic resistance gene chip has been completed. The utility of this method for testing the impact of antimicrobials in the food animal production environment and probiotic products is currently being investigated.

Since there has been concern about the use of antibiotics in agriculture, other approaches are also being evaluated to minimize contamination of animal products with foodborne human pathogens. Reducing colonization of animals by pathogenic bacteria by using competitive exclusion treatments is being considered as an alternative to antimicrobial feed additives. Competitive exclusion products must adhere to FDA regulations that the bacterial mixtures be well defined, pathogen-free, not resistant to antimicrobials and effective. For commercial use, competitive exclusion preparations for poultry must be free from all known human and avian pathogens and from any microorganisms with unusually high resistance to antimicrobials. The FDA has approved a competitive exclusion product designed to prevent the colonization of chicken intestines by pathogenic bacteria, such as *Salmonella* spp., *Campylobacter* spp., and *E. coli*, and also to reduce the use of antimicrobials and the spread of antimicrobial-resistance genes.

These products are reviewed as new animal drugs for safety and efficacy because of claims that they affect the health of the chicken. The composition of these complex mixtures is not simple to define, and in the Division of Microbiology, scientists compared automated conventional techniques, including biochemical analysis and cellular fatty acid analysis, with newer automated molecular methods (16S rRNA sequence analysis) for characterization of the mixtures. Our studies provide the FDA with methods that will help to standardize the identification techniques used to characterize the components of competitive exclusion products. These results will provide guidelines for manufacturers of competitive exclusion products to submit more reliable product information for market approval by regulatory agencies.

In addition, researchers in the Division of Microbiology found that the bacteria in a competitive exclusion product harbor multiple resistance markers against several drugs; including resistance to erythromycin by enterococci and lactobacilli, to fluoroquinolones by *E. coli*, and to vancomycin in *Lactobacillus lactis*. The possibility of interspecies transfer of these resistance mechanisms, and transfer eventually to humans, makes this discovery a matter of concern for food safety.

As part of the NCTR Counterterrorism Initiative, scientists in the Division of Microbiology have collaborated with investigators in the Division of Chemistry on the rapid identification of bacteria by mass spectrometry. They have used this method to fingerprint *Vibrio parahaemolyticus* isolates from seafood and environmental samples and antibiotic resistant *Salmonella* spp. from poultry facilities.

Gastrointestinal Microbiology and Host Interactions

Intestinal microflora play significant roles in human health because they aid in the digestion of food, metabolize drugs and foreign compounds, mediate hormonal activities of phytoestrogens, and help prevent pathogens from colonizing the gastrointestinal tract. Because these bacteria play critical roles in human physiology, scientists have devised many methods for identifying them in fecal samples. Investigators in the Division of Microbiology have studied the variation of intestinal microflora from different individuals in modification of hormonal activities of phytoestrogens. Since identification of GI tract bacteria by traditional methods is time consuming and many molecular methods are limited in their ability to identify bacterial species, the Division of Microbiology, in collaboration with scientists from the University of Arkansas for Medical Sciences, has shown that microarray technology can identify the 40 most common bacterial species in the human gastrointestinal tract. These results demonstrate that microarray methods can reliably and rapidly identify intestinal flora and in most cases are more sensitive than culture methods. The microarray method has many more potential applications, for example, examining bacterial species in various patients clinically treated for intestinal diseases, and for experimental animal studies to determine the effect of food additives, antimicrobial residues, phytoestrogens, and xenobiotics on the intestinal microflora.

In response to FDA's need for assessing the microbiological safety of animal drug residues in food, the Division of Microbiology and CVM have been investigating an *in vitro* culture system that examines the effect of low-level antibiotic residues on the human intestinal microflora by using a continuous culture to model the human intestinal tract. In FY 2003, fed batch culture systems were tested, and molecular methods were used to identify changes in the bacterial populations in response to antimicrobial residues. Recommendations on the methods and protocols for determining the effect of residual levels of antimicrobials on the human intestinal microflora were presented at several meetings of the Microbial Safety Task Force of the Veterinary International Cooperation and Harmonization Safety Working Group. Guidance documents have been drafted on determining the effect of residual levels of antimicrobials on the human intestinal microflora.

Another essential study in the Division of Microbiology is the elucidation of the mechanism of resistance to antimicrobial agents among bacteria from the human gastrointestinal tract. The resistant bacteria are of particular concern, because not only do they act as a reservoir for antimicrobial resistance genes, but also if they establish themselves in other parts of the body, they can cause diseases that cannot be treated. The Division of Microbiology research scientists have detected anaerobic bacteria from the human intestinal tract that are resistant to high concentrations of various fluoroquinolones. They also determined that one of the mechanisms of fluoroquinolone-resistance is due to the bacteria having an active efflux pump, which effectively reduces the intracellular concentration of the antimicrobial and drug efficacy. The antimicrobial

agent metronidazole is the drug of choice for prevention and treatment of many anaerobic infections. Metronidazole is a product that requires conversion by nitroreductases to demonstrate its bactericidal activity. Resistance to the drug is commonly associated with decreased nitroreductase activity in the target bacteria. Scientists in the Division of Microbiology tested *Enterococcus* species bacteria from the feces of an individual who had been treated repeatedly with metronidazole for nitroreductase activity. The drug did not kill the bacteria even though they contained full nitroreductase activity. Further analysis showed that these metronidazole-resistant bacteria likely inactivated the drug by metabolism.

Environmental Biotechnology

The environmental fate of veterinary drugs and factors that influence the persistence and biodegradation of antibiotics used in farm animals and aquaculture have been investigated. Both fundamental and applied studies on the biodegradation pathways of erythromycin and the fluoroquinolones ciprofloxacin, norfloxacin, and sarafloxacin have been conducted. These studies indicate that microorganisms may play an important role in the detoxification and removal of antimicrobials from animal wastes and aquaculture sites. Scientists in the Division of Microbiology and the University of Mississippi determined that the common poultry litter, rice hulls and ground corncobs, supplemented with the nonpathogenic fungus *Pestalotiopsis guepini* degraded the norfloxacin. By comparison, pine shavings did not support the growth of *P. guepini* or degradation of the antimicrobial. These experiments suggest a technique to reduce or eliminate contamination of the environment in agricultural uses of clinically important drugs.

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of organic compounds whose environmental fate is of concern because some PAHs have mutagenic, ecotoxic and carcinogenic potential. Scientists in the Division of Microbiology have elucidated the biodegradative pathways of benzo[*a*]pyrene, benz[*a*]anthracene and 7,12-dimethylbenz[*a*]anthracene and the enzymes involved in PAH metabolism. Proteomic and genomic techniques have been developed to characterize protein expression and the genes involved in the bacterial metabolism of PAHs. This research increases our understanding of the environmental fate of PAHs and in developing practical PAH bioremediation strategies in the future.

Microbial Models of Mammalian Metabolism

Another ongoing research initiative within the Division of Microbiology is to exploit the use of microorganisms as models of mammalian drug metabolism. Studies were completed which determined that the fungus *Cunninghamella elegans* mimics mammalian metabolism of several tricyclic antidepressant drugs. Microbial metabolites of a wide range of drugs can be produced more cost-effectively and in less time than those produced by experimental animals, cell cultures or mammalian enzyme systems for structural elucidation and toxicity evaluation.

Microbiological Surveillance

The primary mission of the Surveillance/Diagnostic Program in the Division of Microbiology is to assure that the experimental animals at NCTR are healthy and free from infections that could compromise research data. The staff also provides researchers critical support in microbial culture identification, contamination investigation, stock culture maintenance, media preparation, and technical assistance. A major initiative in FY 2004 will be to develop molecular biology detection procedures for each of the microorganisms on our potential animal pathogen list and incorporate these methods into our surveillance screening.

FY 2004 Plans

Work will continue on a number of ongoing projects, including:

1) The Importance of Human Intestinal Microflora in Conversion of Phytoestrogens to Estrogenic Compounds – The phytoestrogen metabolites produced by colonic bacteria of different individuals will be characterized and the bacteria involved in the metabolic process will be identified;
2) Studies on Mechanism of Fluoroquinolone Resistance in *Salmonella* spp. Isolated from Animal Feeds (Poultry), Animal Production and the Development of Molecular Methods for Screening the Drug Resistance Genes – We will characterize *E. coli*, *Salmonella* spp. and *Campylobacter*sp. strains from turkey and chicken samples using a variety of molecular biology methods and study the effects of environmental enteric pathogens on intracellular signaling of the host;
3) Studies on the Fluoroquinolone Resistance in *Campylobacter* sp. Isolated from Poultry - Characterization of campylobacters from turkey litter will be continued and data on the correlation of environmental factors on the occurrence of campylobacters in turkey and chicken farms will be conducted;
4) Characterization of Tetracycline and Sulfadimethoxine-Ormetoprim Resistant Pathogenic Bacteria from Catfish Tissues – Molecular probes will be developed and used to localize the DNA fragments that harbor these tetracycline resistance genes. Similar molecular methods will be used to determine the epidemiology of the occurrence of tetracycline-resistant genes in *E. coli*, *Vibrio* spp., *Salmonella* spp., and Citrobacter in catfish tissues;
5) Develop gene array chips for rapid microbial pathogen detection of *Salmonella* spp. and *Vibrio* spp. in ocean-derived products – The method developed will be used as a template for development of a diagnostic array that is capable of simultaneous detection of multiple foodborne pathogens;
6) Microbial Models for Biotransformation of Fluoroquinolones – We will identify the transformation products that are produced by fungi and bacteria from flumequine, norfloxacin, and ofloxacin;
7) *In Vitro* Assay for Perturbation of Colonization Resistance by Antibiotic Residues – We will test the effects of antimicrobial agents on the ability of the new model human intestinal microflora to protect against *Salmonella* spp. and *Campylobacter* sp. invasion of Caco-2 cells and test a new human intestinal cell line in the assay;
8) Determining the Effect of Low Levels of Antibiotic Residues on the Human Intestinal Microflora using an *In Vitro* Continuous Culture System – We will continue to develop and refine methods for detecting specific intestinal bacterial in complex mixtures such as feces

or fecal cultures. *In vitro* cultures of the human intestinal microflora will be exposed to different concentrations of a variety of antimicrobial compounds, and the changes in bacterial populations will be monitored using both qualitative and quantitative methods;

9) Elucidation of the Mechanism of Resistance Development in Anaerobic Bacteria from the Human Intestinal Tract – We will evaluate the roles of alteration of target genes and efflux pumps in the mechanisms of resistance to antimicrobial agents;
10) Probiotic Effects on Host Defense Against Enteric Pathogens – We will acquire germ-free mice, colonize them with the model intestinal microflora and evaluate the colonization of the mice by the intestinal microflora;
11) Proteomic Approaches to Elucidate Biodegradative Pathways – We will identify differentially expressed proteins by N-terminal sequencing and mass spectrometry and determine condition-specific marker proteins, which are part of the response of the bacteria under different conditions. Furthermore, we will elucidate the metabolic pathways for high molecular weight PAHs in *Mycobacterium vanbaalenii* PYR-1 and characterize the PAH degradative genes using molecular techniques;
12) Novel Molecular Approaches for the Detection and Analysis of the Predominant Bacterial Species in the Human Gastrointestinal Tract – We will evaluate microarray-slides to detect 40 intestinal bacterial species from human fecal samples and determine its applicability to clinical samples;
13) Development of a microarray chip for the detection of multiple antibiotic resistance markers - We will correlate microarray results with disk diffusion, broth dilution, and PCR assays to analyze the signal intensity of microarrays with quantification software, and to characterize a vancomycin-resistant mechanism employing wild type, intermediate and high mutator strains of *Enterococcus faecium* ATCC 51559;
14) Characterization of Antimicrobial Drug Resistance Genes from *Lactococcus lactos* P1-79 – We will identify and isolate the genes for multiple antibiotic drug resistances in a *Lactococcus lactis* isolate from a competitive exclusion product; and
15) We will also continue to collaborate with investigators in the Division of Chemistry on the rapid identification of bacteria by mass spectrometry.

We hired four research microbiologists last year and they have developed protocols that will be initiated in FY 2004. Three of the projects will compliment existing studies on antimicrobial resistance. They are on the contributions of membrane-associated efflux systems to antibiotic resistance in *Lactobacillus*, molecular epidemiology and characterization of multiple antibiotic resistant *Salmonella* spp. isolated from the turkey production environment, and development of proteomic approaches to identify *Staphylococcus aureus* extracellular proteins responsible for causing pneumonia. The fourth project is molecular cloning and characterization of genes coding for tattoo degrading and permanent cosmetic pigment degrading enzymes from human skin microflora.

Public Health Significance

The FDA, various national and international committees, and the general public are concerned about the increased multiple antimicrobial resistance that has recently been found among pathogenic microorganisms. This may be due in part to the veterinary use of antimicrobials, which will potentially bring about a general increase in the numbers of antimicrobial-resistant bacteria in food animals and the environment and increased amounts of antimicrobials and their biotransformation products in meat, milk or egg products that could affect consumers via the intestinal microflora. The issue of microbial drug resistance has significance both to health and regulatory agencies. The FDA has expressed an interest in research that would determine whether antimicrobial resistance occurs in bacteria isolated from animal feeds containing antibiotics, the pattern of resistance development in bacteria found in animals fed antibiotics, and differences in survival rates of drug-resistant pathogens compared to nonresistant pathogens. Various antimicrobial drugs are currently approved for growth promotion in food animals by Canada, Mexico, Australia, New Zealand, and the European Union as well as the United States. Furthermore, the Division of Microbiology is involved in basic research for the advancement of biochemical and molecular technology for further understanding of the role of microbial communities in human health. It has taken a multidisciplinary approach to provide fundamental information to the FDA on antimicrobial resistance, environmental biotechnology, and food safety issues.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Cerniglia, Carl

- **Proteomic Approaches to Elucidate Biodegradative Pathways** — **E0711801** — **Method Driven Research**

  Objective(s):

  1) Use proteomic approach to isolate putative catabolic proteins that are over-expressed when microorganisms are grown in the presence of polycyclic aromatic hydrocarbons; and
  2) Develop software to analyze 2-D gels.

  Status: Started/Ongoing

PI: Chen, Huizhong

- **Molecular Cloning and Characterization of Genes Coding for Enzymatic Degradation of Tattoo and Permanent Cosmetic Pigments from Human Skin Microflora** — **E0717901** — **Predictivie Toxicology**

  Objective(s):

  The research will be focused predominately on human skin and intestinal microflora of genera *Staphylococci, Propionibacteria, Clostridia, and Enterococci*. The objectives of the projects are:

  1) Biodegradation and bioconversion of the tattoo, topically applied colorants, and permanent make-up pigments in the selected bacteria;
  2) Clone and over-express genes encoding for azoreductases and nitroreductases, which are able to decolorize the pigments, from the bacteria;
  3) Determine physicochemical properties of the purified native enzyme from the bacteria and the expressed recombinant enzymes cloned in *E. coli*;
  4) Elucidate the role of the microflora with potential genotoxic effects of tattoo and permanent make-up pigments; and
  5) Study effects of sunlight and tanning lights on skin microfloral populations.

  Status: Project Under Review

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Elkins, Christopher

- **Assessment of Membrane-Associated Antibiotic Resistance Mechanisms in *Lactobacilli*** — **E0718001** — **Predictivie Toxicology**

  Objective(s):

  1) Obtain *Lactobacillus* cultures from available commercial or private culture collections and test such cultures for multiple drug resistance;
  2) Compare these "intrinsic resistances" in species used routinely by the food industry to GI tract commensuals;
  3) Search current sequence databases and determine putative membrane efflux transporters in *lactobacilli* based on sequence similarity to functionally identified MDR proteins;
  4) Close and test such genes for MDR phenotype in a MDR-sensitive *E. coli*;
  5) Create genomic libraries of *lactobacilli* to determine the extent of "genetic dedication" to these activities by identifying genes associated with drug efflux; and
  6) Develop a microarray, if feasible, with genes identified in this study and test it with the culture collection to determine the contribution of efflux to the observed resistances in objective 1.

  Status: Project Under Review

PI: Erickson, Bruce

- **Determining the Effect of Low Levels of Antibiotic Residues on the Human Intestinal Microflora Using an *InVitro* Continuous Culture System** — **E0709201** — **Method Driven Research**

  Objective(s):

  Determine the concentration of selected fluoroquinolones that produce no adverse effect on the human intestinal microflora. Hypothesize that an *in vitro* chemostat culture system that mimics the human intestinal tract can be used to detect and characterize the effect of low-level antibiotic residues in food on the human intestinal microflora.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Hart, Mark

- **Development of Proteomic Approaches to Identify *Staphylococcal Aureus* Extracellular Proteins Responsible for *Staphylococcal* Pneumonia** — **E0717501** — **Knowledge Bases**

  Objective(s):

  1) Develop a new, more effective approach to prevent and treat *staphylococcal* pneumonia;
  2) Develop a proteomic approach of identifying proteins by first fractionating proteins in spent media using isoelectric focusing followed by nonporous, reverse phase HPLC. Proteins isolated in this manner will be submitted to protease degradation and peptide profiles will be generated using LC/MS/MS. Peptide profiles will be searched against the public NCBI protein database to identify the proteins; and
  3) Generate a proteomic profile for *S. aureus* RN6390 and its AGR and SAR isogenic mutants. These profiles will be compared to identify differences between strains and thus, preliminarily identify potential proteins responsible for the lethality observed in the mouse model of pneumonia.

  Status: Project Under Review

PI: Khan, Ashraf

- **Studies on Mechanism of Fluoroquinolone-Resistant *Salmonella* spp. Isolated from Animal Feeds (Poultry), Animal Production Environment and the Development of Molecular Methods for Screening the Drug Resistance Genes** — **E0704801** — **Method Driven Research**

  Objective(s):

  1) Isolation, identification and characterization of nalidixic acid and fluoroquinolone-resistant *Salmonella* spp. from chicken farms (animal feed, feces, manure, litters and animals) by biochemical and Polymerase Chain Reaction;
  2) Determination of minimum inhibitory concentration for environmental isolates, development of molecular techniques and its comparison with clinical strains;
  3) Determination of drug-resistance mechanisms in the environmental isolates and their characterization by molecular techniques; and
  4) Determination of influence of seasons and the frequency of isolation of fluoroquinolone-resistant *Salmonella* spp.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Khan, Saeed

- **Molecular Screening Methods for the Determination of Vancomycin-Resistance in Selective Competitive Exclusion Product CF3 (PREEMPT) Bacteria** — **E0705301** — **Method Driven Research**

  Objective(s):

  1) Isolation, identification and biochemical characterization of vancomycin-resistant bacteria present in a commercially available competitive exclusion product CF3;
  2) Development of a rapid PCR method of the detection of vancomycin-resistance determinant genes, namely, the Van A0, Van B, Van C and D-ala-D-lac ligase gene Ddl.;
  3) Characterization of plasmid DNA Profile and plasmid-mediated drug resistance transfer;
  4) Genetic fingerprinting of the vancomycin-resistant microorgnasims present in PREEMPT culture; and
  5) Nucleotide sequence analysis of the PCR products of vancomycin-resistant determinant genes showing interesting restriction profiles.

  Status: Started/Ongoing

- **Development of a Microarray Chip for the Detection of Multiple Antibiotic-Resistance Markers** — **E0715101** — **Method Driven Research**

  Objective(s):

  Develop a microarray-based method for the detection of 150 genes associated with 22 antibiotics; some of which are used to promote growth in poultry and animal farming while others are used to treat infections in both humans and animals. The data generated by the use of the chip in monitoring and tracking the spread of resistance markers may be helpful for the FDA in making regulatory decisions that require banning and/or approving the use of certain antibiotics in poultry and farm animals.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Kurniasih, Dedeh

- **Characterization of Antimicrobial Drug-Resistance Genes from *Lactococcus lactis* P1-79** — **E0716201** — **Knowledge Bases**

  Objective(s):

  1) Determine whether the antimicrobial-resistance genes are encoded on the bacterial chromosome or on episomes;
  2) Screen for the presence of common resistance genes;
  3) Clone the resistance genes in *E. coli* and evaluate their DNA sequence; and
  4) Evaluate the potential for *L. lactis* P1-79 to transfer antimicrobial-resistance genes to *Enterococcus faecium* or *Staphylococcus aureus*.

  Status: Started/Ongoing

PI: Nawaz, Mohamed

- **Studies on the Fluoroquinolone-Resistance in *Campylobacter* sp. Isolated from Poultry** — **E0705001** — **Method Driven Research**

  Objective(s):

  1) Isolation and identification of fluoroquinolone-resistant *Campylobacter jejuni* and *C. coli* from water, feed and litter samples in poultry houses;
  2) Determination of the optimum concentration of nalidixic acid and fluoroquinolone resistance in *C. jejuni* and *C. coli*;
  3) Determination of the influence of various seasons and the frequency of isolation of fluoroquinolone-resistant *C. jejuni* and *C. coli*; and
  4) Molecular characterization of fluoroquinolone-resistance by polymerase chain reaction (PCR), nucleotide sequencing and single-strand conformation polymorphism (SSCP).

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **The Fate and Degradation of Antimicrobials, Oxytetracycline (OTC) and Sulfadimethoxine-Ormetoprim (Romet-30) from Aquaculture Environmental Samples** — **E0707501** — **Method Driven Research**

  Objective(s):

  1) Determine the biodegradation rates and metabolic fate of antimicrobials, oxytetracycline and sulfadimethoxine-ormetrorpim (Romet-30) (SDO), used in fish farming systems;
  2) Isolate, characterize and identify OTC- and SDO-resistant organisms from aquaculture sediment and natural environment samples and conduct molecular characterization of the genes that regulate resistance to the drugs.

  Status: Started/Ongoing

PI: Nayak, Rajesh

- **Molecular Epidemiology and Characterization of Multiple Antibiotic-Resistant *Salmonella* Isolated from Turkey Production Environment** — **E0717301** — **Knowledge Bases**

  Objective(s):

  1) Determine the preharvest sources and/or vectors of horizontal transmission S*almonella* in turkey flocks;
  2) Evaluate the intrinsic resistances of *Salmonella* isolates to multiple antibiotics;
  3) Assess the genetic diversity and epidemiological profiles of *Salmonella* strains isolated in a turkey production environment; and
  4) Develop DNA-based and microarray assays to detect genes in *Salmonella* isolates that are involved in antibiotic-resistance and pathogenicity.

  Status: Project Under Review

- **Animal Husbandry Breeding Support** — **E0002200** — **Center Support (Research)**

  Objective(s):

  Microbiological evaluation of animals and non-animal samples not specifically designated to an ongoing experiment.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Conventional Mice Breeding** **E0010900** **Center Support (Research)**

  Objective(s):

  Determine health status of mice breeding colonies maintained under conventional conditions.

  Status: Started/Ongoing

- **SPF Rat Breeding Colony** **E0011000** **Center Support (Research)**

  Objective(s):

  Determine health status of rats breeding colonies maintained under specified pathogen free conditions.

  Status: Started/Ongoing

- **Conventional Rat Breeding Colony** **E0011100** **Center Support (Research)**

  Objective(s):

  Determine health status of rats breeding colonies maintained under conventional conditions.

  Status: Started/Ongoing

- **Conventional Guinea Pigs Breeding Colony** **E0011200** **Center Support (Research)**

  Objective(s):

  Determine health status of guinea pigs colonies maintained under conventional conditions.

  Status: Started/Ongoing

- **Quarantine Animals** **E0011300** **Center Support (Research)**

  Objective(s):

  Determine health status of animals received at NCTR and held under quarantine condtions.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Diet Prep General Support** — **E0014500** — **Center Support (Research)**

  Objective(s):

  Determine the microbial contamination level in dosed- or control-feed and water lots prepared for animal use but not designated to a specific ongoing experiment.

  Status: Started/Ongoing

- **Primate Colony Surveillance** — **E0023500** — **Center Support (Research)**

  Objective(s):

  Determine the health status of the primate colonies maintained at NCTR.

  Status: Started/Ongoing

- **Microbiological Diagnostic Methods: Development, Testing, & Evaluation** — **E0026200** — **Method Driven Research**

  Objective(s):

  Improve diagnostic and epidemiological capabilities in bacteriology, parasitology, mycology, virology and serology as applicable to NCTR programs and projects.

  Status: Started/Ongoing

- **General Microbiological Support - Bacteriology, Parasitology, Mycology & Virology** — **S00006** — **Center Support (Research)**

  Objective(s):

  Determine health status of animal colony and their environment.

  Status: Started/Ongoing

- **Microbiology Division - Media Preparation** — **S00064** — **Center Support (Research)**

  Objective(s):

  Provide media and reagent preparation to both research and surveillance/diagnostic needs.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

◆ **Special Epidemiology Investigations of Potential Microbiological Contamination Problems** — **S00185** — **Center Support (Research)**

Objective(s):

1) Investigate potential microbiological contamination problems; and
2) Report nonroutine sample time which is not recorded on Sample Collection Report (SCR).

Status: Started/Ongoing

PI: Rafii, Fatemeh

◆ **Importance of Human Intestinal Microflora in Conversion of Phytoestrogens to Estrogenic Compounds** — **E0700701** — **Concept Driven Research**

Objective(s):

1) Detect various metabolites of phytoestrogens, produced by the metabolism of these compounds by pure culture of bacteria typical of that isolated from human microflora, and elucidation of the metabolic pathways of phytoestrogens by human intestinal bacteria;
2) Assess the estrogenic effect of each phytoestrogen metabolite produced by intestinal bacteria;
3) Determine the bacterial species producing estrogenic metabolites from phytoestrogens and elucidation of enzymes involved in various steps of these metabolic processes; and
4) Evaluate the effects of phytoestrogens and their metabolites on the population, composition, metabolic activity and enzyme production of bacteria from the human gastrointestinal tract.

Status: Started/Ongoing

◆ **Elucidation of the Mechanism of Resistance Development in Anaerobic Bacteria from the Human Intestinal Tract** — **E0709301** — **Knowledge Bases**

Objective(s):

The aim of this study is the evaluation of the effect of fluoroquinolones on the resistance development in the bacteria from the human intestinal tract and analysis of the fluoroquinolone resistance mechanism in anaerobic bacteria from the human intestinal tract.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Sutherland, John

- **Biotransformation of Fluoroquinolones by Fungi** — **E0705201** — **Method Driven Research**

Objective(s):

1) Measure the kinetics of biodegradation of veterinary fluoroquinolone drugs in natural matrices;
2) Identify the potential metabolites produced by fungi from fluoroquinolones; and
3) Assess the residual antibacterial activity and potential risks of the metabolites formed from these drugs.

Status: Started/Ongoing

PI: Wagner, Robert

- ***In Vitro* Model and Molecular Analysis of Competitive Exclusion Products** — **E0704901** — **Method Driven Research**

Objective(s):

1) Evaluate individual component bacteria in a defined competitive exclusion (CE) product for exclusion of enteric pathogens from Caco-2 and CRL-2117 cell monolayers;
2) Define the antimicrobial susceptibility patterns of the component bacteria using Minimal Inhibitory concentration measurements;
3) Sequence analysis of 16s rRNA Polymerase Chain Reaction (PCR) products from defined culture component bacteria and development of a database containing the sequences for use in subsequent identification of the organisms in undefined CE products; and
4) Application of the 16s rRNA sequence analysis procedure to detect and identify effective CE component bacteria in undefined CE products.

Status: Started/Ongoing

- **Measurement of Antimicrobial Drug Concentrations that Inhibit Colonization Resistance** — **E0708601** — **Method Driven Research**

Objective(s):

An enterocyte culture model of colonization resistance by enteric microbial flora against *Salmonella* spp. colonization/invasion will be adapted to measure concentrations of antimicrobial drugs as food residues that would inhibit the barrier effect of the consumer's intestinal flora.

Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

◆ **Probiotic Effects on Host Defense Against Enteric Pathogens** — **E0709701** — **Knowledge Bases**

Objective(s):

1) Establish a model intestinal bacteria population in mice that consists of human intestine-derived bacteria;
2) Observe the fate of members of the model bacterial population when probiotic bacteria are fed to the mice;
3) Observe the fate of the probiotic bacteria fed to the human flora-associated mice;
4) Observe the effects of the human-derived flora on the host protective systems of immunodeficient and immunocompetent mice;
5) Observe effects of adding probiotic bacteria to HFA mouse on immunodeficient and immunocompetent host protective systems; and
6) Observe the roles of model host flora and probiotic bacteria to modulate host protective systems of immunodeficient and immunocompetent mice from *Salmonella typhimurium* and *Campylobacter jejuni*.

Status: Started/Ongoing

PI: Wang, Rongfu

◆ **Novel Molecular Approaches for the Detection and Analysis of the Predominant Bacterial Species in the Human Gastrointestinal Tract** — **E0711901** — **Method Driven Research**

Objective(s):

1) Develop a rapid method for quantification of intestinal bacteria;
2) Qualitative analysis of the communities for several major genera and discovering the species which are noncultivated;
3) Isolation and identification of the bacterial species from probiotics used for human or animal health; and
4) Develop microarray method for the detection of intestinal bacteria.

Status: Started/Ongoing

Publications

Cho, B.P., Yang, T., Blankenship**,** L.R., Moody, J.D., Churchwell, M., Beland, F.A., and Culp, S.J. 2003. Synthesis and Characterization of *N*-Demethylated Metabolites Of Malachite Green and Leucomalachite Green, *Chemical Research in Toxicology*. 16:285-294. Accepted 1/2/2003

Elkins, C., Savage, D.C., Cbst2 From Lactobacillus Johnsonii 100-100 is a Transport Protein of the Major Facilitator Superfamily that Facilitates Bile Acid Antipot., *Journal of Molecular Microbiology and Biotechnology*. Accepted: 9/5/2003 (E0718001)

Kim, Y., Kim, S., Cerniglia, C.E. and Heinze, T.M., Adsorption And Clay-Catalyzed Degradation of Erythromycin A on Homoionic Clays, *Journal of Enviornmental Quality*. Accepted: 6/23/2003 (E0690101)

Kim, Y.-H., Engesser, K-H., and Cerniglia, C.E. 2003. Two Polycyclic Aromatic Hydrocarbon *O*-Quinone Reductases from a Pyrene-Degrading *Mycobacterium. Archives of Biochemistry and Biophysics* 416**:**209-217. Accepted: 08/06/2003.

Kim, Y., Pak, K., Pothuluri, J.V. and Cerniglia, C.E., Desorption and Degradation of Erythromycin a in Aquaculture Sediments, *Aquaculture.* Accepted: 6/1/2003 (E0690101)

Mattia, A., Cerniglia, C.E., Safety Evaluation of Certain Food Additives and Contaminants: Natamycin, *WHO Food Additives*, Geneva, Series 48:49-76. Accepted: 11/15/2002 (N/A)

Moody, J.D., Fu, P.P., Freeman, J.P. and Cerniglia, C.E., Regio- and Stereoselective Metabolism of 7,12-Dimethylbenz[A]Anthracene By Mycobacterium Vanbaalenii PYR-1, *Applied and Environmental Microbiology*, 69:3924-3931. Accepted: 4/1/2003 (E0707501)

Nawaz, M.S., Wang, R., Khan, S.A. and Khan, A.A., Detection of Gale Gene by Polymerase Chain Reaction in Campylobacters, *Molecular Cellular Probes.* Accepted: 8/16/2003 (E0705101)

Rafii, F., Heinze, T.M., Beger, R., Park, M. and Davis, C.L., Variation in Metabolism of the Soy Isoflavonoid Daidzein by Human Intestinal Microflora from Different Individuals, *Archives of Microbiology*, 180:11-16. Accepted: 4/14/2003 (E0700701)

Rafii, F., Wynne, R.A., Heinze, T.M. and Paine, D.D., Mechanism of Metronidazole-Resistance in Isolates of Nitroreductase-Producing *Enterococcus Gallinarum* and *E. Casseliflavus* from the Human Intestinal Tract, *FEMS Microbiology Letters*, 225:195-200. Accepted: 6/1/2003 (E0709301)

Roberts, G., Cerniglia, C.E., Toxicological Evaluation of Certain Veterinary Drug Residues in Food, *WHO Food Additives, Series 49*, 49:29-62. Accepted: 11/1/2002 (N/A)

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Shahverdi, A.R., and Rafii, F., Inhibition of Nitrofuratoin Reduction by Menthol Leads to Enhanced Antimicrobial Activity, *Journal of Chemotherapy.* Accepted: 5/5/2003 (E0709301)

Wagner, R.D., Paine, D.D., and Cerniglia, C.E., Phenotypic and Genotypic Characterization of Competitive Exclusion Products, J. *Applied Microbiology*. 94:1098-1107. Accepted 02/21/2003 (E0704901)

Concept Papers

Title	Project Number	Strategic Research Goal

PI: Wang, Rongfu

- **Development of Molecular Methods Including Oligo-Microarray Methods for the Detection and Monitoring of Foodborne Pathogenic Bacteria** — **E0715401** — **Method Driven Research**

  Objective(s):

  1) Development of an oligo-microarray method for the detection of foodborne pathogens based on 16S rDNA sequences;
  2) Development of oligo-microarray methods with multiplexed PCR based on many genes for the detection and genotyping of specific pathogenic bacterial strains; and
  3) Development and modification of PCR methods for the detection of all above pathogens.

  Status: Approved Concept Paper

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Molecular Epidemiology

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Researchers utilizing Transgenomics Wave System for SNP Discovery

Executive Summary

Introduction

The strategic goals of the Division of Molecular Epidemiology are: 1) the identification of genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy; 2) the conduct of epidemiological studies for post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices; 3) human exposure biomonitoring and DNA adduct detection; and 4) the operation of a "Structural Genomics Center" for discovery of single nucleotide polymorphisms (SNPs) and its application to human diagnostics.

FY 2003 Accomplishments

The intent is to better understand the mechanisms of human carcinogenesis; to provide an estimation of human exposure to direct and indirect-acting carcinogens; to assess the importance of interindividual differences in carcinogen and drug bioactivation, detoxification, or induced changes in gene expression; and to suggest intervention strategies for human cancer prevention. Accordingly, our research has provided new knowledge on the identification of subpopulations that are not only more susceptible to chemical carcinogens, but also those that are likely to experience adverse drug reactions or decreased therapeutic drug efficacy. Our research has been focused on the foodborne heterocyclic amines, environmental aromatic amines and polycyclic aromatic hydrocarbons, on widely used drugs, as well as on tobacco usage. Projects on the etiology of human cancers of the colon/rectum, pancreas, esophagus, breast, prostate, lung, and bone marrow are ongoing. These are outlined as follows:

Studies to identify genetic polymorphisms that influence drug, hormone and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy:

Metabolic Polymorphisms and Individual Cancer Susceptibility

a) Polymorphisms of cytochromes P450 1B1 and 3A4, tissue-dependent expression, and hormone metabolism.
b) Polymorphisms of sulfotransferases.
c) Polymorphisms of glutathione S-transferases.
d) Gender-specific variation in drug metabolism.

Chemoprevention

a) Modulation of gene expression by chemopreventive agents and identification of gene targets as surrogate biomarkers of effect (e.g., *COX-2, mdr-2, nFkB, DIA4, MnSOD, ras)*
b) DNA methylation, DNA methyltransferases, methyl donors and cancer risk.

Epidemiology and Post-Market Surveillance for Chemical Toxicants Found in Foods, Drugs, Cosmetics and Medical Devices

a) Etiology of human breast and prostate cancers in African-Americans and Caucasians.
b) Etiology of human pancreatic cancer: role of carcinogen & drug exposures, chronic pancreatitis, and dietary imbalance.

Human Exposure Biomonitoring and DNA Adduct Detection

Biomarkers of exposure and susceptibility to breast, prostate, and esophageal cancers.

a) DNA adduct detection in breast epithelial cells in relation to cancer risk and hair dye use.
b) DNA adduct detection in prostate and esophageal tissue in relation to dietary heterocyclic amine exposure.

Structural Genomics Center

a) In an effort to develop a genome haplotype map (hapmap) for prostate cancer and esophageal cancer susceptibility, 80 polymorphisms of drug/carcinogen metabolism and DNA repair enzymes were genotyped and hapmap construction is underway.
b) Genotyping of 7 polymorphisms of DNA repair enzymes in uniplex and multiplex in a 500-person esophageal cancer case-control study as a showcase for new technology and throughput.
c) Detection of aberrant tumor DNA in plasma of patients with prostate and breast cancer as biomarkers of cancer detection.
d) Detection of aberrant DNA in needle biopsies of patients with prostate cancer as markers of cancer diagnosis and early detection.

Impact on Public Health

Applications utilizing gene diversity can be harvested to define disease susceptibility, predict adverse events and to detect cancer. The ongoing initiatives at the Division of Molecular Epidemiology are targeted to reduce the burden of cancer and to contribute to the regulatory mission of the FDA.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Chen, Junjian

- **Somatic Alterations in Prostate Cancer and Its Precursor Lesions** — **E0711301** — **Concept Driven Research**

Objective(s):

1) Test the hypothesis that homoplasmic mutations in the mitochondrial genome are elevated in human prostate carcinomas as a consequence of increased oxidative stress;
2) Test the hypothesis that at least some of the homoplasmic mtDNA mutations detected in prostate carcinomas are also detectable in evolutionarily related precursor lesions identified in the same prostate biopsies;
3) Test the hypothesis that the incidence and type of homoplasmic mtDNA mutations in benign prostatic hyperplasia differ from those in prostate carcinomas; and
4) Test the hypothesis that homoplasmic mtDNA mutations are more sensitive than nuclear markers in delineation of clonal evolution of prostate cancers.

Status: Started/Ongoing

PI: Coles, Brian

- **Dietary Isothiocyanates, Glutathioine S-Transferases, and Colorectal Neoplasia** — **E0320001** — **Concept Driven Research**

Objective(s):

Explore the relationship between dietary isothiocyanates, glutathione S-transferase induction and colon polyp recurrence. NCTR's direct objective is to quantitate glutathione S-transferases in human plasma.

Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **ADDEND: Project III: Environmental and Genetic Epidemiology of Colorectal Adenomas** — **E0320011** — **Concept Driven Research**

  Objective(s):

  1) Incorporate Project III with respect to the analyses to be performed by NCTR. These analyses are glutathione S-transferase genotype and phenotype as specified in the master project E0320001, plus MTHFR genotype;
  2) Extend the lifetime of E0320001 to five years to incorporate lifetime of Project III; and
  3) Extend the lifetime of the associated CRADA, 300-00-0045, from two to five years to incorporate lifetime of Project III.

  Status: Started/Ongoing

PI: Hammons, George

- **Methylation Profile, Gene Expression, and Enzyme Activity of CYP1A2 in Human Livers** — **E0696201** — **Predictive Toxicology**

  Objective(s):

  This protocol will serve as a preliminary study to determine the possible involvement of epigenetic mechanisms in the regulation of the expression of the *CYP1A2* gene. The methylation status determined for each sample will be correlated with the expression of the *CYP1A2* gene and enzyme activity.

  Status: Completed on 11/4/2002

PI: Kadlubar, Fred

- **A Case-Control Study of Pancreatic Cancer & Aromatic Amines** — **E0694601** — **Predictive Toxicology**

  Objective(s):

  Measure the associations of aromatic amine exposure and metabolism with the risk of pancreatic cancer. The sources of aromatic and heterocyclic amines to be studied are cigarette smoking and diet; the metabolic capabilities to be studied are acetylator status and N-oxidation status.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Role of Acetylation & N-Oxidation in Colorectal Cancer** — **E0694701** — **Predictive Toxicology**

  Objective(s):

  Confirm the initial findings of our pilot study regarding the roles of heterocyclic amine metabolism and exposure as putative risk factors from the diet or the environment. The sources of heterocyclic amines to be studied are cigarette smoking, diet and cooking methods; the metabolic pathways to be studied include heterocyclic amine N-oxidation status and O-acetylation status.

  Status: Started/Ongoing

- **Chemical Carcinogenesis: Epithelial Cells in Breast Milk** — **E0697801** — **Predictive Toxicology**

  Objective(s):

  1) Develop and refine a methodology for separation of luminal epithelial cells from human breast milk for DNA extraction;
  2) Detect and quantify aromatic/hydrophobic-DNA adducts in luminal epithelial cells derived from human breast milk;
  3) Detect genetic polymorphisms in carcinogen-metabolizing genes derived from DNA extracted from epithelial cells in human breast milk; and
  4) Evaluate the relationships between carcinogen-DNA adducts and smoking status, and adduct levels with polymorphisms in *NAT1, NAT2, CYP1A1*, and *GSTM1*.

  Status: Started/Ongoing

- **ADDEND: Chemical Carcinogenesis: Epithelial Cells in Breast Milk** — **E0697811** — **Predictive Toxicology**

  Objective(s):

  1) Measure the levels of aromatic amines in human breast milk;
  2) Evaluate the mutagenicity of human milk and milk fat in an Ames *Salmonella* test highly sensitive to aromatic amines; and
  3) Evaluate relationships between aromatic amines in milk, mutagenicity, and carcinogen-DNA adduct levels in ductal epithelial cells with exposure and susceptibility factors.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Breast Cancer in African-American Women: Metabolic Modification of Dietary and Hormonal Risk Factors** — **E0701501** — **Method Driven Research**

  Objective(s):

  Examine the role of interindividual variability in response to exogenous agents as it may relate to breast cancer risk in African-American women. By evaluating risk associated with exposure to oral contraceptives, hormone replacement therapy, and modification of that risk by genetic variability in their metabolism, the effects of substances regulated by the FDA on breast cancer risk in African-American women may be further elucidated. Additionally, a successful model to increase African-American participation in research studies would greatly assist in future studies related to FDA-regulated substances in African-American populations.

  Status: Started/Ongoing

- **ADDEND: The Role of Glutathione S-Transferase Genetic Polymorphisms in Breast Cancer Sensitivity to Radio- and Chemotherapy** — **E0701511** — **Predictive Toxicology**

  Objective(s):

  1) Determine expression of enzymes (phenotype) in tumor tissue from women who received adjuvant therapy for breast cancer, using biopsy or surgical tissue specimens, using immunohistochemistry, and evaluate associations between phenotypes in tumor tissue and risk of breast cancer recurrence;
  2) Determine inherited *GSTM1, GSTT1* and *GSTP1* genotypes in normal tissue from these same women, and determine associations of *GSTM1, GSTT1* and *GSTP1* genotype with phenotype in tumor tissue; and
  3) Evaluate if GST genotypes predict breast cancer recurrence following treatment, controlling for other factors that may relate to prognosis.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Lyn-Cook, Beverly

- **The Effects of Nicotine and Other Cigarette Components on Normal and Neoplastic Human Pancreatic Cells: The Role of Low Zinc Levels on *Ras*, *Mdr-1* Genes Activation and Metabolizing Enzyme Activities as a Possible Risk Factor for Pancreatic Cancer** — **E0701701** — **Predictive Toxicology**

  Objective(s):

  1) Determine the effects of nicotine and other cigarette components on exocrine and endocrine human pancreatic cells *in vitro*; and
  2) Examine *ras, mdr-1*, *CYP1A1* and *CYP1A2* expression in normal and neoplastic human pancreatic tissue grouped according to race and sex obtained from a human tissue bank.

  Status: Started/Ongoing

- **Mechanistic Actions of Chemopreventive Agents in Pancreatic Cancer** — **E0707601** — **Predictive Toxicology**

  Objective(s):

  Screen a number of agents found in natural products and establish mechanistic data on their potential as anticancer agents against pancreatic cancer.

  Status: Started/Ongoing

PI: McClure, Gail

- ***In Vivo* Modeling of Steroid-Mediated Gender Effects in Drug Metabolism** — **E0704301** — **Predictive Toxicology**

  Objective(s):

  1) Characterize the activity of *CYP1A2* in female subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive usage, menopause, and HRT;
  2) Characterize the activity of *CYP1A2* in male subjects with regard to age;
  3) Measure estradiol, progesterone, testosterone, cortisol, Il-1, IL-6 and IL-10 levels in female and male subjects studied for *CYP1A2* activity;
  4) Correlate the activity of *CYP1A2* with circulating levels of cytokines and/or circulating levels of steroid hormones; and
  5) Statistically assess the impact of each of the measured variables on the *CYP1A2* phenotype.

  Status: Completed on 10/29/2002

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **ADDEND: Part II of Somatic Alterations in Prostate Cancer and Its Precursor Lesions** — **E0704311** — **Predictive Toxicology**

  Objective(s):

  1) Determine the activity of *CYP2D6* and 3A4 in female and male subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive usage, menopause, and HRT;
  2) Measure estradiol, progesterone, testosterone, cortisol, IL-1, IL-6 and IL-10 levels in female and male subjects studied for CYP activity;
  3) Correlate the activity of *CYP2D6* and 3A4 with circulating levels of cytokines and/or circulating levels of steroid hormones; and
  4) Statistically assess the impact of each of the measured variables on the *CYP2D6* phenotype and *CYP3A4* activity level.

  Status: Completed on 10/29/2002

- **Chemical Carcinogens: DNA-Adducts in Breast Epithelial Cells** — **E0714801** — **Predictive Toxicology**

  Objective(s):

  1) Develop and refine methodology for the separation of luminal epithelial cells from samples obtained from the ductal lavage procedure for use in DNA extraction;
  2) Characterize DNA adducts in breast tissue from women at high risk for breast cancer undergoing ductal lavage to identify dominant mutagenic agents;
  3) Characterize the most common types of recent exogenous carcinogen exposure in high-risk patients receiving ductal lavage;
  4) Evaluate variability in metabolism and susceptibility to carcinogen exposure, as measured by phenotypic and genotypic variability in carcinogen-metabolizing enzymes, and to evaluate the interaction of these factors with the exposure data obtained in Objective 2;
  5) Obtain DNA adduct profiles from ductal lavage samples in women at normal risk for comparison with high-risk women; and
  6) Compare DNA adduct profiles with respect to exposure levels and genotypic and phenotypic variability in high risk and normal risk women.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Nowell, Susan

- **Sulfotransferase 1A1 (SULT1A1) Genotype and Phenotype in Relation to Efficacy of Tamoxifen Treatment** — **E0714401** — **Predictive Toxicology**

  Objective(s):

  1) Determine whether induction of SULT1A by 4-OH TAM results in an increase in expressed protein and enzymatic activity toward environmental estrogens in tamoxifen treated breast cancer patients;
  2) Determine the effect of 4-OH TAM on SULT1A1 activity in breast cancer cell lines;
  3) Determine SULT1A1 Genotype in Tamoxifen Treated Women and Genotype-Phenotype Correlations; and
  4) Archiving of blood samples, administration of the Block 98 Questionnaire, and determining survival data for future studies.

  Status: Started/Ongoing

PI: Poirier, Lionel

- **Colorectal Adenoma Study - Task 1** — **E0707101** — **Predictive Toxicology**

  Objective(s):

  Provide analytical support for the analysis of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in red blood cell specimens for an intramural study being conducted by the Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Ratnasinghe, Luke

- **Prostate Cancer: Exposure, Susceptibility and DNA Adducts** — **E0702101** — **Method Driven Research**

Objective(s):

1) Determine levels of carcinogen exposure in African-Americans and Caucasians with histologically confirmed prostate cancer using a case-control design;
2) Evaluate variability in hormone metabolism and susceptibility to carcinogen exposure, as measured by phenotypic and genotypic variability in carcinogen metabolism, and evaluate the interaction of these factors with the exposure data obtained in Specific Aim 1; and
3) Characterize DNA adducts in prostate tissue from men with prostate cancer to identify mutagenic agents and evaluate levels of adducts in relation to carcinogen exposure data and susceptibility factors obtained in Specific Aims 1 and 2.

Status: Started/Ongoing

PI: Tang, Yong

- **The Role of Human Cytochrome *CYP1B1* in Drug Metabolism and Carcinogenesis** — **E0699001** — **Predictive Toxicology**

Objective(s):

To elucidate the role of human cytochrome P450 IB1 (*CYP1B1*) in drug metabolism and carcinogenesis. Specific aims are:

1) Design and develop peptide-specific antibodies against human *CYP1B1*;
2) Determine the levels of *CYP1B1* protein in various human tissues;
3) Evaluate *CYP1B1* expression as a biomarker for tumorigenesis;
4) Identify *CYP1B1* inducers among the most common drugs and carcinogens;
5) Identify *CYP1B1* substrates, including the endogenous steroid hormones, as well as drugs and carcinogens known to be metabolized by the closely related cytochromes P450 IA1 and IA2;
6) Find specific enzyme inhibitors for *CYP1B1*;
7) Develop a sensitive, convenient, and specific assay method for *CYP1B1* enzyme activity *in vitro*; and
8) Evaluate genetic polymorphism(s) for *CYP1B1* as an epidemiological marker for cancer risk.

Status: Completed on 10/16/2002

Title	Project Number	Strategic Research Goal

◆ **ADDEND: The Role of Human Cytochrome *CYP1B1* in Drug Metabolism and Carcinogenesis** — **E0699011** — **Predictive Toxicology**

Objective(s):

Request to add in situ hybridization as an additional approach to investigate the expression of *CYP1B1* in various human tissues. This will be performed in addition to the immunohistochemistry of the protocol. Request for inclusion of Pathology support in the performance of these studies.

Status: Completed on 10/16/2002

PI: Wise, Carolyn

◆ **Methylation Status and Cancer Risk** — **E0704601** — **Predictive Toxicology**

Objective(s):

Determine whether methylation status, determined by noninvasive procedures, may be a biomarker of cancer risk in humans. The methylation status will be assessed by measurement of SAM, SAH and homocysteine in blood, and of DNA hypomethylation in lymphocytes. Two-thirds of the work will be supported under the terms of an IAG from NCI.

Status: Started/Ongoing

Publications

Chen, J., Kadlubar, F.F., A New Clue to Glaucoma Pathogenesis, *American Journal of Medicine*, 114:697-698. Accepted: 4/30/2003 (E0711301)

Chen, J., Lang, N.P. and Kadlubar, F.F., Extensive Somatic Mitochondrial Mutations in Primary Prostate Cancer: A Laser Capture Microdissection Based Approach, *Cancer Research: Advance Brief*, 62:6470-6474. Accepted: 10/1/2002 (E0711301)

Guozheng, G., Yan, Y., Oberley, L., Ogi, J., Lyn-Cook, B.A., Khaletskiy, A., Weydert, C., Longmate, J.A., Spitz, D.R. and Li, J.J., Radiation-Inducible Genes are Modulated by Manganese-Containing Superoxide Dismutase, J *Molecular and Cellular Biology*, 7 Accepted: 3/1/2003 (NA)

Kadlubar, F.F., Kimura, S., Kawabe, M., Hammons, G., Carcinogenesis of the Food Mutagen, Phip, in Mice is Independent of CYP1A2, *Carcinogenesis*, 24:583-587. Accepted: 11/11/2002 (N/A)

Ramirez, L., Wise, C.K., Garcia-Montalvo, V., Poirier, L.A., Ostrosky-Wegman, P., Herrera, L.A. and Cea-Olivares, R., Effects of S-adenosyl-L-methionine on the Micronucleus Frequency Induced by Sodium Arsenite in Cultured Human Lymphocytes, *Mutation Research*, 528:61-67. Accepted: 4/10/2003 (NA)

Ratnasinghe, L., Tangrea, J.A., Barrett, M.J. and Albanes, D., Polymorphisms in th DNA Repair Genes XPD, XRCC1, XRcc3 and APE/ref-1 and the Risk of Lung Cancer Among Male Smokers in Finland, *Cancer Letters*, 191(2):171-178. Accepted: 2/4/2003 (NA)

Ratnasinghe, L., Yao, S., Qiao, Y., Anderson, M.R., Barrett, M.J., Giffen, C.A., Erozan, Y., Tockman, M.S. and Taylor, P.R., Gene-environment Interactions Between Codon 194 Polymorphisms of XRCC1 and Antioxidants Influence Lung Cancer Risk, *Anti-Cancer Research*, 23:627-632. Accepted: 12/4/2002 (NA)

Santella, R.M., and Lin, D., Evaluation of 4-aminobiphenyl-DNA Adducts in Human Breast Cancer: The Influence of Tobacco Smoke, *Carcinogenesis*, 24:719-725. Accepted: 12/1/2002 (NA)

Sweeney, C., Ambrosone, C.B., Stone, A., Kadlubar, F.F. and Coles, B.F., Association Between a Glutathine-S-transferase A1 Promoter Polymorphism and Survival after Breast Cancer Treatment, *International Journal of Cancer*, 103:810-814. Accepted: 12/5/2002 (E0701521)

Turesky, R., Freeman, J.P., Holland, R.D., Nestorick, D.M., Miller, D.W., Kadlubar, F.F. and Ratnasinghe, L., Identification of Aminobiphenyl Derivatives in Commercial Hair Dyes, *Chemical Research in Toxicology*, 16:1162-1173. Accepted: 6/27/2003 (E0714801)

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Wiese, F.W., Thompson-carino, P., Albers, D.S. and Kadlubar, F.F., Variation in COX Expression Levels Within the Colorectum, *Molecular Carcinogenesis*, 37:25-31. Accepted: 11/15/2002 (NA)

Xia, Q., Chou, M.W., Kadlubar, F.F., Chan, P.C. and Fu, P.P., Human Liver Microsomal Metabolism and DNA Adduct Formation of the Tumorigenic Pyrrolizidine Alkaloid, Riddelline, *Chem. Res. Toxicol.*, 16:66-73. Accepted: 10/30/2002 (E0710401)

Title	Project Number	Strategic Research Goal
PI: Chen, Junjian		
◆ **CONCEPT - Molecular Profiling of Metabolic and Repair Capacities of Breast Cancer Patients by Pathway-Based Tissue cDNA Macroarray** **Objective(s):** **Status:** Approved Concept Paper	**E0712301**	**Predictive Toxicology**
PI: Coles, Brian		
◆ **CONCEPT - Genetic Variation as a Factor in Asthmatic Response to Environmental Tobacco Smoke in Children** **Objective(s):** Explore the impact of specific genetic polymorphisms of asthma incidence of severity using the population of children 3-5 years of age in the Arkansas Head Start Program of Pulaski County, AR. **Status:** Approved Concept Paper	**E0717001**	**Concept-Driven Research**
PI: Hammons, George		
◆ **CONCEPT - Assessment of Interindividual Variability in Expression of DNA Methyltransferases, DNMT 3a and DNMT 3b, in Liver and Identification of Factors Influencing Expressions** **Objective(s):** 1) Determine levels of expression of DNMT 3a and DNMT 3b in liver samples from a pool of donors selected according to smoking status, gender, and age; and 2) Provide mechanistic data on the regulation of expression of DNMT 3a and DNMT 3b. **Status:** Approved Concept Paper	**E0716701**	**Predictive Toxicology**

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Kadlubar, Fred

- **Dietary Heterocyclic Amines, DNA Adducts and Polymorphic Variants in Metabolizing Enzymes** — **C30032** — **Predictive Toxicology**

  Objective(s):

  1) Determine if dietary intake of HCAs by humans produces DNA adducts in the esophagus and stomach;
  2) Determine if the level of DNA adduct formation is affected by the amount of HCAs in the diet, either the "usual" level or the amount eaten 24 hours before biopsy collection;
  3) Determine if the level of DNA adduct formation is affected by polymorphic ariants in key activation, detoxification or DNA repair enzymes; and
  4) Determine if there is gender-specific variation in DNA adduct production.

  Status: Pending Concept Paper

- **CONCEPT - The Influence of Preeclampsia on Hormonal and Anthropometric Status in Boys and Girls at 10 and 13 Years of Age: Follow-up of the Stavanger Population-Based Nested Case-Control Study** — **E0719101** — **Predictive Toxicology**

  Objective(s):

  Among women previously diagnosed with preeclampsia and their offspring, and two sets of normotensive controls and their offspring, hormonal and anthropometric status and Tanner Stage will be compared when the boys and girls are 10 and 13 years.

  Status: Approved Concept Paper

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **CONCEPT - Early Puberty, Higher Obesity, and Lower Energy Expenditure in African-American than Caucasian Girls: Can Health Disparities in the Young Lead to Increased Cancer Burden?** — **E0719201** — **Predictive Toxicology**

  Objective(s):

  1) Examine ethnic group differences in energy expenditure among females of prepubertal, pubertal, and reproductive age;
  2) Evaluate whether family history of obesity, birth weight, diet, and endogenous hormone levels contribute to ethnic group differences in energy expenditure; and
  3) Test potential biomarkers of early onset of puberty, which may be indicators of higher estradiol/testosterone levels and earlier age at menarche in contrast with classical assessment by Tanner Developmental Stage.

  Status: Approved Concept Paper

- **CONCEPT - Prostate Cancer in High-Risk and Low-Risk Populations: Role of Food-Derived Heterocyclic Amine Carcinogens and Chemopreventive Agents** — **E0719301** — **Predictive Toxicology**

  Objective(s):

  1) Examine the correlation between meat/food preparation, meat/food intake, and prostate cancer in high-risk and low-risk populations in Australia and the U.S. and China and Saudi Arabia, respectively;
  2) Assess any effect of genetic polymorphisms in the genes encoding enzymes involved in the pathways carrying out the activation and detoxification of the food borne heterocyclic amines in humans;
  3) Determine the correlation between high-temperature cooked meat/food intake and genetic susceptibility in specific ethnic groups with the high-risk populations;
  4) Measure the level of heterocyclic amine-carcinogen DNA adducts in prostate tissue from high-risk and low-risk populations;
  5) Obtain proteomic profiles of serum proteins as biomarkers of heterocyclic amine exposure in high-risk and low-risk populations;
  6) Examine the incidence of "signature" mutations in the APC and b-catenin genes in prostate cancer tissue; and
  7) Examine the risk of prostate cancer in a cohort from the Busselton Health Studies with respect to other risk factors, other diseases, and heterocyclic amine-serum protein adducts.

  Status: Approved Concept Paper

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Lyn-Cook, Beverly

- **CONCEPT - *CYP1B1* Polymorphisms in Uterine Leiomyomas: Frequency in African-American Women and Response to Therapy** — **P00443** — **Predictive Toxicology**

  Objective(s):

  Determine the frequency of the polymorphic variant and others of cytochrome P450 *CYP1B1* in human uterine leiomyoma cases compared with the frequency in patient-matched controls.

  Status: Approved Concept Paper

PI: Ning, Baitang

- **CONCEPT - Evaluation of Effects of Genetic Polymorphisms in Oxidative-Stress-Related Genes HO-1, iNOS and eNOS on the Incidence and Severity of Colorectal Cancer** — **E0716601** — **Predictive Toxicology**

  Objective(s):

  Status: Approved Concept Paper

PI: Nowell, Susan

- **CONCEPT - Effect of Genetic Variation on Efficacy of Tamoxifen or Toremifine Adjuvant Therapy in the NAFTA Trial** — **E0717401** — **Predictive Toxicology**

  Objective(s):

  1) Obtain a blood specimen from each participant in the North American Fareton versus Tamoxifen Adjuvant (NFTA) trial; and
  2) Isolate DNA from the samples and perform genotype analyses for polymorphisms in genes involved in the metabolism of each drug. These studies could result in the identification of patients likely to respond to different adjuvant therapies for breast cancer.

  Status: Approved Concept Paper

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Ratnasinghe, Luke

- **CONCEPT - Phase I - SNP (Single Nucleotide Polymorphisms) Discovery using Denaturing HPLC and Phase II - Mutation Analysis - Work for Center for Structural Genomics Center** — **E0714301** — **Method-Driven Research**

  Objective(s):

  Status: Funded Concept Paper

- **CONCEPT - Development of Breast, Lung and Esophageal Cancer Proteomics and Genomic Signatures for Cancer Early Detection** — **E0715501** — **Predictive Toxicology**

  Objective(s):

  Develop breast, lung and esophageal cancer proteomic and genomic signatures for cancer early detection with the use of plasma pooling and high-resolution, multidimensional proteome analyses and mass spectrometry-based pooled genomic DNA analyses.

  Status: Approved Concept Paper

- **CONCEPT - Association Between Aspirin Use and Chronic Disease in the NHANES I and II Cohorts** — **E0715601** — **Predictive Toxicology**

  Objective(s):

  Status: Approved Concept Paper

- **Concept - Influence of Polymorphisms in Enzymes Involved in Carcinogen Detoxification and DNA Repair on Lung Cancer Risk and Survival** — **E0715701** — **Predictive Toxicology**

  Objective(s):

  1) Examine the association between the codon 198 polymorphism of hGPX1, and other important polymorphisms and lung cancer risk; and
  2) Evaluate the influence of polymorphisms on lung cancer survival post cancer diagnosis.

  Status: Approved Concept Paper

Neurotoxicology

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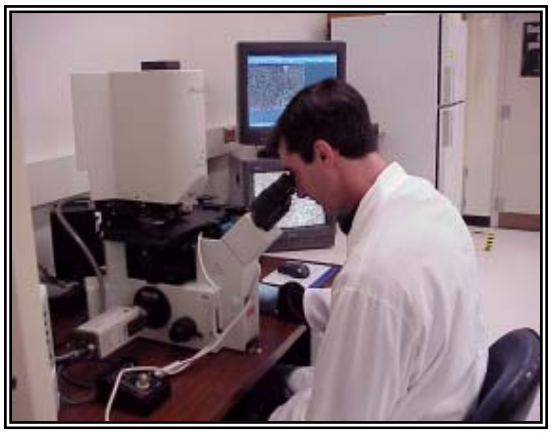

The laser capture microdissection instrument can be used to collect individual cells from tissues sections. Then the cells can be analyzed for genomic and proteomic

Executive Summary

Introduction

In the United States, brain-related disorders account for more hospitalizations than any other major disease group. One of four Americans will suffer a brain-related disorder during their life and the estimated cost to the national economy for treatment, rehabilitation, and related consequences is in excess of $400 billion annually. At no time in the past, however, have researchers been better poised to increase our understanding of brain-related disorders and reduce the risks associated with neurotoxic events.

According to a report from the Congressional Office of Technology Assessment, "Neurotoxicity: Identifying and Controlling Poisons of the Nervous System," the known or suspected causes of brain-related disorders include exposures to chemicals such as therapeutic drugs, food additives, foods, cosmetic ingredients, pesticides, and naturally occurring substances. The number of potential neurotoxicants that require FDA regulation is estimated to be in the thousands and yet guidelines for neurotoxicity risk assessment remain vague and underdeveloped compared with those for cancer. Chemicals from the categories listed above are vital to the national economy and our quality of life. The challenge is to determine at what dose and under what conditions a specific chemical may produce nervous system-related toxicity.

The overall goals of the Division of Neurotoxicology are to develop and validate quantitative biomarkers and precursor events of neurotoxicity and to use these to elucidate modes of action. This will increase the certainty of assumptions underlying human risk assessments for neurotoxicants. The strategy for achieving these goals has been to develop a multidisciplinary approach integrating neurochemical/neurobiological (including genomics and proteomics), neuropathological, neurophysiological, and behavioral assessments to determine adverse effects and explore modes of neurotoxic action. Unique features of the NCTR's neurotoxicology research efforts are the capabilities to determine target-tissue chemical concentrations and cellular level interactions of neurotoxicants and to reduce the uncertainty associated with extrapolating findings across species by effectively using rodent and nonhuman primate animal models--as well as humans--whenever possible.

FY 2003 Accomplishments

Research protocols were developed to provide data in three main focal areas: 1) monoamine (dopamine and serotonin) neurotransmitter systems as a target for neurotoxicity; 2) mitochondrial dysfunction and oxidative stress as mechanisms of neurotoxicity; and 3) the NMDA receptor complex as a mediator of adult and developmental neurotoxicity.

In support of our earlier work on disruption of monoamine neurotransmitter systems, methylenedioxymethamphetamine (MDMA) was shown (as were methamphetamine and fenfluramine before) to produce neuronal cell death in animals that also become hyperthermic as a result of drug treatment. This neurodegeneration was demonstrated with Fluoro-Jade B (FJ), a fluorescent tracer recently developed within the Division. As the initial part of an ongoing project determining the proteomic response of neurodegeneration, the two most active components of FJ were identified.

The genomic response to methamphetamine was also systematically described in regional brain areas of the rat which were neither significantly hyperthermic nor exhibited overt seizure-like activity during amphetamine exposure. Genes with increased expression (mRNA levels) and documented with RT-PCR included neuropeptide Y precursor protein in the parietal cortex, insulin-like growth factor binding protein 1 in the amygdaloid cortex while decreases in nerve growth factor inducible protein IA & IB and activity-regulated cytoskeletal protein expression were seen in the parietal cortex. While regional and acute gene expression changes were documented, long-term alterations in gene expression were less robust but there was a significant 1.4-fold increase in Annexin V in both cortical regions 14 days after amphetamine exposure. Collection of individual cell types or groups by laser capture microdissection (LCM) may be needed to observe larger fold-changes in gene expression induced by the selective effects of these indirect-acting monoaminergic agents. Furthermore, this recently reported data suggest that hyperthermia and seizures, as well as strokes, are not necessary for amphetamine to produce neurodegeneration. However, the neurodegeneration that is produced in the absence of these physiological factors is restricted to very discrete areas of the cortex and involves parvalbumin and GABA containing inhibitory neurons, not excitatory pyramidal neurons as might be expected.

In support of our focus on the study of mitochondrial dysfunction and oxidative stress as mechanisms of neurotoxicity, a combination of laser capture microdissection and genomic approaches was developed to identify gene expression profiles associated with aging and mitochondrial dysfunction. Memory regulation throughout life is controlled by the presence and absence of specific transcription factors (TFs). Because the hippocampus plays a critical role in memory formation and retention, and a decrease in this function occurs with aging, the age-related function of TFs in the hippocampus was examined by evaluating the differential expression of TFs in young (3 month) and aged (2 yrs) C57BL/6N mice. Frozen brain sections were mounted on glass slides and stained cells from selected regions of the hippocampus were collected using LCM. Nuclear extracts containing TFs that are thought to control levels of oxidative stress, apoptotic pathways and to impact memory were prepared from these cells. TF/DNA complexes were isolated and DNA was then analyzed with a Protein/DNA array with 54 different consensus-binding sequences. Each consensus sequence corresponds to a specific transcription factor. In young mice, several TFs responsible for anti-apoptotic pathways were up-regulated, such as: AP-1, AP-2, GATA, Brn-3, c-Myb, Ets/PEA3, IRF-1, NFκB, MRE, and

TFIID. However, in aged mice these anti-apoptotic TFs were down-regulated. In addition, aged mice demonstrated an up-regulation of pro-apoptotic TFs, such as: MEF-2, PPAR, Stat6, and Smad ¾. This approach allows us to test the hypothesis that age-related memory degradation in the hippocampus is associated with age-related changes in gene expression affecting mitochondrial function, apoptosis and levels of oxidative stress, and could also help elucidate the molecular mechanisms of age-related memory disorders, such as Alzheimer's disease. In related studies, the mitochondrial toxicant, 3-nitropropionic acid (3-NPA), was used to test the hypothesis that an enhancer of mitochondrial metabolism, L-carnitine, can also normalize a related set of biomarkers of neurotoxicity including the activity of free radical scavenging enzymes and hypothermia induced by 3-NPA.

An important long-term study was completed in which it was observed that the response of the rat model to chronic exposure to the NMDA receptor antagonist, MK-801 was markedly different from that seen in earlier studies in monkeys. In monkeys, chronic exposure to MK-801 was shown to have negligible effects on the ability of subjects to learn to perform several complex brain function tasks whereas in rats, MK-801 caused severe, long-lasting disruption of such processes. These findings could have far reaching implications should it be confirmed that the rodent model is fundamentally different from the primate model.

In collaboration with CDER staff, experiments were conducted and a manuscript was written that confirmed that administration of ketamine during the brain growth spurt results in widespread neuronal apoptosis in the rat. The need to provide confirmatory evidence in another animal model more closely resembling the developing human was documented.

FY 2004 Plans

Work will continue on these three focal areas in the coming year. A recently approved protocol has allowed us to expand our work in the focal areas of the monaoamine neurotransmitter system and oxidative stress. We will address the extent to which the disruption of the monoaminergic system and oxidative stress are involved in the progression of Parkinson's disease. Proteomic analyses will be conducted on samples of both mouse and human tissue to develop profiles of the various proteins that are affected by neurotoxic insults producing Parkinsonism or Parkinson-like symptoms. Post mortem brains of Parkinson's Disease subjects and protein extracts from the substantia nigra and the striatum isolated from methamphetamine- and MPTP-treated mice will be used to measure post-translational protein modifications using a Phosphoprotein Isotope-coded Solid-phase Tag (PhIST) technique. This recently developed technique utilizes stable isotopes and a solid-phase reagent to more efficiently label and isolate phosphorylated peptides from complex peptide mixtures. Quantification and identification can then be accomplished by matrix-assisted laser desorption/ionization (MALDI)-mass spectrometry. In addition, protein/DNA arrays will be used to examine specific transcription factors involved in methamphetamine- and MPTP-induced neurodegeneration. Recent data from our laboratory utilizing PC12 cell cultures indicate changes in dopamine content correlate with selective alterations in specific transcription factors that regulate monoaminergic systems. Nigrostriatal regions from MPTP-treated mice are currently being evaluated to determine if these changes in transcription factor expression are present and if they correlate with alterations in the dopaminergic system.

Another recently approved protocol will measure the neurochemical and behavioral alterations associated with Accutane (13-cis-retinoic acid) treatment. These studies, specifically requested by CDER, will use validated tests to determine depression-related behaviors in the typical laboratory rat strain, Sprague-Dawley. A subsequent study will ascertain if the specific depression-prone strain (the Flinders Sensitive Line) shows increased sensitivity to the effects of Accutane treatment.

To further compare the rat model with that of the monkey, studies have begun wherein the effects of chronic exposure to remacemide (a sodium channel blocker with relatively weak NMDA receptor blocking properties) will be determined. The rat data will then be compared to monkey data demonstrating that remacemide causes profound and long-lasting deficits in cognitive function. It is hoped that such comparisons will help to define important neurodevelopmental similarities and/or differences between the two species.

In the mitochondrial dysfunction and oxidative stress focal area, a newly approved protocol will allow us to define the gene expression profile (genomic approach) associated with 3-NPA exposure in the rat. The genomic data will be directly compared to already established biomarkers of 3-NPA neurotoxicity including electrophysiological, histopathological, and biochemical endpoints.

The NMDA-mediated excitotoxic response in the adult rat will be used to isolate and characterize the "neurodegeneration protein" expressed by FJ positive neurons following neurotoxic insult. The role of apoptosis versus necrosis as a pathway of neuronal death will be more clearly defined with the use of the cytotoxic marker, FJ, and proteomic approaches. The NMDA receptor complex as a mediator of developmental neurotoxicity is the focus of a new protocol in collaboration with CDER. This protocol will specifically determine whether the ketamine-induced neuronal apoptosis observed in the developing rat is also observed in the immature nonhuman primate, an animal model more closely related to the developing human. Control and ketamine-treated animals will be assessed using histochemical, functional, genomic and proteomic approaches whenever possible.

Public Health Significance

Over the last decade, increasing expertise, technologically advanced equipment, and improved facilities have been interwoven to pursue the overall goals of neurotoxicology research through three primary research areas. These focal areas were developed and based on prevailing scientific understanding and the importance of each area to regulatory concerns. They include mechanistically-based approaches for defining and understanding the potential of a broad range of drugs and other chemicals to produce neurotoxic effects during developmental, adult, or senescent life stages.

Staff will build on our strong base of dose-dependent biomarkers of effect and our unique assessment tools to focus on mechanistically-based and fundamental research projects. The use of DNA array expression and proteomic tools will be further developed. Key personnel are being recruited and extensive training is being provided for existing staff so that new technologies can be incorporated into our research approach.

An interdisciplinary approach, the use of multiple established animal models and innovative biomarkers, and an in-depth working knowledge of and experience with mechanistically-based focal areas of research enable the Division of Neurotoxicology to be responsive to FDA regulatory needs in a timely fashion. Several ongoing or planned studies, many in conjunction with other FDA centers, exemplify the application of the group's approach to providing critical research information applicable to FDA's regulatory concerns.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Ali, Syed

- **Effects of Ibogaine on Neurotransmitter Systems, Generation of Free Radicals and Nitric Oxide Synthase Activity: Correlation with Neurohistological Evaluations in Mouse and Rat Brain** — **E0698301** — **Agent Driven Research**

  Objective(s):

  1) Determine the effects of ibogaine on dopamine, serotonin and their metabolite concentrations in different regions of mouse and rat brain;
  2) Determine the effects of ibogaine on reactive oxygen species (ROS) and lipid peroxidation in different regions of mouse and rat brain;
  3) Determine the effects of ibogaine on the activities of several antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione levels in different regions of mouse and rat brain;
  4) Evaluate the effects of ibogaine on the activity of nitric oxide synthase (NOS) in different regions of mouse and rat brain;
  5) Determine the levels of ibogaine, noribogaine and neurohormone, prolactin and corticosterone in plasma of mouse and rat; and
  6) Evaluate the neurohistorical effects of ibogaine in different brain regions in the mouse and the rat and to correlate them with any neurochemical alterations.

  Status: Completed on 8/15/2003

- **ADDEND: The Effects of Ibogaine on Neurotransmitter Systems, Generation of Free Radicals and Nitric Oxide Synthase Activity: Correlation w/Neurohistological Evaluations in Mouse and Rat Brains** — **E0698311** — **Agent Driven Research**

  Objective(s):

  Investigate if direct infusion of compounds into the brain produces similar changes in the neurotransmitter system in rats. Ibogaine, noribogaine and the structurally-related compound harmaline will be injected directly into the brain. The changes in neurotransmitter levels will be evaluated. Requesting an additional 24 male adult Sprague-Dawley rats.

  Status: Completed on 8/15/2003

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **ADDEND: The Effects of Ibogaine on Neurotransmitter Systems: Correlation with Body Temperature and Electroencephalogram (EEG)** — **E0698321** — **Agent Driven Research**

  Objective(s):

  Investigate what effect ibogaine might have on the electroencephalogram profile along with the time course of temperature changes in rats exposed to this compound. Inject ibogaine (50 mg/kg, i.p.) in five male adult Sprague-Dawley rats instrumented for the EEG and temperature recording as described in the protocol P00404. Additional resources will be labor for Dr. Binienda as a CO-PI - no other additional resources required.

  Status: Completed on 8/15/2003

- **Acute Toxicity of Iron Compounds in Young Mice and Rats** — **E0703801** — **Agent Driven Research**

  Objective(s):

  1) Compare acute toxicity in young animals using two forms of iron commonly used in iron supplements and one form that is to be used in fortification;
  2) Determine if high doses of iron compounds produce reactive oxygen species, an alteration in the lipid peroxidation and changes in antioxidant enzymes in different regions of brain and liver of young mice and rats;
  3) Determine the effect of high doses of iron compounds on complete blood counts. CBC, MCV, MCHC, TIBC and the distribution of iron and iron-binding proteins in different regions of brain and other visceral organs in young animals;
  4) Determine if high doses of iron compounds produce significant changes in neurotransmitter concentrations and activity of nitric oxide synthase in different regions of brain in young mice and rats; and
  5) Determine if high doses of iron compounds produce pathological alteration in brain and other visceral organs in young mice and rats.

  Status: Completed on 8/15/2003

Title	Project Number	Strategic Research Goal
◆ **Neurotoxicity Assessment of Ephedra-Containing Dietary Supplements: Application of cDNA Array and Neurochemical Approaches**	**E0708801**	**Agent Driven Research**

Objective(s):

1) Evaluate the neurotoxicity of the eight most popular ephedra-containing dietary supplements sold in the market place and consumed by the public;
2) Determine the IC-50 of these dietary supplements using PC-12 cultured cells;
3) Determine if *in vitro* exposure to these dietary supplements selectively induces a specific genomic changes in PC-12 cultured cells using cDNA arrays;
4) Determine if multiple doses of these dietary supplements selectively induce specific genomic changes in different regions of mouse brain using cDNA arrays;
5) Determine if multiple doses of these compounds produce significant changes in neurotransmitter concentrations in different regions of the brain in mice;
6) Determine if multiple doses of these compounds produce significant changes in the formation of 3-nitrotyrosine, an *in vivo* biomarker for oxidative stress, in different regions of the mouse brain;
7) Determine if multiple doses of these dietary supplements produce reactive oxygen species, alteration in the lipid peroxidation, and changes in antioxidant enzymes in different regions of the mouse brain; and
8) Determine if multiple doses of these dietary supplements produce pathological alteration in brain and other visceral organs in the mouse.

Status: Project Under Review

Title	Project Number	Strategic Research Goal

- **Evaluation of Novel Genetic Changes and Post-Translational Modification in the Protein Products of Specific Genes in Parkinson's Disease and in Substituted Amphetamine Neurotoxicity Using Quantitative Proteome Analysis in Mice Models and Human Subjects** — **E0712101** — **Agent Driven Research**

  Objective(s):

  1) Determine the post-translational protein modifications in the protein extracts of nigral and striatal tissues in substituted amphetamines and MPTP-treated mice;
  2) Evaluate the effect of various nNOS inhibitors and peroxynitrite decomposition catalysts on the post-translational protein modifications in the protein extracts of nigral and striatal tissues in substituted amphetamines and MPTP-treated mice;
  3) Determine the protein-DNA interaction in the nuclear extracts from the nigral and striatal tissues in substituted amphetamines and MPTP-treated mice for the evaluation of novel post-translational changes in the proteins mediated by various transcription factors;
  4) Determine the effect of various nNOS inhibitors on substituted amphetamine and MPTP-induced free radical production and monoamine concentration in mouse brains;
  5) Determine the nitrated protein on tyrosine hydroxylase by immunoprecipitation of tyrosine hydroxylase and co-localization of 3-nitrotyrosine in the presence or absence of nNOS inhibitors in order to correlate the physiological effects paradigm with the protein changes paradigm from objectives 1, 2 & 3; and
  6) Determine the post-translational protein modifications in the protein extracts and protein-DNA interaction in the nuclear extracts of nigral and striatal tissues obtained from human subjects of Parkinson's Disease.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Binienda, Zbigniew

- **The Role of Mitochondrial Energy Disruption in the Mechanism of Neurotoxicity: Neurophysiological, Neurochemical, and cDNA Microarray Approaches** — **E0711001** — **Concept Driven Research**

  Objective(s):

  1) Define neurophysiological and neurochemical phenotypes associated with brain exposure to 3-NPA and L-carnitine;
  2) Define changes in patterns of gene expression induced by 3-NPA and L-carnitine in the rat brain;
  3) Assess the attenuation of energy deficit by L-carnitine using enzymatic and neurochemical biomarkers of neurotoxicity in the rat model of 3-NPA-induced histotoxic hypoxia;
  4) Establish the relationship between 3-NPA-induced physiological, neurochemical phenotypes and transcriptome profiling in the rat brain model.

  Status: Started/Ongoing

- **ADDEND: The Role of Mitochondrial Energy Disruption in the Mechanism of Neurotoxicity: Neurophysiological, Neurochemical, and cDNA Microarray Approaches** — **E0711011** — **Concept Driven Research**

  Objective(s):

  Include an EEG experiment with another compound, malonic acid. Requesting additional 20 rats over two years.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Bowyer, John

- **Multiple cDNA Array Analysis of the Temporal Changes in mRNA Species after Neurotoxic Events** — **E0707301** — **Predictive Toxicology**

Objective(s):

1) Develop the use of cDNA arrays as a means of detecting mRNA changes that are potential indicators of subtle and severe neurodegeneration at time points of several days up to months after neurotoxic insult;
2) Use cDNA arrays to examine changes in mRNA species that may play a role in changes in the phenotypic expression of neuronal populations in selected brain regions;
3) Expose both neuronal cell line cultures and the brain *in vivo* to neurotoxic insults, and compare the changes in mRNA in the cultured cells versus specific regions of brain using cDNA arrays; and
4) Compare differences in mRNA changes in specific brain regions of adult versus neonatal rats.

Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Determining the Neurotoxic Profile - Specific Changes in Cortical Gene Expression Resulting from Amphetamine Exposures: A Laser Capture Microdissection- and cDNA Array-Assisted Research** — **E0713401** — **Concept Driven Research**

  Objective(s):

  1) Determine the importance of the innervation of the dopaminergic and glutamatergic neurotransmitter systems in the neurodegeneration produced in the interneurons in parietal cortex layers II and IV using specific antagonists and agonists to these two systems;
  2) Determine the gene expression pattern changes that occur in parietal cortex layers II and IV when AMPH-induced neurodegeneration is produced under normothermic, 2-day AMPH exposure, conditions using cryostat-assisted dissection;
  3) Analyze the changes in gene expression in parietal cortex layers II and IV in the same manner as Objective 2 but in animals that are given an acute neurotoxin exposure to AMPH and become extremely hypothermic;
  4) Using cryostat-assisted dissection, determine the changes in gene expression that occur in layer III of the parietal cortex under conditions that do not produce neurodegeneration, and compare this expression pattern to that produced from an acute AMPH exposure where severe hyperthermia occurs and extensive degeneration occurs in pyramidal cells of layer III; and
  5) Using LCM, determine whether astrocytes and microglia respond differentially to the 2 dosing paradigms in the absence or presence of neurodegeneration.

  Status: Started/Ongoing

PI: Chelonis, John

- **Complex Brain Function in Children as Measured by Performance in the NCTR Operant Test Battery** — **E0703301** — **Agent Driven Research**

  Objective(s):

  A battery of automated tests (games) will be given to measure aspects of learning, short-term-memory and attention, motivation, time perception, and color and position discrimination.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Ferguson, Sherry

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Genistein Exposure on Sexually Dimorphic Behaviors** — **E0212213** — **Agent Driven Research**

  Objective(s):

  Determine whether pre/neonatal exposure to genistein, a compound with estrogenic properties, will alter imprinting of sex differences in behavior.

  Status: Completed on 1/10/2003

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Nonylphenol Exposure on Sexually Dimorphic Behaviors** — **E0212513** — **Agent Driven Research**

  Objective(s):

  Determine whether pre/neonatal exposure to nonylphenol, a compound with estrogenic properties, will alter sex differences in behavior.

  Status: Started/Ongoing

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Vinclozolin Exposure on Sexually Dimorphic Behavior** — **E0212613** — **Agent Driven Research**

  Objective(s):

  Determine whether pre/neonatal exposure to vinclozolin, a compound with potential estrogenic properties, will alter sex differences in behavior.

  Status: Started/Ongoing

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Ethinyl Estradiol Exposure on Sexually Dimorphic Behaviors** — **E0212913** — **Agent Driven Research**

  Objective(s):

  Determine whether pre/neonatal exposure to ethinyl estradiol, a compound with potential estrogenic properties, will alter sex differences in behavior.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **ADDEND: The Effects of Developmental/Chronic Genistein Exposure over Multiple Generations on Maternal, Play, Mating/Reproductive Behaviors and Neurochemical Measures** — **E0213213** — **Agent Driven Research**

  Objective(s):

  Determine whether chronic exposure of rats over multiple generations to genistein, a compound with potential estrogenic properties, will alter maternal behavior, play behavior of either sex, the female lordosis response, male mating behavior or the amphetamine-induced release of striatal dopamine, which is known to be estrogen-modulated.

  Status: Started/Ongoing

- **ADDEND: The Effects of Developmental/Chronic Nonylphenol Exposure over Multiple Generations on Sexually Dimorphic Behaviors, and Neurochemical Measures** — **E0213513** — **Agent Driven Research**

  Objective(s):

  Determine whether chronic exposure of rats over multiple generations to nonylphenol, a compound with potential estrogenic and/or androgenic properties, will alter maternal behavior, the female lordosis response, male mating behavior, sodium solution intake, amphetamine-induced release of the striatal dopamine, or serum levels of testosterone and estradiol in males.

  Status: Started/Ongoing

- **ADDEND: The Effects of Nonylphenol Exposure over Multiple Generations on Cognitive Functions and Hippocampal Structure in Female Rats** — **E0213521** — **Agent Driven Research**

  Objective(s):

  Determine whether chronic exposure over multiple generations to nonylphenol, a compound with estrogenic properties, will alter performance on learning/memory tasks and/or hippocampal structure in young adult and middle aged female rats.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal
◆ **Validity of Developmental Cerebellar Stunting in the Rat as a Model for Attention Deficit Hyperactivity Disorder: Behavior and Neurochemistry**	**E0704001**	**Concept Driven Research**

Objective(s):

1) Identify treatments which cause developmental cerebellar stunting, specifically those which decrease the granule cell population with few effects on Purkinje cells;
2) Confirm the increase in locomotor activity caused by developmental cerebellar stunting and determine the degree to which this hyperactivity resembles human ADHD;
3) Identify other behavioral alterations associated with developmental cerebellar stunting and determine the degree to which these resemble those associated with human ADHD;
4) Identify the neurochemical alterations in different brain regions resulting from the developmental insult; and
5) Compare these neurobehavioral and neurochemical alterations to those exhibited by the most common rodent model of ADHD: the Spontaneously Hypertensive Rat (SHR).

Status: Completed on 7/2/2003

Title	Project Number	Strategic Research Goal

◆ **Assessment of Depression Risk Associated with Accutane (13-Cis-Retinoic Aid or Isotretinoin) and All-Trans-Retinoic Acid Treatment: Measurement of Behavioral and Neurochemical Alterations in Adult Sprague-Dawley and Flinders Sensitive and Insensitive Line Rats** — **E0714501** — **Agent Driven Research**

Objective(s):

1) Establish the necessary oral doses of 13-cis-retinoic acid and all-tran-retinoic acid in rats that produce peak plasma levels similar to those of humans prescribed 13-cis-retinoic acid;
2) Measure the toxicity and pathology associated with long-term oral treatment with 13-cis-retinoic acid and all-trans-retinoic acid in rats;
3) Describe the behavioral alterations associated with chronic 13-cis-retinoic acid and all-trans-retinoic acid treatment in adult male and female Sprague-Dawley rats;
4) Determine if such alterations resemble those described in humans treated with 13-cis-retinoic;
5) Measure sex differences in behavioral response to 13-cis-retinoic acid and all-trans-retinoic acid treatment;
6) Evaluate the reversibility of the 13-cis-retinoic acid induced and/or all-trans-retinoic acid-induced alterations;
7) Assess if genetic predisposition to depression determines the frequency and/or magnitude of the behavioral alterations associated with 13-cis-retinoic acid and/or all-trans-retinoic acid treatment; and
8) Quantitate the neurochemical alterations induced by 13-cis-retinoic acid and/or all-trans-retinoic acid treatment.

Status: Started/Ongoing

PI: Gillam, Michael

◆ **Procedure for Ambulation Exercise of Nonhuman Primates Using the Controlled Ambulation Device (CAD)** — **S00173** — **Method Driven Research**

Objective(s):

Provide training of nonhuman primates in the CAD apparatus.

Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Hotchkiss, Charlotte

- **ADDEND: An Efficient Regulatory Method for Evaluating Chromosomal Damage: Analysis Of Micronuclei In The Rhesus Monkey By Flow-Cytometry** — **E0714021** — **Method Driven Research**

  Objective(s):

  A two-year project with funding from FDA's Office of Science for the first year was proposed and approved last year (E0714001). The second year of this project has now been funded. This addendum covers the non-human primate experiments to be performed at NCTR.

  Status: Started/Ongoing

PI: Patterson, Tucker

- **Neurotoxicological and Behavioral Assessment of the Human Immunodeficiency Virus (HIV) Suppressors 2',3'-Dideoxycytidine (ddC) and Thalidomide in Rhesus Monkeys** — **E0250201** — **Predictive Toxicology**

  Objective(s):

  Assess the neurotoxicity and neurobehavioral effects of chronic treatment with the anti-HIV agents 2',3'-dideoxycytidine (ddC) and thalidomide in Rhesus monkeys.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Analyses of the Rat Hippocampus via DNA Microarrays and a Novel Antibody Array, Coupled with Laser Capture Microdissection (LCM) - Evaluation of the Effect of Aging on Gene and Protein Expression Associated with Learning** — **E0713901** — **Concept Driven Research**

  Objective(s):

  1) Measure gene and protein expression in regions of the hippocampus to determine regional distribution;
  2) Determine the effect of aging on regional distribution of hippocampal proteins in three strains of rats;
  3) Determine if aging, behavioral performance and alterations in gene and protein expression in the hippocampus are related; and
  4) Correlate the differences in gene and protein expression with behavioral performance of young adult and aged rats in a learning task previously shown to be sensitive to changes in protein expression.

  Status: Started/Ongoing

PI: Paule, Merle

- **Developmental Neurotoxicity Assessment of Acrylamide in Rats: Range-finding Studies** — **E0214801** — **Agent Driven Research**

  Objective(s):

  Determine acrylamide doses to be used in subsequent long-term developmental neurotoxicity studies by identifying those that will not result in overt toxicity as determined by alterations in body weight gain and a variety of physiological, developmental and behavioral parameters of either pups or dams.

  Status: Started/Ongoing

- **ADDEND: Developmental Neurotoxicity Assessment of Acrylamide in Rats: Range-finding Studies - Chemistry Support** — **E0214811** — **Agent Driven Research**

  Objective(s):

  Addendum submitted to include chemistry support for analysis of acrylamide test agent, dosage forms, and irradiated chow for this study.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **ADDEND: Developmental Neurotoxicity Assessment of Acrylamide in Rats: Range-finding Studies** — **E0214821** — **Agent Driven Research**

  Objective(s):

  Request additional pathology support for thyroid analysis to determine whether acrylamide produces direct effects on thyroid morphology and on thryoid hormone levels during a critical developmental period.

  Status: Started/Ongoing

- **Development of a Nonhuman Primate Model for Studying the Consequences of Long-Term Anticonvulsant Medication on Complex Brain Functions (97032)- ASTRA CRADA** — **E0280001** — **Predictive Toxicology**

  Objective(s):

  1) Establish acquisition curves for several operant behaviors in juvenile Rhesus monkeys during chronic oral exposure to two anticonvulsant agents and vehicle;
  2) Determine whether such exposure results in any significant changes in the acquisition and performance of these operant and other observable behaviors;
  3) Determine whether such exposure results in any significant changes in clinical chemistry or ophthalmic parameters; and
  4) Determine plasma distribution profiles and concentrations for each of these agents at various stages of chronic exposure.

  Status: Completed on 5/13/2003

- **ADDEND: Development of a Nonhuman Primate Model for Studying the Consequences of Long-Term Anticonvulsant Medication on Complex Brain Functions** — **E0280011** — **Predictive Toxicology**

  Objective(s):

  1) Determine whether the effects of chronic ramacemide treatment are due to reversible effects linked to daily drug exposure or are due to irreversible CNS toxicity;
  2) Monitor behavioral acquisition in subjects during six months of reduced drug exposure; and
  3) Request extension of project requiring additional housing/maintenance and research time.

  Status: Completed on 5/13/2003

Title	Project Number	Strategic Research Goal

- **ADDEND: Development of a Nonhuman Primate Model for Studying the Consequences of Long-Term Anticonvulsant Medication on Complex Brain Functions - Rodent Equivalent Addendum** — **E0280041** — **Predictive Toxicology**

  Objective(s):

  1) Examine the effects of acute and chronic exposure to dizocilpine and/or phenytoin on neuronal degeneration and cell death;
  2) Establish acquisition curves for several operant behaviors in rats during chronic oral exposure to 2 different anticonvulsant agents;
  3) Determine whether such exposure results in any significant changes in the acquisition and performance of these operant behaviors; and
  4) Address the relationship between drug-induced cell death and drug-induced changes in behavioral acquisition.

  Status: Complete on 9/18/2003

- **ADDEND: Development of Nonhuman Primate Model for Studying the Consequences of Long-Term Anticonvulsant Medication on Complex Brain Functions (97032)/Rodent Equivalent: Estrous Cycle Assessment And Tissue Collection** — **E0280051** — **Agent Driven Research**

  Objective(s):

  1) Determine whether disruptions in reproductive function might also have been affected in previous experiment (E0280041). Daily estrous cycle assessments will be made over a three-week period to determine whether the cycles of experimental subjects differ from those of controls; and
  2) Request an additional 10 retired female breeders needed to ensure the collection of 5 ml blood plasma in order to assess phenytoin blood levels from stored samples collected throughout the previous study. Blank rat plasma will be needed to serve as an analytical matrix for HPLC analysis.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **ADDEND: Preliminary Studies for Determining the Effects of Chronic Cocaine Exposure during Pregnancy on the Behavior of Offspring in Monkeys** — **E0663306** — **Agent Driven Research**

  Objective(s):

  1) Increase the number of offspring in the total gestational exposure (TGE) group to ten;
  2) Request 10 nonpregnant animals be maintained under chronic cocaine treatment while they are in the breeding program until at least 10 viable offspring are available; and
  3) Request additional 7 animals for inclusion in control group to bring the total to 10.

  Status: Completed on 9/11/2003

- **Effects of Prenatal Cocaine on Behavioral Plasticity** — **E0663307** — **Agent Driven Research**

  Objective(s):

  Determine whether chronic exposure to cocaine in utero results in long-term or residual functional consequences in Rhesus monkey offspring as adults. Systematically explore how long affected subjects must be exposed to specific reinforcement contingencies before reversals of those contingencies manifest as behavioral problems.

  Status: Started/Ongoing

- **Effects of Chronic Methylphenidate (Ritalin) Administration on 'Cognitive' Functions in the Rhesus Monkey** — **E0683700** — **Agent Driven Research**

  Objective(s):

  Determine whether chronic treatment with relevant doses of the therapeutic agent methylphenidate (Ritalin) will result in detectable changes in specific 'cognitive' abilities in a nonhuman primate model of complex brain function.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Use of the NCTR Nonhuman Primate Operant Test Battery (OTB) as a Predictor of Acute Neurobehavioral Toxicity: Pharmacological Manipulation at Specific Neurotransmitter Receptor Subtypes** — **E0697901** — **Predictive Toxicology**

  Objective(s):

  1) Further explore the extent to which the use of operant behavioral techniques in nonhuman primates can serve to reliably model the effects of compounds selected to act on specific neurotransmitter systems;
  2) Determine the acute dose-effect relationships of several drugs believed to act primarily as subtypes of specific neurotransmitter receptors using Rhesus monkey OTB performance;
  3) Characterize the relative sensitivities of the various behavioral endpoints in the NCTR OTB to pharmacological manipulation of specific neurotransmitter systems and to add new tasks to the NCTR OTB;
  4) More thoroughly characterize the role of specific neurotransmitter systems in the expression of complex brain functions through the pharmacological manipulation of specific receptor subtypes of some of the known major neurotransmitter systems; and
  5) Determine if the acute behavioral effects of the exogenous compounds of interest differ with regard to gender in the Rhesus monkey.

  Status: Started/Ongoing

- **Pharmacological Countermeasures for Space Motion Sickness** — **E0712401** — **Predictive Toxicology**

  Objective(s):

  Establish effectiveness and quantify side effects for potential drug countermeasures for Space Motion Sickness (SMS).

  Status: Started/Ongoing

- **Automated Cognitive Assessment of Persons with Alzheimer's Disease** — **E0715301** — **Predictive Toxicology**

  Objective(s):

  Investigate whether performance on a variety of behavioral tests that measure timing ability, memory, and learning is different between persons with mild to moderate Alzheimer's Disease (AD) and persons who have no diagnosis of AD. This research will also determine which of these tasks is most sensitive to disease severity.

  Status: Started/Ongoing

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Evaluation of Changes in Gene Expression in the Brain Associated with Normal Development and the Behavioral Toxicity Caused by Developmental Exposure to the N-Methyl-D-Aspartate (NMDA) Receptor Antagonists, Sodium Channel Blockers, and Combinations** — **E0716501** — **Agent Driven Research**

  Objective(s):

  1) Determine the differences in gene expression between control and treated subjects from earlier rat studies, which entailed chronic treatment with MK-801, phenytoin, and combinations of the two;
  2) Establish acquisition curves for several operant behaviors performed by rats during chronic oral exposure to ketamine or remacemide;
  3) Determine the differences in gene expression between control subjects and subjects treated with ketamine and remacemide at times during behavioral training and performances that coincide with the expression of treatment-related effects;
  4) Establish "normal" gene-expression profiles during a variety of developmental stages in the Sprague-Dawley rat brain; and
  5) Determine the differences in gene expression between control subjects and subjects acutely treated with ketamine during a sensitive brain growth spurt period, and to compare gene expression associated with the ketamine-induced apoptosis with that expressed later in life after chronic ketamine exposure.

  Status: Started/Ongoing

- **Complex Brain Function Study in Children with and Without Major Depression** — **E0717701** — **Concept Driven Research**

  Objective(s):

  Determine if children diagnosed with major depression according to the Diagnostic and Statistical of Mental Disorders (DSM-IV) criteria perform differently than children without such a diagnosis on tests of motivation, simple visual discrimination, timing ability, memory, and learning.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

PI: Scallet, Andrew

- **ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds During Development: II. Genistein** — **E0212215** — **Agent Driven Research**

  Objective(s):

  1) Determine whether developmental exposure to genistein may modify the sexually dimorphic areas of the adult rodent brain; and
  2) Compare neurochemical and neurohistological biomarkers of genistein exposure for their relative sensitivity and concordance.

  Status: Completed on 1/10/2003

- **ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds During Development: III. Nonylphenol** — **E0212515** — **Agent Driven Research**

  Objective(s):

  1) Determine whether developmental exposure to nonylphenol may modify the sexually dimorphic areas of the adult rodent brain; and
  2) Compare neurochemical and neurohistological biomarkers of nonylphenol exposure for their relative sensitivity and concordance.

  Status: Started/Ongoing

- **ADDEND: Neurotoxicological Effects of Exposure to an Anti-Androgenic Compound During Development: Vinclozolin** — **E0212615** — **Agent Driven Research**

  Objective(s):

  1) Determine whether developmental exposure to vinclozolin may modify the sexually dimorphic areas of the adult rodent brain; and
  2) Compare neurochemical and neurohistological biomarkers of vinclozolin exposure for their relative sensitivity and concordance.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds During Development: V. Ethinyl Estradiol** — **E0212915** — **Agent Driven Research**

  Objective(s):

  1) Determine whether developmental exposure to ethinyl estradiol may modify the sexually dimorphic areas of the adult rodent brain; and
  2) Compare neurochemical and neurohistological biomarkers of ethinyl estradiol exposure for their relative sensitivity and concordance.

  Status: Started/Ongoing

- **ADDEND: Multigenerational Exposure to Estrogenic Compounds: I. Genistein Effects on Volume of the Sexually Dimorphic Nucleus** — **E0213215** — **Agent Driven Research**

  Objective(s):

  Evaluate the hypothesis that multigenerational exposure to genistein may produce a reduction in the volume of the male sexually dimorphic nucleus of the medial preoptic area of the hypothalamus.

  Status: Started/Ongoing

- **ADDEND: Multigenerational Exposure to Estrogenic Compounds: II. Nonylphenol Effects on Volume of the Sexually Dimorphic Nucleus** — **E0213515** — **Agent Driven Research**

  Objective(s):

  Evaluate the hypothesis that multigenerational exposure to nonylphenol may produce a reduction in the volume of the male sexually dimorphic nucleus of the medial preoptic area of the hypothalamus.

  Status: Started/Ongoing

- **Estimating Quantitative Neurotoxicity Risk from Domoic Acid Exposure** — **E0693001** — **Agent Driven Research**

  Objective(s):

  1) Correlate pharmacokinetic profiles of single and multiple doses of domoic acid with associated quantitative neurohistological and behavioral effects in non-human primates;
  2) Identify genetic factors modulating domoic acid sensitivity in Wistar rats; and
  3) Identify neurochemical biomarkers of domoic acid exposure and damage.

  Status: Completed on 8/11/2003

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Schmued, Laurence

- **Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanisms of Action of Neurotoxicants** — **E0701301** — **Predictive Toxicology**

  Objective(s):

  1) Develop and validate a battery of conventional and novel histochemical techniques for resolving the nature, distribution and underlying mechanisms of brain damage resulting from exposure to FDA-relevant neurotoxicants;
  2) Localize throughout the central nervous system histochemical and pathological changes resulting from exposure to different classes of neurotoxicants; and
  3) Develop the ability to predict the neuroanatomical regions at risk and the potential functional consequences of exposure to the neurotoxicant of interest by correlating a compound's putative mode of action with a characteristic histochemical profile.

  Status: Completed on 8/11/2003

- **ADDEND: Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanisms of Action of Neurotoxicants** — **E0701311** — **Predictive Toxicology**

  Objective(s):

  Addendum submitted to add compound strychnine to be used in this project. No additional resources requested.

  Status: Completed on 8/11/2003

- **ADDEND: Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanisms of Action of Neurotoxicants** — **E0701321** — **Predictive Toxicology**

  Objective(s):

  Requesting to add formaldehyde-fixed human brain autopsy tissue to the list of neurotoxicant-exposed brains in the test battery associated with this project. No additional costs expected with this addendum.

  Status: Completed on 8/11/2003

Title	Project Number	Strategic Research Goal

- **ADDEND: Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanism of Action of Neurotoxicants** — **E0701331** — **Predictive Toxicology**

  Objective(s):

  Request for additional compounds to be added to the histochemical test battery: Aurothioglucose, Pilocarpine, and Beta-amyloid peptide fragment 1-40. No additional costs associated with this addendum.

  Status: Completed on 8/11/2003

- **Proteomics of Toxicant-Induced Neuronal Degeneration** — **E0711101** — **Concept Driven Research**

  Objective(s):

  1) Resolve the chemical identity of the endogenous protein(s) associated with neuronal cell death as identified by Fluoro-Jade B binding;
  2) Determine if the same proteins are expressed regardless of the mechanism of neurodegeneration;
  3) Resolve the chemical identity of the fluorescent component in Fluoro-Jade B responsible for the high affinity labeling of degenerating neurons; and
  4) Resolve the metabolic pathway by which the "degeneration protein" is generated.

  Status: Started/Ongoing

PI: Slikker, William

- **Quantitative Procedures for Neurotoxicity Risk Assessment** — **E0310001** — **Predictive Toxicology**

  Objective(s):

  Determine the necessary parameters for a biologically-based dose-response model to predict neurotoxic adverse effects following exposure to cholinesterase inhibiting pesticides. Such information would improve the ability of risk assessments to evaluate toxicological data for potential human health risk and address a specific need identified by the Neurotoxicity Risk Assessment Guidelines.

  Status: Started/Ongoing

Title	Project Number	Strategic Research Goal

- **Preliminary Studies for the Effects of Chronic Dexfenfluramine Administration in the Rhesus Monkey** — **E0702601** — **Predictive Toxicology**

  Objective(s):

  1) Determine if the Rhesus monkey demonstrates cardiac valve changes due to chronically administered dexfenfluramine; and
  2) Determine if the Rhesus monkey demonstrates neurobiological changes due to chronically administered dexfenfluramine.

  Status: Started/Ongoing

- **Assessment of Ketamine in the Developing Nonhuman Primate** — **E0718901** — **Predictive Toxicology**

  Objective(s):

  1) Determine, using neurohistochemical approaches, if, and at what developmental stages, ketamine exposure increases neuronal apoptosis/proliferation;
  2) Determine, using neurohistochemical approaches, the dose-response for ketamine to produce apoptosis at the most sensitive developmental stage;
  3) Determine the reversibility or permanence of the response using behavioral, imaging and neurohistochemical approaches; and
  4) Determine, at the most sensitive stage and dose, genomic and proteomic responses to ketamine treatment.

  Status: Started/Ongoing

PI: Wang, Cheng

- **Application and Development of Standard Operating Procedures Required for Studies of NMDA Antagonists/GABA Agonists in Developing Rats - Preliminary Ketamine Project** — **P00636** — **Method Driven Research**

  Objective(s):

  1) Establish SOP's for an *ex vivo* postnatal day PND 7 rat brain organotypic slice culture system;
  2) Develop SOP's for genomic analysis of PND 7 ketamine-treated brains and slices; and
  3) Compare the neurotoxic effects of ketamine exposure on PND 7 brains and on the *ex vivo* slices.

  Status: Project Under Review

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Xu, Zengjun

- **Adolescent Nicotine Administration Effects on CNS Serotonergic Systems** — **E0709801** — **Agent Driven Research**

  Objective(s):

  1) Determine whether adolescent nicotine administration elicits axonal/terminal damage in 5HT systems;
  2) Determine if adolescent nicotine administration alters 5HT presynaptic activity;
  3) Determine 5HT receptor and signaling activity and functions induced by adolescent nicotine exposure; and
  4) Determine if adolescent nicotine administration produces changes in cAMP-medicated signal transduction, 5HT metabolic enzymes and/or 5HT receptors

  Status: Started/Ongoing

Publications

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Veterinary Services

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Division of Veterinary Services provides research support through on-site contractor for histopathology.

Executive Summary

Introduction

The Division of Veterinary Services (DVS) provides professional and technical support to the various NCTR research divisions and Centers of Excellence in their efforts to conduct peer-reviewed scientific research that supports and anticipates the FDA's current and future regulatory needs. The Division provides administration for the Center's Animal Care and Use Program, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). Included within the Division are the contracted services for animal care, diet preparation, and pathology, all of which are staffed by on-site contract employees.

FY 2003 Accomplishments

Immediate Office

The Division provided oversight and management of all laboratory animal facilities at NCTR. Divisional personnel were responsible for breeding, rearing, and/or acquiring and quarantining all experimental animals used on-site including the establishment of a breeding colony of transgenic rats in support of future work on hepatotoxicity. Personnel submitted annual reports assuring compliance with Federal regulations and NIH guidelines relative to our Animal Care and Use Program and participated in semi-annual program reviews, facility inspections, and experimental protocol reviews as part of the NCTR Institutional Animal Care and Use Committee proceedings. Divisional personnel served as government project officers for the pathology services, animal care and diet preparation, and rodent bedding contracts for the Center. Divisional personnel also planned and implemented the NCTR Laboratory Animal Care Technician Recognition Week and the Annual Arkansas Branch AALAS Meeting; and they participated as instructors in the on-site AALAS technician certification program. As a member of the FDA Research Animal Committee the director performs peer reviews of the Animal Care and Use Program Description Documents of each Center and provides "mock" AAALAC site visits to those facilities. The director is also a member of the interagency working group on nonhuman primate resources and during 2003 became a certified manager of animal resources (CMAR).

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Animal Care/Diet Preparation Services

During 2003, the average number of experiments supported per month by contract animal care personnel was 29. Those experiments entailed as a minimum the daily animal care support of an average of 6,000 rodents, many individually housed, and 78 Rhesus monkeys. Technical manipulations for those studies included one or more of the following procedures: tattooing (6,200 animals), vaginal lavages (5,000), tumor palpations (14,000), injections (2,000 SQ, IM or IV), oral gavage [including development and implementation of oral gavage procedures for neonatal mice] (15,000), behavioral assessments (15,000), topical cream applications (136,000), and blood collection [including development and implementation of cardiac puncture of neonatal mice] (500). Three additional animal care employees became certified assistant laboratory animal technicians, three supervisors became Certified Managers (CM), and eight supervisors and management personnel became Certified Managers of Animal Resources (CMAR).

Contract diet preparation personnel provided consultation and nutritional support and diet preparation for several studies including *Aloe vera*, acrylamide and glucidamide, and retinyl palmitate funded through an Interagency Agreement with the National Institute for Environmental Health Sciences (NIEHS). Additionally, personnel autoclaved 26,000 kg of rodent chow and prepared 1,700 liters of dosed water and 2,200 vials of dosed cream for topical applications. Quality assurance personnel performed 4,700 quality control audits of contractor-performed procedures and updated all animal care and diet preparation SOPs. During 2003, animal care/diet preparation contract employees authored or co-authored 6 publications or presentations.

Pathology and Pathology-related Services

During 2003, six trainees completed PAI's Laboratory Technician apprenticeship training program and became eligible to take the histotechnician registry exam. Implementation of the "paperless" pathology system for collecting and reporting of pathology data and tracking of specimens through the pathology system is projected for Fall of 2003. A new laser capture microdissection system along with a cryostat was purchased and installed to support new procedures and methodologies at the Center. A new Virtual Microscopy/Pathology System (ScanScope) was purchased and installed. The ScanScope will allow digital storage of an entire microscope slide at diagnostic resolution. The system will allow a Pathology Working Group (PWG) to be conducted over the Internet where members of the PWG are able to view images of study slides while sitting at their home computer. All members of the PWG are able to view the same slide simultaneously, if desired. In the clinical chemistry laboratory, the hematology analyzer was upgraded to be able to perform automated differentials suitable for nonhuman primates and rodents. New assays developed include: mouse adiponectin, rodent IGF-1, rat leptin, and IGF-BP's. Several protocol-related *in situ* hybridization procedures and immunohistochemistry procedures were also developed. A laboratory was established and a protocol developed to perform genetic monitoring of NCTR's rodent breeding colonies using microsatellite DNA analysis. Personnel prepared for peer review for Glycolic and Salicylic Acid on the Photocarcinogenicity of Simulated Solar Light in SKH-1 mice and the Chronic

Carcinogenicity study on Genistein. During 2003, pathology contract employees authored or co-authored 27 publications or presentations.

FY 2004 Plans

- Continue to support the research mission of NCTR including future BSL/ABSL work.
- Procure a new Pathology Services Contract for the Center.
- Continue supplying methods development and support, both technical and professional, needed to accomplish the NIEHS IAG work at NCTR.
- Add genomic microarray design and analysis to the pathology services provided.
- Continue a quality laboratory animal care program that is consistent with State and Federal laws, regulations, and guidelines.
- Continue to assist FDA's Centers in maintaining AAALAC accreditation of their laboratory animal care and use programs.

Public Health Significance

FDA's mission is to protect and promote the nation's public health. Animal-related studies such as those being conducted by the NCTR research community greatly enhance the Agency's ability to meet this public health mission. The Division of Veterinary Services (DVS) has the facilities, equipment and personnel to actively support this vital interdisciplinary research.

The "gold standard" for laboratory animal care and use programs is accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). Such accreditation is widely accepted by the scientific community and indicates that the accredited organization conforms to all government policies and regulations and that it endorses the highest quality care for the animals involved in their animal use activities. DVS personnel oversee the NCTR Laboratory Animal Care and Use Program, which has been accredited by AAALAC International since 1977. The DVS director, working through the FDA Research Animal Council (FRAC), has assisted, and will continue to assist other FDA Centers in obtaining and maintaining accreditation of their animal care and use programs.

Research Projects

Title	Project Number	Strategic Research Goal
PI: Muskhelishvili, Levan		
◆ **Non-Isotopic *In Situ* Hybridization for Histone mRNA in Detection of S-phase Cells**	**P00438**	**Method Driven Research**
Objective(s): Validate applicability of non-isotopic *in situ* hybridization for histone mRNA technique for detection of S-phase cells in rat non-hepatic proliferating tissues by comparing counts of S-phase cells detected using the histone mRNA and BrdU procedures. **Status:** Started/Ongoing		
PI: Witt, William		
◆ **Pathology Training Animals**	**S00000**	**Center Support (Research)**
Objective(s): **Status:** Started/Ongoing		
◆ **NCTR's Building 6 Rodent Breeding Program for IACUC-Approved Biomedical Research Activities**	**S00221**	**Center Support (Research**
Objective(s): Establish an approved project number for the Building 6 Breeding Colony to comply with Public Health Service Policy. **Status:** Started/Ongoing		

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Publications

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Project Number Codes:
E–Ongoing P–Preliminary S–Support

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Interagency Agreements (IAGs)

NCTR has been fortunate in establishing Interagency Agreements (IAGs) with other government agencies to conduct research on problems of common interest to the FDA and the collaborating agency. The most significant, in terms of size, is the IAG between FDA/NCTR and the National Institute of Environmental Health Sciences (NIEHS).

In May 1992 the Food and Drug Administration (FDA) submitted a proposal for an interagency agreement (IAG) with the National Institute for Environmental Health Sciences (NIEHS). The design for this agreement concentrated on FDA's priority National Toxicology Program (NTP) nominations and utilized the unique resources and facilities at the National Center for Toxicological Research (NCTR). The research conducted under the IAG provided FDA the ability to better assess risk/risk-benefit analysis and perform a confident level of research in a more timely fashion. The IAG between NIEHS and FDA was finalized in December 1992.

The 1992 agreement provided support for five FDA priority chemical/agent NTP nominations. The success of the IAG resulted in an open-ended IAG (no IAG time limit and no monetary limit per compound) in 1995. Since that time the agreement between the NIEHS and the FDA has expanded to include collaborative research on five putative endocrine disrupter compounds, which included three multigeneration studies and two chronic cancer studies. A Phototoxicity Research and Testing Laboratory opened in 1998 at the NCTR under the IAG. The facility is state of the art, testing compounds applied to the skin in simulated solar light. The IAG has expanded to include AIDS therapeutic research as it relates to the carcinogenicity of drug combinations used to prevent mother-to-child transmission of HIV.

The IAG continues to expand and provide resources for toxicity, cancer and mechanistic data. The IAG manages resources from public funds and retains exceptional scientific expertise to provide the best possible assessment of product safety and significantly reduce human risk in order to provide the greatest health benefit for the American public.

In addition to the IAG with NIEHS/NTP, NCTR has received support from other governmental agencies. For example, the Environmental Protection Agency (EPA) has supported NCTR in conducting a broad area of research on neurotoxicity risk assessment, risk assessment associated with waterborne and foodborne pathogens, and support for the development of an endocrine disruptor computerized knowledge base. The Federal Aviation Administration (FAA) has entered into an IAG with scientists at the Center to develop rapid sensor detection methods to screen for explosives in counterterrorism. Additionally, the National Institutes of Health (NIH) and the National Cancer Institute (NCI) are supporting studies at the NCTR into Agent Orange exposure and the mechanism of colorectal cancer, respectively.

Although not an IAG in the strict sense, NCTR has received generous support from the FDA's Office of Women's Health (OWH) for a number of research programs. These include studies to: 1) develop the methodologies to assay hydroxylation of endogenous estrogens as that process relates to the risk of developing breast cancer; 2) identify the effects of dietary supplements on

women's health issues; 3) develop *in vitro* model systems for the study of mechanisms of toxicity in humans from different genders and/or ethnic populations; 4) determine if tamoxifen (currently being used in clinical trials as a chemoprotective agent against breast cancer) is acting through a genotoxic mechanism by characterizing DNA adducts from suspected tamoxifen metabolites; 5) investigate whether the $Tk^{-/-}$ genotype in mice is lupus prone to establish an association of Tk deficiency and lupus; and 6) develop a human hepatocyte cell line to analyze gender differences in the metabolism of drugs.

NCTR has received support from both the FDA's Office of Women's Health and the U.S. Department of Defense (DOD) to conduct molecular epidemiology studies designed to determine the variability in metabolic phenotype and genotype in women with respect to their recurrence of breast cancer following high-dose radiation and chemotherapy.

Collaborative Research and Development Agreements (CRADAs)

NCTR's Division of Neurotoxicology concluded a study that had received financial support from AstraZeneca to study the effects of long-term blockage of glutamate receptors and/or sodium channel blockage on neurobehavioral endpoints in the non-human primate. AstraZeneca is supporting another study to determine whether ketamine, an NMDA receptor antagonist frequently used as an anesthetic in children, and remacemide, an antiepileptic agent with both NMDA receptor antagonist and sodium channel blocking properties, cause adverse effects similar to those noted in previous rat and monkey studies. Researchers have determined that administration of ketamine during the brain growth spurt results in widespread neuronal apoptosis in the rat. Further confirmatory evidence in another animal model closely resembling the human was documented.

NCTR's Division of Chemistry has developed small disks called Food Quality Indicators (FQIs), rapid, chemical sensors to assess food quality. These FQIs have been evaluated by The Canadian Center for Fisheries Innovation (CCFI), St. Johns, Newfoundland, Canada. Their findings show that FQI is rapid, sensitive, rugged, and simple enough that multiple analysts can obtain results of equal quality. A CRADA has been developed with Litmus for a commercial outlet and partial support for extension of the FQI technology.

A CRADA with Argus Research Laboratories provides that historical data from the Argus Research Laboratories of the tumor incidence in SKH-1 mice treated with simulated solar light (SSL) is entered into the NCTR MultiGen database. This data will be summarized and analyzed for tumor incidents. NCTR will share with Argus Research Laboratories the incidence data on SKH-1 mice treated with SSL at the NCTR and any statistical methods developed for analyzing the data.

Experiments have shown that animals exposed to cocaine during gestation fail to adapt to important changes in their environment. Researchers at the University of Arkansas at Little Rock (UALR) and in the Division of Neurotoxicology at NCTR are expanding upon these findings by examining additional aspects of behavioral adaptability by changing 'the rules of the game' for a variety of behavioral tasks.

University Interactions

Many NCTR scientists hold adjunct faculty positions and collaborate with individuals and departments of universities. This practice has been instrumental in leveraging both the intellectual and infrastructure capabilities of NCTR. NCTR scientists have developed research collaborations with more than 20 universities and many scientists have been granted adjunct academic positions. This arrangement permits NCTR staff to develop close collaborative efforts with various university staffs to solve problems of mutual interest to FDA and the respective university. Academic collaborations include mutual use of specialized equipment, sharing of research samples to maximize the gain of information from a project, and the exchange of staff between the institutions for lectures, seminars, and conduct of research.

Of particular importance are the close collaborations between NCTR and the University of Arkansas for Medical Sciences (UAMS) in Little Rock, AR. In addition to the adjunct positions held by NCTR scientists at the UAMS, NCTR participates in the UAMS Interdisciplinary Toxicology Program through which graduate students receive a Ph.D. in toxicology. Many of the graduate students perform research for their dissertations in an NCTR laboratory under NCTR staff supervision.

One example of leveraging with local institutions is that NCTR staff in the Division of Neurotoxicology has access to a Complex Brain Function Laboratory at the Arkansas Children's Hospital (ACH). Results of behavioral studies obtained in animals using the Operant Test Battery at NCTR are verified in humans at ACH.

NCTR Staff members serve as collaborators on a number of grants with area universities. These include a NASA-funded UAMS (Department of Otolarynology) project designed to provide information on the efficacy of several drugs used as anti-space motion sickness therapies and their effects on cognitive function as assessed using the NCTR Operant Test Battery. Grant funding via UAMS/UALR and NIH has provided support for studies to examine the ability of the NCTR Operant Test Battery to detect and monitor cognitive dysfunction in Alzheimer’s patients.

Other collaborations with UAMS scientists include: 1) investigate the biological effects of ephedrine, ethanol, baby diets, cardiovascular disease and aging on health using the metabonomics approach; 2) investigate the influence of biotin on the developing embryo; 3) identify the brain tumor diagnostic method to achieve tissue characterization; 4) assess DNA from breast epithelial cells for the presence of carcinogen adducts, 5) develop methodology and determine the critical biotransformation pathways involved in adduct formation and assess possible differential sensitivity in normal-risk women as opposed to women at high risk for breast cancer; and 6) conduct the first LCM-based study on genetic changes of the mitochondrial genome in prostate cancer and precursor lesions.

Recent projects that include collaborations with area universities and hospitals include those with UAMS, Central Arkansas Veterans Healthcare System (CAVHS), ACH, and the University of Arkansas. NCTR scientists are collaborating with UAMS scientists to develop a microarray-based method for the detection of 150 genes associated with 22 antibiotics, some of which are used to promote growth in poultry and animal farming while others are used to treat infections in

both humans and animals. A new project with UAMS and CAVHS will identify the measurement of cancer-associated gene mutation in colon tumor and non-tumor tissue. Collaborations with UAMS/UALR/ACH have begun on a project to determine if children diagnosed with major depression perform differently than children without such a diagnosis on tests of motivation, simple visual discrimination, timing ability, memory, and learning. In addition, the University of Arkansas and NCTR scientists will address the extent to which the disruption of the monoaminergic system and oxidative stress are involved in the progression of Parkinsonism or Parkinson-like symptoms.

NCTR scientists collaborating with universities in the U.S. and abroad have resulted in, at no cost to FDA, a number of visiting scientists who come to NCTR to pursue research in areas developed by NCTR scientists. Thus far, NCTR has hosted more than 36 visiting scientists from the U.S. and 15 foreign countries. These visiting scientists not only contribute valuable scientific expertise to NCTR research programs, but many return to their respective institutions to continue research on problems of interest to FDA and NCTR.