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Preface

The National Center for Toxicological Research (NCTR), plays a critical role in the U.S. Food and Drug Administration (FDA) and Department of Health and Human Services (DHHS) mission to 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 is dedicated to the conduct of fundamental and applied research to provide FDA with a stronger scientific base for making regulatory decisions.

The mission of NCTR is to conduct peer-reviewed scientific research that supports the FDA's current mission and anticipates future regulatory needs. This involves research specifically designed to define biological mechanisms of action underlying the toxicity of products regulated by the FDA and the development of improved methods for assessment of human exposure, susceptibility and risk. In addition to its FDA support, NCTR leverages its resources by conducting integrated research programs with other FDA centers, the Office of Regulatory Affairs (ORA) and through collaborative agreements with other government agencies, academia, and industry. NCTR receives guidance and advice on the relevance and quality of its research programs from an extramural Science Advisory Board, liaison members from each of the other FDA centers, the ORA, and other stakeholders.

The NCTR views its FDA 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, 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. Additionally, the NCTR is a participant in national and international consortia that are developing standards for using emerging genomic technologies and standards for interpreting the data derived from these technologies. Examples of how NCTR is supporting and meeting standard setting challenges of the FDA include:

- Initiating an on-line scientific journal entitled *Regulatory Research Perspectives*, which highlights some of the latest research topics in the scientific regulatory arena.
- Refining rapid detection methods for potential bioterrorism agents.
- Developing microarray/proteomic technology to provide high volume screening of biomarkers for susceptible subpopulations and evaluate the effects of chemical toxicants on gene expression and protein profiles.
- Introducing the knowledge of new genetic systems, specifically transgenic systems and data into the application review process.

- Developing computer-based models to predict the impact of increased exposure to toxic compounds on public health.
- Conducting studies on FDA-regulated compounds to relate the mechanism(s) by which a chemical causes toxicity to the biological outcome.
- Developing and/or modifying standards to better suit the regulatory needs of the DHHS/FDA for food safety.
- Developing methods and building biological dose-response models to quickly and accurately predict risks associated with antimicrobial resistance and foodborne pathogens/contaminated foods, dietary supplements, and genetically-modified foods.

Other important areas of research supported in part by external funding include identification of the effects of anticonvulsants on complex brain functions in non-human primates, antibiotic resistance associated with competitive exclusion products, and development of risk assessment tools to better extrapolate animal toxicity data to humans.

Perhaps of greater importance to our research accomplishments was the benefit gained by sharing knowledge through collaborations with scientific staff of other government, academic, and industrial institutions. I am proud to present this report that summarizes these and other NCTR research accomplishments and plans for the fiscal years 2001-2002.

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

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 2001 Accomplishments

A full meeting of the Board was held June 11-12, 2001.

Dr. Daniel Acosta, Jr., was named Chairperson. Dr. Bernard Schwetz, Acting Principal Deputy Commissioner, in addressing the SAB, commented that the SAB has had a greater impact on NCTR planning than any other boards of scientific counselors or SABs in other centers. He indicated that peer review of Agency operating components was becoming more formalized, especially at the center level, and that formal peer reviews of the FDA centers have been initiated at the Agency level. These Agency-level reviews are focused on the overall scientific programs of the centers, and are less in-depth than the NCTR reviews.

Responses to previous SAB site visit reports were provided by the following programs: Dr. Daniel Sheehan on behalf of the Endocrine Disruptor Knowledge Base Program, Dr. Ashraf Khan on behalf of the Division of Microbiology, and Dr. William Slikker on behalf of the Division of Neurotoxicology. The Board was also provided with updates by the following: Dr. William Allaben on NTP activities at the Center, Dr. Frederick Beland on the Division of Biochemical Toxicology, Dr. Lionel Poirier on the Division of Molecular Epidemiology, Dr. Martha Moore on the Division of Genetic and Reproductive Toxicology, Dr. Robert Turesky on the Division of Chemistry, and Dr. Ralph Kodell on the Division of Biometry and Risk Assessment. The site visits reports and the minutes of the SAB meetings can be accessed at http://www.fda.gov/nctr/science/committees/committees.htm.

Dr. James MacGregor provided the Board with an overview of a proposed Subcommittee on Scientific Opportunities to Improve Regulatory Approaches. He addressed the concept and related activities of the existing Nonclinical Studies Subcommittee of the Advisory Committee for Pharmaceutical Science, and recommended to the Board that this Subcommittee become a subcommittee of the NCTR SAB. The Board will be asked to vote on this issue at the next full meeting.

Science Advisory Board Membership Roster

NAME/TITLE	AFFILIATION	TERM ENDS	EXPERTISE
Dr. Daniel Acosta, Jr.* Dean, School of Pharmacy	University of Cincinnati	6/30/03	Pharmacology and Toxicology
Dr. Catherine W. Donnelly Associate Dean, College of Agriculture & Life Sciences	University of Vermont Burlington, VT	6/30/02	Microbiology/Food Science
Dr. Nancy Ann Gillett Sr. Vice President Sierra Biomedical	Charles River Laboratories	6/30/03	Veterinary Medicine and Pathology
Dr. Stephen S. Hecht Wallin Land Grant Professor of Cancer Prevention	University of Minnesota Cancer Center Minneapolis, MN	06/30/02	Chemistry
Dr. Jerry Kaplan Associate Dean for Research	University of Utah School of Medicine	06/30/04	Molecular Biology
Dr. Marcy Rosenkrantz Director, Library Systems	Cornell University	06/30/02	Computational Chemistry
Dr. Kenneth R. Tindall Senior Vice President for Science and Business Development	North Carolina Biotechnology Center	06/20/04	Biomedical Sciences/Genetics
Dr. Leonard M. Schechtman Executive Secretary, Associate Deputy Director for Washington Operations, NCTR	FDA/NCTR Rockville, MD	Ongoing	Research Administration

*Chair
There are currently two vacancies on the Board.

FDA Coordination Activities—Safety Testing

Function

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

The ICCVAM coordinates interagency issues on test method development, validation, regulatory acceptance, and national and international harmonization. Congress recently enacted the ICCVAM Authorization Act (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 is revising its operating procedures to improve the efficiency of the validation and acceptance of new test methods for regulatory purposes. The Scientific Advisory Committee for the Validation of Alternative Methods (SACATM), which advises ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), has been established and chartered.

FY 2001 Accomplishments

- The murine Local Lymph Node Assay (LLNA) gained international regulatory acceptance by the 30 member countries of the OECD.

- A training workshop for the LLNA was held to train regulatory scientists and industry toxicologists on how to perform the LLNA and interpret data in accordance with regulatory testing requirements.

- ICCVAM completed evaluation of a revised up-and-down procedure (UDP) that can be used in place of the conventional acute toxicity test for hazard identification and classification. The revised UDP was subsequently accepted by the OECD as an internationally harmonized test guideline.

- The *Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity* was published and made available to the public.

- A *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity* was drafted and published.

- A report was published describing an International Workshop on *In Vitro Methods for Assessing Acute Systemic Toxicity.*

- ICCVAM has developed an expedited review process for use with methods that have undergone validation assessment by other comparable organizations, e.g., the European Centre for the Validation of Alternative Methods (ECVAM), to avoid duplication of effort and unnecessary delays in recommending potentially useful test methods for regulatory purposes.

- Three alternative *in vitro* test methods for assessing skin corrosivity, i.e., EpiDerm™, EPISKIN™, and the Rat Skin Transcutaneous Electrical Resistance (TER) assay, are being evaluated by ICCVAM using the expedited review process.

Biochemical Toxicology

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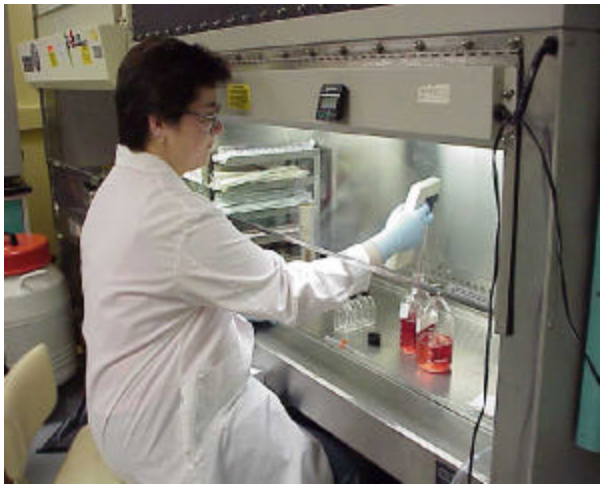

Marta Pogribna setting up cell culture for DNA methylation studies

Executive Summary

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 risks associated with specific chemicals and gene-nutrient interactions, and the introduction of new techniques to assess toxicities and carcinogenic risks. 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.

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 staff of scientists with strong multi-disciplined mechanistic research experience. As such, the Center has the capability to conduct sub-chronic 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.

The Division's initial investigations for the NIEHS/NTP included fumonisin B_1, a mycotoxin found in corn, which was nominated by the FDA Center for Food Safety and Applied Nutrition (CFSAN); chloral hydrate, a pediatric sedative, in response to a request made by the Center for Drug Evaluation and Research (CDER); malachite

green, a therapeutic agent used in aquaculture that was nominated by the Center for Veterinary Medicine (CVM); urethane, a contaminant of certain food products, at the request of CFSAN to assist this center to establish regulatory levels for this carcinogen; and riddelliine, a pyrrolizidine alkaloid present in various herbal preparations. More recently the scope of the NIEHS/NTP investigations has been expanded to include a series of endocrine-active compounds, including genistein, ethinyl estradiol, nonylphenol, methoxychlor, and vinclozolin. In addition, a major effort has started in the area of phototoxicity, with emphasis on the potential interaction between ultraviolet (UV) light and substances found in over-the-counter cosmetics. Initial studies have focused on alpha- and beta-hydroxy acids, and the scope has been expanded to include *Aloe vera* and retinyl palmitate. Studies have also been initiated to include toxicological evaluations on antiretroviral drugs, including zidovudine, lamivudine, nevirapine and nelfinavir, both alone and in combination.

Traditional chronic carcinogenicity bioassays are both very expensive and lengthy; thus, the development of alternative methods of assessing carcinogenic potential should be of great value. One approach that is currently being investigated is the neonatal mouse tumorigenicity assay. The advantages of this method are that only limited amounts of test material are required, a direct assessment is obtained as to whether or not the agent acts through a genotoxic mechanism, and less time is required to elicit a carcinogenic response. This alternative bioassay has been applied to benzodiazepines, antihistamines, lipid peroxidation products, estrogens, antiestrogens, peroxisome proliferators, lipid peroxidation inducers, proton pump inhibitors, mycotoxins, antiretroviral drugs, and a series of known human carcinogens.

An ongoing goal within the Division is to exploit both the immunogenicity and the antigenicity of toxicants, metabolites, and DNA adducts to develop and apply immunochemical methods combined with mass spectral techniques to address problems of regulatory concern. This technology has been applied to fumonisin B_1, fumonisin B_2, and fumonisin B_3, aromatic amine DNA adducts, nucleoside analogues of anti-HIV drugs, and etheno-type DNA adducts formed by urethane. More recently, studies have focused on the preparation of antibodies against UV photo products in support of the Division's phototoxicity effort. In addition, Division investigators have developed methodologies to assay catechol formation from endogenous estrogens.

A major emphasis of the Division is to develop analytical methodology based on mass spectrometry to measure biomarkers of exposure and toxicity in animals and humans in conjunction with studies that define mechanisms of toxicity. Toward this end, liquid chromatography-mass spectrometry (LC/MS) methods have been developed and applied to analyze genistein and daidzein, compounds found in soy products. Additional LC/MS methods have been developed for the sensitive and selective detection of DNA adducts formed through metabolic activation of exogenous chemical carcinogens and by reactive oxygen species that are byproducts of normal aerobic metabolism.

A strong emphasis within the Division has been in the area of nutritional folic acid deficiency. As part of this program, Division investigators have evaluated the progression of global DNA hypomethylation and promoter region hypermethylation in the tumor suppressor genes, p53 and p16. They have also assessed thiol metabolites associated with folate-dependent homocysteine metabolism and have shown that increased plasma homocysteine, a risk factor for cardiovascular disease and certain birth defects, is associated with a parallel increase in S-adenosylhomocysteine. In further work they demonstrated that abnormal folate metabolism was associated with polymorphisms in the methylene tetrahydrofolate reductase and methionine synthase reductase genes in mothers of children with Down Syndrome.

FY 2001 Accomplishments and FY 2002 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Beland, Frederick

- **Tumorigenicity of Chloral Hydrate in $B_6C_3F_1$ Mice** — E0211601 — CDER — Agent-Driven

  Objective(s):

  To determine the effect of animal age and duration of exposure upon the tumorigenicity of chloral hydrate in female $B_6C_3F_1$ mice.

  FY 2001 Accomplishments:

  NTP final report completed.

  FY 2002 Plans:

  None.

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

  Objective(s):

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

  FY 2001 Accomplishments:

  1) Completed pathology, including audit of pathology results.
  2) Initiated molecular analyses of tumor mutations.
  3) Continued DNA adduct analyses, with emphasis on the quantities of adducts formed in specific types of liver cells.
  4) Began preparation of draft NCTR/NTP final report.

  FY 2002 Plans:

  1) Continue molecular analyses of tumor mutations.
  2) Complete NCTR/NTP final report.

- **Perinatal Carcinogenicity of Drug Combinations used to Prevent Mother-to-Child Transmission of HIV** — E0214111 — None — Agent-Driven

  Objective(s):

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

  FY 2001 Accomplishments:

  Prepare protocol.

  FY 2002 Plans:

  Initiate studies.

Title	Project Number	Collaborator	Strategic Research Goal

- **ADDEND: The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bio-assay and in Transgenic Human Lympho-blastoid Cells** — **E0687923** — **None** — **Agent-Driven**

  Objective(s):

  Continue the work initially outlined in the protocol E0687917 with selected reverse transcriptase inhibitors used in the treatment of AIDS.

  FY 2001 Accomplishments:

  1) Evaluated the mutagenicity and induction of micronuclei of the following reverse transcriptase inhibitors used in the treatment of AIDS: zidovudine (AZT), didanosine (ddI), lamivudine (3TC), and stavudine (d4T). Similar experiments were conducted on the following combinations of reverse transcriptase inhibitors: AZT/3T3 and AZT/ddI.
  2) Conducted molecular analyses on the type of mutations induced by the reverse transcriptase inhibitors.
  3) One manuscript was submitted for publication.

  FY 2002 Plans:

  Evaluate the mutagenicity and induction of micronuclei of the reverse transcriptase inhibitors nivirapine and zalcitabane (ddC).

- **DNA Adducts of Tamoxifen** — **E0701101** — **None** — **Agent-Driven**

  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.

  FY 2001 Accomplishments:

  1) Assessed the role of *N, N*-didesmethyltamoxifen and its α-hydroxy derivative in the genotoxicity of tamoxifen.
  2) Conducted the Big Blue mutagenesis assay to assess the mutagenicity of tamoxifen and its suspected metabolites in various tissues.
  3) Developed a LC/MS method to quantify tamoxifen DNA adducts.
  4) Initiated studies to assess the presence of tamoxifen DNA adducts in women being treated with the drug.
  5) Initiate studies to assess the presence of tamoxifen DNA adducts in nonhuman primates administered tamoxifen.
  6) Submitted two papers.

  FY 2002 Plans:

  1) Submit two manuscripts.
  2) Continue DNA adduct studies in women receiving tamoxifen.

Title	Project Number	Collaborator	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** — **None** — **Agent-Driven**

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.
5) Determine the influence of *Aloe vera* components on simulated solar light-induced tumor formations in mice.

FY 2001 Accomplishments:

1) Prepared protocol to examine whether *Aloe vera* potentiates simulated sunlight-induced skin damage and tumor formation.
2) Initiated experiments to examine the dose and temporal effects of topical *Aloe vera* treatment on the structure of mouse skin in the absence of simulated solar light.
3) Examined by microscopy SKH-1 mice skin samples to determine the duration of treatment to use in subsequent experiments.

FY 2002 Plans:

1) Complete the initial experiments to establish the dose and temporal effects of topical application of *Aloe vera* on the structure of mouse skin.
2) Conduct the range-finding study for *Aloe vera* test substances on mouse skin.
3) Begin experiments to examine the dose and temporal effects of simulated solar light exposure on mouse skin in the presence and absence of topical application of *Aloe vera*.
4) Prepare draft review article on *Aloe vera*.
5) Initiate experiments to evaluate *in vitro* expression of ornithine decarboxylase and cyclooxygenase following exposure to UV light.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Chou, Ming

- **A Study of Genotoxic and Secondary Mechanisms of Riddelliine Carcinogenesis** — **E0213301** — **None** — **Predictive Toxicology**

  Objective(s):

  1) To study the mechanisms of direct-acting genotoxicity (involving exogenous DNA adduct formation) of riddelliine.
  2) To analyze riddelliine-derived DNA adducts in target tissues of rats treated with riddelliine as part of the NTP chronic study, and from male and female rats to be treated at the NCTR for a shorter period of time with riddelliine and its reactive metabolite, dehydroriddelliine.
  3) If a dehydroretronecine-modified DNA adduct is detected in the liver tissues of animals treated with riddelliine, propose to determine whether or not this DNA adduct is also formed in animals treated with other tumorigenic pyrrolizidine alkaloids.
  4) To compare the metabolic activation pathways and DNA adduct formation of the tumorigenic pyrrolizidine alkaloid, riddelliine, retrorsine, and monocrotaline, and a non-tumorigenic pyrrolizidine alkaloid, retronecine, in rat and human liver microsomal systems.

  FY 2001 Accomplishments:

  1) Determined that riddelliine-induced liver tumors in F344 rats occur through a genotoxic mechanism.
  2) Established that set of eight DHP-derived DNA adducts is responsible in part, if not all, for the liver tumor development.
  3) Conducted studies to characterize the blood toxicokinetics and DHP-derived DNA adducts in rat and mouse parenchymal and endothelial cells *in vivo*.
  4) Completed NCTR/NTP final report.
  5) Published three papers.

  FY 2002 Plans:

  None.

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

  Objective(s):

  1) Test the hypothesis that a chemically induced tumor incidence is a function of the accumulated lifetime exposure.
  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.

  FY 2001 Accomplishments:

  Project completed.

  FY 2002 Plans:

  Prepare NCTR technical report.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

- **Effects of Dietary Restriction on the Post-Initiation Stages in Aflatoxin B_1(AFB_1)-Induced Carcinogenesis on Male F344 Rats Fed Methyl-Deficient Diets** — **E0695201** — **None** — **Concept-Driven**

Objective(s):

To 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.
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.

FY 2001 Accomplishments:

A manuscript describing relationships between methyl deficiency, dietary restriction, hepatic cell proliferation, and telomerase activity was published.

FY 2002 Plans:

None.

PI: Culp, Sandra

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

Objective(s):

To determine the risk associated with exposure to malachite green or leucomalachite green.

FY 2001 Accomplishments:

Completed In-life phase of bioassay with malachite green/leucomalachite green in mice.

FY 2002 Plans:

Complete NCTR/NTP final report.

- Two-Year Bioassay in Rats Administered Malachite Green or Leucomalachite Green in the Diet — E0212801 — CVM — Agent-Driven

Objective(s):

To determine the risk associated with exposure to malachite green or leucomalachite green.

FY 2001 Accomplishments:

1) Completed In-life phase of bioassay with malachite green/leucomalachite green in rats.

Title	Project Number	Collaborator	Strategic Research Goal

2) Completed Big Blue Rat experiments with leucomalachite green.
3) A manuscript describing the results was accepted for publication.

FY 2002 Plans:

Complete NCTR/NTP final report.

PI: Delclos, K. Barry

- **Range Finding Study for the Evaluation of the Toxicity of Genistein Administered in the Feed to CD (Sprague-Dawley) Rats** — E0212201 — None — Agent-Driven

Objective(s):

To 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.

FY 2001 Accomplishments:

1) Two manuscripts were submitted and accepted for publication.
2) One additional manuscript was drafted describing down-regulation of estrogen receptor beta in the prostate by genistein.
3) Data from this study and the other dose range-finding studies played an important role in the NTP/EPA meeting on Low Dose Effects of Endocrine Disruptors held in October, 2001.

FY 2002 Plans:

Upon receiving the audited support reports, a final report will be prepared.

- **Range Finding Study for the Evaluation of the Toxicity of Methoxychlor Administered Feed to CD (Sprague-Dawley) Rats** — E0212301 — None — Agent-Driven

Objective(s):

To determine the doses of methoxychlor for use in a multigeneration bioassay for assessing the effects of this pesticide on the development of the reproductive tract, reproduction, cancer of the reproductive organs, and neurological and immunological function.

FY 2001 Accomplishments:

Analysis of In-life data was completed. A decision was made to discontinue the pathology on this study.

FY 2002 Plans:

Summary of the data collected in this aborted experiment will be prepared to close out this experiment.

Title	Project Number	Collaborator	Strategic Research Goal

- **Range Finding Study for the Evaluation of the Toxicity of Nonylphenol Administered in the Feed to CD (Sprague-Dawley) Rats** — **E0212501** — **None** — **Agent-Driven**

  Objective(s):

  To 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.

  FY 2001 Accomplishments:

  1) Two manuscripts were submitted and accepted for publication.
  2) Data from this study and the other dose range-finding studies played an important role in the NTP/EPA meeting on Low Dose Effects of Endocrine Distruptors held in October, 2001.

  FY 2002 Plans:

  Upon receiving the audited support reports, a final report will be prepared.

- **Range Finding Study for the Evaluation of the Toxicity of Vinclozolin Administered in the Feed to CD (Sprague-Dawley) Rats** — **E0212601** — **None** — **Agent-Driven**

  Objective(s):

  To 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.

  FY 2001 Accomplishments:

  1) Analysis of In-life data and pathology were completed.
  2) Data from this study and the other dose-range-finding studies played an important role in the NTP/EPA meetings on Low Dose Effects of Endocrine Disruptors held in October, 2001.

  FY 2002 Plans:

  1) Data will be presented at the 2002 Society of Toxicology meeting.
  2) A manuscript will be drafted and, upon receiving the audited support reports, a final report will be prepared.

- **Range Finding Study for the Evaluation of the Effects of Ethinyl Estradiol Administered in the Feed to CD (Sprague-Dawley) Rats During Development** — **E0212901** — **None** — **Agent-Driven**

  Objective(s):

  To 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.

  FY 2001 Accomplishments:

  1) Analysis of In-life data and pathology were completed.
  2) One manuscript was submitted and accepted for publication. Preparation of a second manuscript began.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

A manuscript will be submitted and, upon receiving the audited support reports, a final report will be prepared.

- **Genistein: Evaluation of Reproductive Effects Over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages** — E0213201 — None — Agent-Driven

Objective(s):

1) To determine the effects of genistein, a naturally occurring isoflavone, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations.
2) To determine if subtle effects observed in the dose-range-finding study are magnified through multiple generations.
3) To evaluate the reversibility of any observed effects.
4) To 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).

FY 2001 Accomplishments:

1) Data analysis on the reproductive phase of the study began and pathology started.
2) In-life portion of the chronic phase of the study was completed.
3) Completed analyses of sex steroid receptor changes induced by genistein in male reproductive tract. Confirmed down-regulation of estrogen receptor beta in the prostate that had been observed in the dose-range-finding study.

FY 2002 Plans:

1) Pathology and statistical analyses of both the reproductive and chronic phases of this study should be completed during this fiscal year. Data interpretation and the preparation of manuscripts and reports will be started.
2) Assess the modulation of sex steroid receptors by dietary genistein in the reproductive organ.

- **para-Nonylphenol: Evaluation of Repro- ductive Effects over Multiple Generations** — E0213501 — None — Agent-Driven

Objective(s):

1) To 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) To determine if subtle effects observed in the dose-range-finding study are magnified through multiple generations.
3) To evaluate the reversibility of any observed effects.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

1) In-life portion of the experiment was completed. Pathology started late in the year.
2) A manuscript on the effects of nonylphenol on serum testosterone and testicular steroidogenic enzymes was prepared and submitted.

FY 2002 Plans:

Pathology and statistical analyses of the data from this study will continue. Data interpretation and the preparation of manuscripts and reports will be started.

- **Ethinyl Estradiol: Evaluation of Reproductive Effects over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages** — E0213801 — None — Agent-Driven

Objective(s):

1) To 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) To determine if subtle effects observed in the dose-range-finding study are magnified through multiple generations.
3) To evaluate the reversibility of any observed effects.
4) To 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.

FY 2001 Accomplishments:

1) Both the reproductive and chronic toxic assessment phases of this experiment were started.
2) Experiments were conducted to examine the effects (stimulation of male mammary, increased prostate weight, acceleration of puberty) observed in the dose range-finding as low-dose effects. The stimulation of the male mammary gland appeared to be a reproducible effect that persisted into adulthood (the dose range-finding study animals had been terminated during puberty).

FY 2002 Plans:

1) The reproductive phase of this study will end, while the chronic phase will continue through the year.
2) Data collection on the prostate, testis, and mammary gland from addendum animals will continue and should be completed. Presentation of a portion of the data at the annual Society of Toxicology is anticipated.

Title	Project Number	Collaborator	Strategic Research Goal

- **ADDEND: Effect of Diet and Genetic Background of Sprague Dawley Rats on the Expression and Activity of Cytochrome P4501B1** — **E0702721** — **None** — **Agent-Driven**

  Objective(s):

  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 F896 diet up-regulates CYP1B1 activity in the rat adrenal or that the NCTR CD (SD) rat contains higher CYP1B1 activity than Sprague Dawley rats from common commercial suppliers (Charles River and Harlan).

  FY 2001 Accomplishments:

  The data from the pilot study was evaluated and it was determined that the experiment as it had been planned would be terminated. Data from the pilot study suggested that a particular isozyme of cytochrome P450 might be expressed at a higher level in the test animals used either due to genetic background or the diet used. An addendum to the protocol was written to evaluate this possibility.

  FY 2002 Plans:

  Experiments will begin. In addition, we are considering expanding this experiment to include males and to evaluate differences in gene expression due to diet and genetic factors utilizing array technology.

- **Effects of Endocrine-Active Agents on Bone** — **E0710601** — **CFSAN** — **Agent-Driven**

  Objective(s):

  We hypothesize that the administration of the endocrine-active agents genistein and ethinyl estradiol (EE2) will alter bone growth and remodeling and that the direction and extent of the effect will depend on the window of exposure to the compounds. Utilize the experience of Bionetics staff and tissues available from the on-going endocrine disruptor studies to address an important health concern.

  FY 2001 Accomplishments:

  Protocol was written and submitted for review. Some preliminary analyses of bone collected from the genistein and ethinyl estradiol experiments was completed.

  FY 2002 Plans:

  Analyses of bones that have been collected from animals treated with ethinyl estradiol and genistein will continue.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Doerge, Daniel

- **Toxic Hazards from Anti-thyroid Chemicals** — **E0692001** — **None** — **Concept-Driven**

Objective(s):

1) To determine inhibition mechanisms for environmental goitrogens using purified thyroid peroxidase and lactoperoxidase.
2) To 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) To 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) To determine the structure-activity relationship for uptake of goitrogens into the thyroid and inhibition of thyroid hormone synthesis in rats.

FY 2001 Accomplishments:

Published manuscript describing the effects of dietary genistein on the rat thyroid.

FY 2002 Plans:

None.

- **Development of Methods for Analysis and Confirmation of *b*-Agonists** — **E0694501** — **None** — **Method-Driven**

Objective(s):

1) To 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) To develop synthetic procedures to produce authentic β-agonists standards for use in regulatory screening. These methods will provide the flexibility to adapt to the potential for "designer drug" modifications by clandestine laboratories.
3) To explore the use of packed column supercritical fluid chromatography (SFC) coupled with APCI/MS as a more efficient technique for chromatographic separation in the screening of large numbers of β-agonists in livestock tissues.

FY 2001 Accomplishments:

1) Developed a sensitive and selective LC/MS/MS method for the analysis in several important livestock species of ractopamine, a recently approved β-agonist growth promoter with significant trade implications.
2) A manuscript describing this work is in final stages of preparation.
3) In addition, a multiresidue confirmatory LC/MS/MS method has been validated foruse in livestock tissues.

FY 2002 Plans:

Write a final manuscript for the multiresidue method.

Title	Project Number	Collaborator	Strategic Research Goal

- **Measurement of Oxidative DNA Damage in Normal and Hepatitis C-Infected Human Liver** — **E0706401** — **None** — **Method-Driven**

  Objective(s):

  1) To develop simple synthetic methods to produce stable labeled analogs of 8-oxo-dG, etheno-dA, etheno-dC, and M1-dG.
  2) To 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 chromatography and tandem mass spectrometry.
  3) To apply methodology to the analysis of hepatic DNA from humans and animals.
  4) To determine feasibility for application to clinical trials of therapeutic agents and toxicity/carcinogenicity testing in experimental animals.

  FY 2001 Accomplishments:

  1) A sensitive LC/MS/MS method was validated for use in rodent and human DNA.
  2) The method has been applied to normal and diseased human liver, rat liver and mammary DNA, and human leukocyte DNA to better understand the role of diet and disease state on the production of oxidative DNA damage.
  3) A manuscript describing the initial phases of this work is currently undergoing journal review.

  FY 2002 Plans:

  Apply this methodology to hypothesis-driven studies of humans and rodents to establish a role for oxidative DNA damage in cancer etiology.

- **ADDEND: Evaluation of Serum Nonylphenol in CD (Sprague-Dawley) Rats Exposed to Dietary Nonylphenol** — **E0213531** — **None** — **Agent-Driven**

  Objective(s):

  Utilize excess animals from the parent experiment (E0213501) to assess serum and tissue nonylphenol levels (F2 generation only) by high pressure liquid chromatography and atmospheric pressure chemical ionization mass spectrometry (LC-APCI/MS). The data obtained will be important in the interpretation of the biological data gathered in the study.

  FY 2001 Accomplishments:

  1) Mass spectrometry was used to quantify nonylphenol (NP) and metabolites in serum and endocrine-responsive tissues from dietary exposure in Sprague-Dawley rats.
  2) Tissue accumulation of NP aglycone was observed despite the predominance of glucuronidation in blood. Serum toxicokinetics of total NP, measured following gavage administration showed rapid absorption and elimination.
  3) NP was similarly administered by gavage to pregnant dams and total and aglycone NP were measured in dam serum and fetuses to show placental transfer into serum and brain.
  4) These data provide a basis for future correlations of biological effects observed following dietary exposure in rats with those predicted from environmental exposures to humans.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

None.

- **ADDEND: Evaluation of Ethinyl Estradiol Pharmacokinetics in CD (Sprague-Dawley) Rats** — **E0213821** — **None** — **Method-Driven**

Objective(s):

Utilize excess animals from parent protocol (E0213801) to assess pharmacokinetics of ethinyl estradiol by measuring serum levels using high pressure liquid chromatography and atmospheric pressure chemical ionization tandem mass spectrometry following an oral gavage dose.

FY 2001 Accomplishments:

1) Completed the development and validation of a sensitive LC-ES/MS/MS method for measurement of ethinyl estradiol (EE2) serum pharmacokinetics in the rat following oral gavage.
2) Pharmacokinetic analyses showed significant gender-specific differences that may be important in understanding the differences in susceptibility to the estrogenic effects of EE2 observed.

FY 2002 Plans:

Complete a manuscript describing this work.

PI: Fu, Peter

- **The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells** — **E0687901** — **CDER** — **Predictive Toxicology**

Objective(s):

1) To determine if the neonatal mouse bioassay can be employed to evaluate the tumorigenic potential of therapeutic drugs.
2) To examine concurrently as positive controls the genotoxic carcinogens: 4-aminobiphenyl, benzo(a)pyrene, 6-nitrochrysene, and aflatoxin B_1.
3) To 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) Employ transgenic human lymphoblastoid cell lines expressing appropriate CYP isozymes to study the mutations and DNA binding of the subject drugs.

FY 2001 Accomplishments:

Twenty-one (21) compounds were tested in the neonatal $B_6C_3F_1$ mouse bioassay including ochratoxin A, fumonisin B_1, malachite green, leucomalachite green, riddelliine, dehydroretronecine, benzidine, 2-napthylamine, 4,4'-methylenebis(2-chloroaniline), 4-nitrobiphenyl, styrene oxide, N'-nitrosonornicotine, benzene, glycidol, aristolochic acid, carboplatinum, mitomycine C, procarbazine hydrochloride, pantoprazole, lansoprazole and omeprazole.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

1) Pathology analyses on the compounds listed in the accomplishments section will be completed.
2) An addendum will be prepared to (a) conduct a range-finding study to establish a dose for i.p. administration of the one proton pump inhibitor (PPI), rabeprazole, that was not received in time to accommodate inclusion in the tests loaded under E687919 and establish additional doses of the four PPIs, lansoprazole, pantoprazole, omeprazole, and rabeprazole; (b) assess the tumorigenicity of i.p. administered rabeprazole and gavage-administered lansoprazole, pantoprazole, omeprazole, and rabeprazole.

PI: Howard, Paul

- **Chronic Tumor Study of Fumonisin B_1 in Male and Female $B_6C_3F_1$ Mice** — E0210601 — None — Agent-Driven

Objective(s):

Determine if dietary fumonisin B_1 is tumorigenic to male and female $B_6C_3F_1$ mice following chronic dietary exposure.

FY 2001 Accomplishments:

1) NCTR final report completed.
2) NTP technical report completed
3) Three manuscripts completed.

FY 2002 Plans:

None.

- **Chronic Tumor Study of Fumonisin B_1 in Male and Female F344 Rats** — E0210801 — None — Agent-Driven

Objective(s):

Determine the tumorigenicity of fumonisin B_1 in male and female F344 rats following chronic dietary exposure.

FY 2001 Accomplishments:

1) NCTR final report completed.
2) NTP technical report completed.
3) Three manuscripts completed from study.

FY 2002 Plans:

These projects will close, however, one more publication is in preparation.

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

Objective(s):

1) To determine the effect of fumonisin B_1 on signal transduction pathways in cultured human esophageal epithelial tissues.
2) To 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 technology.

Title	Project Number	Collaborator	Strategic Research Goal

3) To determine the pharmacokinetics of fumonisin B_1 in $B_6C_3F_1$ mice and F344 rats under conditions similiar to those used in the chronic bioassay and in non-human primates.

FY 2001 Accomplishments:

1) Demonstrated that a unique DNA adduct is present following incubation of *Fusarium* fungi extracts with DNA, and that this same DNA adduct is present in human esophageal tumor tissue from South Africa.
2) The results, although preliminary, suggest that this adduct, in concert with fumonisin or other mycotoxins, may participate in the high incidence of esophageal cancer in South Africa.
3) A presentation was given at the annual Society of Toxicology meeting on the occurrence of *Fusarium* DNA adducts in human esophageal tumor tissue.

FY 2002 Plans:

1) A manuscript concerning the presence of *Fusarium* DNA adducts in human esophageal tissue will be submitted.
2) A manuscript on the pharmacokinetics of fumonisin B_1 in the Rhesus monkey will be submitted.

- **Comparative Toxicity of Fumonisin Derivatives in Female $B_6C_3F_1$ Mice** — E0212401 — None — Agent-Driven

Objective(s):

To compare the toxicity of several fumonisin derivatives in female $B_6C_3F_1$ mice.

FY 2001 Accomplishments:

1) Examined the toxicity in $B_6C_3F_1$ mice (28-day feeding study) of several fumonisin derivatives including fumonisin B_1, fumonisin B_2, fumonisin B_3, hydrolyzed fumonisin B_1, fumonisin P_1, N-(acetyl) fumonisin B_1, and N-(carboxymethyl) fumonisin B_1.
2) The toxicity of these derivatives was determined by analyses of serum analytes, histopathology of tissues, and tissue levels of spinglipid cha.
3) Fumonisin B_1 was essentially the only toxic derivative, and the data suggest that monitoring fumonisin B_1 levels, and not necessarily all fumonisin derivatives, is an accurate predictor of the overall toxic potential of the fumonisins.

FY 2002 Plans:

Complete final report and manuscript.

- **The Effects of Chemoexfoliation Using a- and b-hydroxy Acids on Cell Proliferation and DNA Adduct Formation in SKH-1 Mice Exposed to Simulated Solar Light** — E0213101 — CFSAN — Agent-Driven

Objective(s):

The NIEHS/FDA/NCTR Phototoxicity Research and Testing Laboratory is designed to address the effects of compounds on the induction of skin cancer in mice using light sources that are relevant to humans. The facility is also designed for expansion to allow simultaneous examination of the toxicity or co-carcinogenicity of compounds in the presence of either simulated sunlight or fluorescent UVB light. The mechanistic

Title	Project Number	Collaborator	Strategic Research Goal

studies in this proposal will provide the data necessary to design and interpret properly the future alpha-hydroxy acid and simulated solar light co-carcinogenicity studies.

FY 2001 Accomplishments:

The phototoxicology facility that was required for conducting the studies was completed is one of the three National Toxicology Program Centers for Research Excellence (NTP Center for Phototoxicology). The studies on the mechanism of action of alpha- and beta-hydroxy acids and impact of short-term treatment of mice with creams containing the alpha- and beta-hydroxy acids were completed. Several manuscripts have been submitted/published concerning the effects of the acids on the mice. Treatment of mice with the acid containing creams results in increased epidermal thickness and increased epidermal basal cell proliferation. When mice are exposed to UV-containing light, the epidermal change elicited by the creams results in a higher amount of UV light to induce a sunburn, or in other words, the adaptations in the skin protect the mice from UV light. This effect is not as apparent when mice are additionally exposed to six weeks of low levels of UV-containing light. Analysis of the DNA damage is still in progress.

FY 2002 Plans:

Complete submission of manuscripts and final report on this study.

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

Objective(s):

To 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.

FY 2001 Accomplishments:

1) The photocarcinogenesis study was conducted.
2) Mice were exposed to light (none, low, medium and high level) with and without topical application of creams (none, control, 4% glycolic acid, 10% glycolic acid, 2% salicyclic acid, 4% salicylic acid).
3) Skin lesions that are consistent with skin tumors were monitored to determine if the application of the creams affected the formation of UV-light-induced skin tumors.

FY 2002 Plans:

1) Complete the pathological analysis of the skin tumors in the mice.
2) Complete statistical analysis of the occurrence of skin tumors in the mice.
3) Submit NCTR final report and the NTP technical report.

Title	Project Number	Collaborator	Strategic Research Goal

- **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** — **None** — **Agent-Driven**

  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.

  FY 2001 Accomplishments:

  1) Mice were treated with the following for 6 weeks: no treatment; 8-methoxypsoralen (8-MOP); UVA light; 8-MOP and UVA light.
  2) Skin was isolated from the mice 2 days after the last treatment, and isolated by techniques required for microarray analysis. Control-matched samples were sent to the NIEHS Microarray Center for analysis.
  3) The mRNA samples were not usable for microarray analysis, although RT-PCR analysis indicated viable mRNA for low-abundance gene products. Analysis of the mRNA has been ongoing to determine why the samples were inadequate for microarray analysis.

  FY 2002 Plans:

  1) Continue to evaluate the integrity of the mRNA samples using real-time RT-PCR and other techniques.
  2) Work with the NIEHS Microarray Center to develop additional methodologies for isolation of mouse skin mRNA.

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

  Objective(s):

  1) Determine the chemicals in tattoo pigments and investigate 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 photocarcinogenic in the SKH-1 hairless mouse using simulated solar light.

  FY 2001 Accomplishments:

  This protocol was prepared and submitted.

  FY 2002 Plans:

  1) Development of methods for identification and quantification of tattoo pigment constituents.
  2) Investigate metabolism of tattoo pigments, and photostability of tattoo pigments.

Title	Project Number	Collaborator	Strategic Research Goal

PI: James-Gaylor, Sandra

- **Nutritional Modulation of Apoptosis and Chemosensitivity: A Novel Anticancer Strategy** — **E0700301** — **None** — **Concept-Driven**

  Objective(s):

  1) In nitroso methylurea (NMU)-initiated mammary epithelial cells, 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.
  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.

  FY 2001 Accomplishments:

  Histological processing of samples was completed.

  FY 2002 Plans:

  Prepare final report.

- **Molecular and Metabolic Determinants of Maternal Risk and Progression of Down Syndrome: Potential for Nutritional Interventions** — **E0701601** — **None** — **Concept-Driven**

  Objective(s):

  1) To define abnormalities in one-carbon metabolism in mitogen-stimulated lymphocytes from women who have had a child with Down Syndrome and to determine whether appropriate folate/methyl supplementation can normalize these metabolic abnormalities.
  2) To define the biochemical and molecular consequences of abnormal one-carbon metabolism in mitogen-stimulated lymphocytes from Down Syndrome children and to determine whether these metabolic abnormalities can be normalized with targeted nutritional intervention.

  FY 2001 Accomplishments:

  1) Published a paper on a second polymorphism in the folate pathway that interacts with methylene-tetrahydrofolate reductase (MTHFR) to further increase risk of Down Syndrome.
  2) Continued development of HPLC/EC methodology to evaluate folate metabolites in plasma and tissue.

  FY 2002 Plans:

  1) Use expression array technology to determine tissue-specific gene expression in various tissues obtained at autopsy from infants with Down Syndrome.
  2) Submit for publication a newly developed method for multiplexing MTHFR C667T and A1298C polymorphisms.

Title	Project Number	Collaborator	Strategic Research Goal

- **DNA Damage with Dietary Methyl Donor Deficiency** — E0706501 — None — Concept-Driven

Objective(s):

To further the understanding of the mechanisms by which diet, as an environmental variable, can alter the susceptibility to cancer.

FY 2001 Accomplishments:

1) Used cDNA array technology to establish nutritionally induced alterations in expression of DNA repair genes during various stages of neoplasia.
2) Completed evaluation and demonstrated that oocytes from folate-deficient dams have reduced pericentromeric DNA methylation.
3) Published paper on alterations in methylation in the p53 promoter region.
4) Determined methylation changes in the CpG island of the p16 gene during tumor progression.
5) Submitted protocol E7085 for external review to extend our studies.
6) Speaker (2 sessions), Experimental Biology 2001; Invited session chair, 12th International Symposium on the Chemistry and Biology of Pteridines and Folates; Speaker (2 sessions), Third International Conference on Homocysteine; Invited moderator for session on "Methyl metabolism and Biochemistry," Trans-NIH Conference on Diet, DNA Methylation and Health.

FY 2002 Plans:

Develop techniques for metaphase spreads in human lymphocytes to examine alternations in chromatin conformation and methylation at the centromeric region in folate-deficient media.

PI: Roberts, Dean

- **Antigenic Biomarkers of Estrogen Catechol Metabolism for Use in Epidemiological Studies** — E0705701 — None — Predictive Toxicology

Objective(s):

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

FY 2001 Accomplishments:

1) Developed improved extraction techniques for preparing large volumes of urine for determination of estrogen metabolites.
2) Published one manuscript and began preparation of another.
3) Made presentation at the American Association of Cancer Research (AACR).

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

Complete experiments and prepare final report.

PI: Tolleson, William

- **The Role of Human Metabolism in Endocrine Disruption** — **E0702301** — **None** — **Method-Driven**

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 P450, UDP-glucuronosyltransferases, and sulfotransferases in the antiestrogens. The relative abilities of 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.

FY 2001 Accomplishments:

1) Examined the competence of human hepatic and extrahepatic microsomal enzymes to catalyze reactions involving biochanin A and formononetin previously attributed to microorganisms endogenous to the gastrointestinal tract.
2) Determined that human liver microsomal enzymes are capable of isoflavone 4'-O-demethylation to liberate genistein and daidzein from biochanin A and formononetin, respectively.
3) Identified three monohydroxylated and two dihydroxylated formononetin metabolites by HPLC-ES-MS/MS. Further characterized by ^{1}H-NMR (Proton Nuclear Magnetic Resonance) and identified as 6-, 8-, and 3'-hydroxyformononetin derivatives. HPLC-ES-MS/MS also identified two monohydroxybiochanin A metabolites.
4) Prepared a manuscript.

FY 2002 Plans:

Continue investigation of altered estrogen metabolism via substrated competition by the common isoflavones found in legumes, namely genistein, daidzein, formononetin, and biochanin A.

- **Purification of Ceramide Synthase** — **E0705901** — **None** — **Concept-Driven**

Objective(s):

1) To isolate rat ceramide synthase.
2) To identify the gene coding for rat ceramide synthase.
3) To develop antibodies to rat ceramide synthase.
4) To use the antibodies to study tissue-specific expression of ceramide synthase.

FY 2001 Accomplishments:

Detergent extraction, polyethylene glycol precipitation, and strong cation exchange chromatography were used to enrich ceramide synthase activity from rat liver homogenate.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

1) Complete the final purification of ceramide synthase.
2) Obtain additional amino acid sequences to supplement data already obtained.
3) Search protein and nucleic acid data bases for correlations. If none is found, then oligonucleotide probes will be developed from the sequences of the derived peptides for screening cDNA libraries.
4) Upon obtaining the coding sequence for ceramide synthase has been determined, this material will be used to develop ceramide synthase-specific antibodies to study the expression of these enzymes in various tissues.

- **Photoinduction of Cutaneous Malignant Melanoma in TP-ras/ink4A (+/-) Transgenic Mice** — E0708901 — CFSAN — 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 loci in tumor tissues.
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.

FY 2001 Accomplishments:

Protocol prepared, submitted, and approved to evaluate the utility of a transgenic mouse melanoma model for photocarcinogenesis studies.

FY 2002 Plans:

Verify the genotypes of the transgenic breeder mice as they are received from NCI-MMHCC.

FY 2001 Publications

Akerman, G.S., Tolleson, W.H., Brown, K.L., Zyzak, L.L., Mourateva, E., Engin, T., Basaraba, A., Coker, A.L., Creek, K.E. and Pirisi, L., Human papillomavirus type 16 E6 and E7 cooperate to increase epidermal growth factor receptor (EGFR) mRNA levels, overcoming mechanisms by which excessive EGFR signaling shortens the life span of normal human keratinocytes, *Cancer Research*, 61:3837-3843. Accepted: 3/15/01 (Z9999906)

Al-Gazali, L.I., Padmanaban, R., Melnyk, S.B., Yi, P., Pogribny, I.P., Pogribna, M.V., Bakir, M., Hamid, Z.A., Abdulrazzaq, Y., Dawodu, A. and James-Gaylor, S.J., Down syndrome and neural tube defect associated with abnormal folate metabolism and genetic polymorphisms in the folate pathway, *Am. J. Med. Genet.*, 103:128-132. Accepted: 7/1/01 (E0701601)

Boudreau, M.D., Baker, D.G., Taylor, H., Barker, S.A. and Means, J.C., Suppression of Arylamine Toxicity in the Fischer-344 Rat following Ingestion of a complex Mixture, *Toxicologic Pathology*, 29:333-343. Accepted: 1/1/01 (NA)

Boudreau, M.D., Sohn, K.H., Rhee, S.H., Lee, S.W., Hunt, J.D. and Hwang, D.H., Suppression of tumor cell growth both in nude mice and in culture by n-3 polyunsaturated fatty acids: mediation through cyclooxygenase-independent pathways, *Cancer Research*, 61(4):1386-91. Accepted: 12/13/00 (NA)

Caudill, M.A., Melnyk, S.B., Pogribny, I.P., Jernigan, S.L., Swendseid, M.E. and James-Gaylor, S.J., Intracellular S-adenosylhomocysteine concentrations predicts global DNA hypomethylation in tissues of methyl deficient cystathionine Beta synthase deficient mice, *J. Nutrition*, 131:2811-2818. Accepted: 8/1/01 (NA)

Cha, C., Doerge, D.R. and Cerniglia, C.E., Biotransformation of malachite green by the fungus *Cunninghamella elegans*, *Applied and Environmental Microbiology,* 67:4358-4360. Accepted: 6/28/01 (N/A)

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Biometry and Risk Assessment

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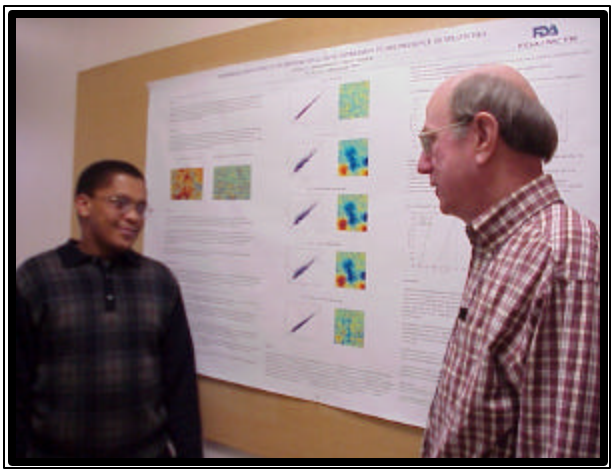

Drs. Molefe and Kodell discuss statistical methods for cDNA microarray data.

Executive Summary

The Division of Biometry and Risk Assessment conducts research to address FDA's regulatory need for new and improved methods for the assessment of human exposure, susceptibility and risk.

FY 2001 Accomplishments

During FY 2001, scientists in the Division of Biometry and Risk Assessment engaged in research projects addressing a variety of risk-assessment issues associated with exposures to toxic chemicals, microbiological pathogens, and radiation.

Projects related to chemical exposures included development of quantitative methods for assessing the cumulative risk of mixtures of chemicals, utilization of PBPK modeling for animal-to-human prediction, development of estimation methods for mutation studies in transgenic mice, and development of improved analytical procedures for standard bioassay tumorigenicity data. Projects related to pathogen exposure included the investigation of dose-response modeling for microbial pathogens in food and water and the investigation of models to explain the transmission of microbial agents in populations composed of a number of subpopulations of varying susceptibility. Projects related to radiation exposure included the analysis of atomic-bomb survivor data and the development of statistical methods for estimation and testing in photocarcinogenicity studies.

FY 2002 Plans

For FY 2002, strong emphasis is being placed on research stimulated by both the explosion of information in the post-genomic era and by the looming threat of bioterrorism. Scientists in the Division will be conducting research related to the statistical analyses of cDNA gene expression microarrays, research addressing issues in bioinformatics and data mining, and research on the spread and detection of microbiological agents of terrorism.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Issues related to microarrays include normalization of gene-expression data in the pre-processing stage; testing for differential expression of genes among comparison groups; and identifying precursor genes, co-expressed genes and target genes for constructing genetic profiles and networks. Issues related to bioinformatics and data mining include reliability of methods for nucleotide sequence matching and prediction of organ-specific toxicity using multiple inputs based on chemical structures and spectra. Issues related to microbiological agents of terrorism include the detection of such pathogens based on protein spectra and the spread of such pathogens through a population.

Experiments are either planned or already underway in all of these areas, with appropriate interaction and collaboration with scientists in FDA's product centers. Of course, work will continue on all ongoing Division projects and on collaborative projects with scientists in other NCTR divisions. Consultation on statistical and pharmacokinetic problems will continue, as will the provision of oversight to contract activities associated with statistical analyses and experimental support.

FY 2001 Accomplishments and FY 2002 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Chen, James

- **Analysis of Multiple Tumor Sites** **E0700901** **CDER** **Method-Driven**

  Objective(s):

  1) To develop analytical and numerical techniques for computing the experiment-wise error rate in testing of multiple tumor sites.
  2) To 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) To evaluate the experiment-wise error rate and power of global statistics for an overall test of carcinogenicity.
  4) To recommend optimal procedures which control the experiment-wise error rate and still maintain the power for the analysis of multiple tumor sites.

  FY 2001 Accomplishments:

  One paper, "Testing for treatment effects on subsets of endpoints", (with S.J. Wang, CDER) was accepted for publication in *Biometrical Journal*.

  FY 2002 Plans:

  Continue collaboration with Dr. S.J. Wang (CDER) and Dr. Hueymiin Hsueh on procedures for testing multiple endpoints/genes.

- **Tests of Equivalence for Dichotomous Endpoints** **E0706201** **CDER** **Predictive Toxicology**

  Objective(s):

  1) To investigate the size of the asymptotic and unconditional exact tests for assessing equivalence between two binomial proportions in 2×2 tables.
  2) To propose an approximate exact test for assessing equivalence between two binomial proportions.
  3) To compare the approximate exact test with the asymptotic and unconditional exact tests in terms of the size and power.
  4) To develop the asymptotic, unconditional, and approximate exact tests of equivalence for the logistic regression trend test in $2\times k$ tables.
  5) To develop the asymptotic, unconditional, and approximate exact tests of equivalence for two multinomial proportions in $k\times 2$ tables.

  FY 2001 Accomplishments:

  1) One paper published in *Biometrics* and one paper accepted for publication in *Statistics in Medicine*.
  2) One manuscript (with J.P. Liu and H.M. Hsueh) submitted for publication.
  3) Presented a paper at the 53rd Session of the International Statistical Institute in Seoul, S. Korea.
  4) Final report was submitted.

  FY 2002 Plans:

  None.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

- **Cumulative Risk Assessment for Chemical Mixtures** — **E0708701** — **None**

Objective(s):

FY 2001 Accomplishments:

1) One paper was published in *Regulatory Toxicology and Pharmacology*.
2) One manuscript was accepted for publication.

FY 2002 Plans:

Continue development of methodology and computational algorithm for calculating cumulative risk.

PI: Kodell, Ralph

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

Objective(s):

1) To 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) To 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) To develop a procedure for estimating the lag time between onset of, and death from, an occult tumor when cause-of-death data are unavailable.
4) To illustrate the new procedures using data from the Project on Caloric Restriction (PCR) studies.

FY 2001 Accomplishments:

1) Paper published on attributing tumor lethality when cause of death not assigned.
2) Paper on modified Peto test based on imputed "cause of death" revised and resubmitted for publication.
3) Invited to write a letter to the editor of *Tox. Path.* commenting on the Society of Toxicologic Pathology's position on Peto's cause-of-death test.

FY 2002 Plans:

1) Submit manuscript on estimating tumor latency.
2) Investigate the estimation of *k* for the Poly-*k* test.
3) Publish paper on modified Peto test using imputed "cause of death."

- **Dose-Response Modeling for Microbial Risk Assessment** — **E0704501** — **None** — **Predictive Toxicology**

Objective(s):

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

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

Paper on statistical models for microbial risk assessment accepted for publication.

FY 2002 Plans:

Continue to investigate issues related to dose-response modeling for microbial risk assessment, including incorporation of model uncertainty.

- **Interagency Agreement on Developing and Evaluating Risk Assessment Models for Key Waterborne and Foodborne Pathogens and Chemicals** — **P00422** — **None** — **Predictive Toxicology**

Objective(s):

To develop and to evaluate 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.

FY 2001 Accomplishments:

1) Developed protocol for an animal experiment to collect data for modeling the transmission dynamics of *Cryptosporidium parvum* (E0708201).
2) Developed protocol for a relative-potency-factor approach to risk assessment for mixtures of chemicals (E0708701).

FY 2002 Plans:

Report progress on E0708201 and E0708701 to EPA and FDA.

- **Interagency Projects** — **S00032** — **None** — **Concept-Driven**

Objective(s):

FY 2001 Accomplishments:

1) Mouse Lymphoma Assay Working Group.
2) ILSI Genetic Toxicology Working Group.
3) ILSI Dose Response Steering Committee.
4) NRC Subcommittee on SWEGs.
5) NTP/EPA Low Dose Endocrine Disruptor Workshop (manuscript published).
6) EPA/CMA/SOT Chemical Mixtures Working Group (manuscript published).
7) NIOSH Workshop on Research Opportunities in Dose-Response Modeling (manuscript submitted).
8) BELLE Symposium Advisory Committee on Non-Linear Dose-Response Relationships.

FY 2002 Plans:

Continue to participate in interagency projects.

- **Risk Assessment (General)** — **S00116** — **None** — **Concept-Driven**

Objective(s):

Efforts in the improvement of Risk Assessment.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

1) Manuscript on tumor analysis adjusted for both body-weight and survival differences accepted and published.
2) Manuscript on risk-based reference doses submitted for publication.
3) Manuscript on change-point dose-response models for continuous variables submitted for publication.
4) Manuscript on interaction between two carcinogens in the two-stage clonal-expansion model accepted and published.

FY 2002 Plans:

Continue risk assessment research efforts.

◆ **Modification and Application of Quantitative Risk Assessment Techniques to FDA-regulated Products** — **S00174** — **CDER CDRH CFSAN CVM** — **Method-Driven**

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.

FY 2001 Accomplishments:

1) Published two manuscripts on fumonisin B_1 risk assessment.
2) Participated in Interagency Risk Assessment Consortium activities, including helping to organize and contributing financial support for the 1st International Conference on Microbial Risk Assessment.
3) Presented talk on risk assessment at CBER/CDER/PhRMA workshop on biostatistics and data management.

FY 2002 Plans:

Continue to collaborate with FDA protocol centers on risk assessment issues.

◆ **Application of Biometrical Procedures for NTP Projects** — **S00175** — **None** — **Concept-Driven**

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.

FY 2001 Accomplishments:

1) Assisted principal investigators and contract statisticians in determining most appropriate statistical analyses to perform on various NTP studies.
2) Assisted principal investigators and contract experimental liaisons with the experimental design and allocation of animals for various NTP studies.

FY 2002 Plans:

Continue to provide statistical support for NTP projects on an *ad hoc* basis.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Molefe, Daniel

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

Objective(s):

1) To 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) To develop a 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) To develop tests for dose-related trend with respect to the expected number of induced tumors and the distribution of time to observation.
4) To extend the model to situations involving multiple or continuous dosing, and situations in which there is a background of spontaneous tumors.
5) To conduct a Monte Carlo simulation study to compare the new methodology to conventional analytical approaches, and to evaluate its robustness and identifiability.
6) To develop user-friendly software for easy implementation of the proposed analytical procedures.

FY 2001 Accomplishments:

1) Developed two software packages for performing between-group differences and the testing for dose-related trends.
2) Used simulations to evaluate and compare the newly developed software for analyzing between-group differences to other existing software.
3) Prepared a manuscript for publication.

FY 2002 Plans:

1) Analyze the CRADA using the developed software.
2) Make user-friendly programs available for use at NCTR.
3) Prepare a manuscript on testing for dose-related trend.
4) Give a poster presentation at the 2002 FDA Science Forum in February, 2002.
5) Present another poster at the 41st Annual Meeting of the Society of Toxicology in Nashville on March, 2002.

PI: Young, John

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

Objective(s):

Using modern SAR technology and statistical approaches, an expert system can be developed to predict rodent carcinogenicity.

FY 2001 Accomplishments:

1) Developed and implemented a research protocol.
2) Interacted with CFSAN.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

3) Presented a paper on method at the Computational Intelligence Methods and Application (CIMA) meeting, February, 2001, Bangor, Wales.
4) Designed and populated a database in Microsoft Access; various subdivisions of the database have been implemented.
5) Constructed 1D and 2D NMR spectra of 1190 chemicals in our database; projected 240 descriptors of these same chemicals.

FY 2002 Plans:

1) Initiate analysis of liver-specific toxicity.
2) Look for patterns for prediction of liver toxicity.

◆ **Species Comparison Utilizing a PBPK Model** **P00393** **None** **Predictive Toxicology**

Objective(s):

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

FY 2001 Accomplishments:

1) A major methyl mercury PBPK model paper was published.
2) Methyl mercury whole body and blood elimination half-life across species was analyzed utilizing rough set and neural network methodology.
3) Cocaine database was constructed and preliminary PBPK modeling was initiated.

FY 2002 Plans:

1) Continue development of the PBPK model for methyl mercury conversion to inorganic mercury.
2) Continue development of the PBPK model for cocaine.
3) Finish the methyl mercury neural-net manuscript.

PI: Zheng, Qi

◆ **Combining Carcinogenesis Models with Pharmacokinetic Models** **E0703001** **None** **Method-Driven**

Objective(s):

1) To explore methods for using physiologically based pharmacokinetic models as tools for allowing target dose to be directly incorporated into stochastic carcinogenesis models, and hence improve risk assessment for various kinds of carcinogenic chemicals.
2) Within the context of using combined models, to investigate the feasibility of estimating certain biological parameters from data, if such parameter values are not readily available in the literature.

FY 2001 Accomplishments:

1) Investigated a plausible mechanism for U-shaped dose-response relationships by using a carcinogenesis model that mimics DNA adduct formation. Results of this study have been published in the journal *Human and Ecological Risk Assessment.*

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

2) Proposed a computational feasible method for computing dispersion indices for the number of base substitutions. The method was presented at the 2001 Annual Meeting of the American Statistical Association, and was subsequently reported in the journal *Mathematical Biosciences.*

FY 2002 Plans:

To explore the possibility of constructing a carcinogenesis model that explicitly uses genomic data.

- **Developing a Computational Package for Fluctuation Analysis** — **E0710801** — **None** — **Predictive Toxicology**

Objective(s):

1) To develop methods for computing Fisher's information for the expected number of mutations accumulated in a broth medium before plating.
2) To compare critically various methods for estimating mutation rats.
3) To write a comprehensive computer package in Mathematica that will encompass the new methods developed by the author and many existing methods.

FY 2001 Accomplishments:

A draft proposal was submitted to solicit expert comments, and some encouragement was received from reviewers.

FY 2002 Plans:

Finish writing and testing the package mentioned in the title of the protocol.

FY 2001 Publications

Ahn, H., Moon, H., Kim, S. and Kodell, R.L., A Newton-Based Approach for Attributing Tumor Lethality in Animal Carcinogenicity Studies, *Computational Statistics and Data Analysis*. Accepted: 4/24/01 (E0689601)

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Chen, J.J., Dose Response Modeling of Cluster Data, *Encyclopedia of Environmetrics*. Accepted: 10/3/00 (S00116)

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Kodell, R.L., Kang, S. and Chen, J.J., Statistical Models of Health Risk Due to Microbial Contamination of Foods, *Environmental and Ecological Statistics*. Accepted: 3/1/01 (E0704501)

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Liu, J., Hsueh, H., Hsieh, E. and Chen, J.J., Tests for Equivalence or Non-inferiority for Paired Binary Data, *Statistics in Medicine*. Accepted: 4/17/01 (E0706201)

Malling, H. and Delongchamp, R.R., Direct Separation of *in vivo* and *ex vivo* am3 Revertants in Transgenic Mice Carrying the PhiX174 am3, cs70 Vector, *Environmental and Molecular Mutagenesis*, 37:345-355. Accepted: 2/1/01 (S00032, E0709501)

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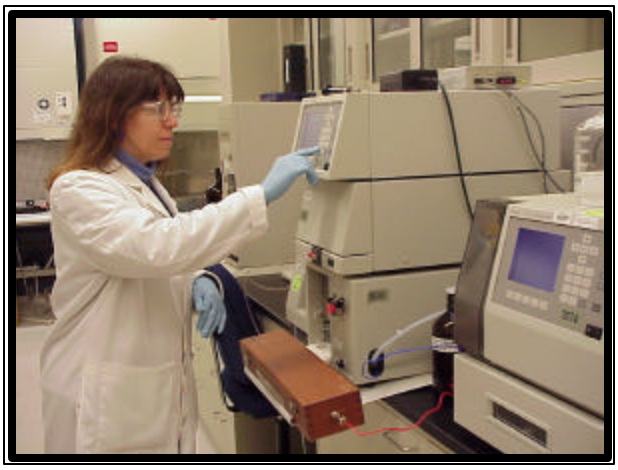

Chemist Beth Brown performs a dose certification HPLC analysis in support of an FDA-nominated NTP study.

Executive Summary

The Division of Chemistry has made significant advances in food quality and safety, risk assessment, predictive toxicology, sensors, mass spectrometry, artificial neural networks, pattern recognition and other research initiatives in support of FDA.

FY 2001 Accomplishments

Fresh Tag™, a rapid chemical sensor to assess freshness and quality of food, has advanced into commercial and consumer test versions. The National Oceanic and Atmospheric Administration/National Marine Fisheries and Canadian Centre for Fisheries Innovation, St. John's, NF, compared two rapid techniques to measure shrimp freshness and selected Fresh Tag™ as the most reliable. Fresh Tag™ will be incorporated into a commercial processing plant for quality control. An interagency agreement was established with the Federal Aviation Administration to develop rapid sensor-based detection methods to screen for explosives in cargo using Fresh Tag™.

Metabolism studies were initiated on heterocyclic aromatic amines (HAAs), carcinogens formed in cooked meats, alcoholic beverages and tobacco smoke. Human liver enzymes are significantly more active than the rat counterparts in the bioactivation of HAAs to genotoxins, suggesting that the toxicity data of experimental rodent models currently used for risk assessment and establishment of acceptable daily intake levels may underestimate the health risk of HAAs.

Methods to measure bioactive ingredients in St. John's Wort were developed and applied to functional foods to provide baseline data that may be used by the FDA for assessing safety of functional foods. Key constituents of St John's Wort, including hypericin and hyperforin, are unstable in some functional foods and also degrade under the acidic conditions of the stomach. Studies are underway to elucidate the structures of these degradation products and their uptake in humans.

In computational chemistry, a novel spectrometric data-activity relationship (SDAR) approach was established that utilizes the interrelationship between chemical

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

structure, spectra and activity for the predictive biological endpoints. ^{13}C nuclear magnetic resonance (NMR) spectra were used to establish quantitative spectrometric data-activity relationships involving binding to the corticosteroid globulin, aromatase enzyme, and aryl hydrocarbon receptors. The correlations had higher cross-validations than previous quantitative structure-activity relationship (QSAR) models using the same endpoints. Advance Chemistry Development in Toronto, Canada, has been negotiating a license with NIH for the original SDAR patent. A High Speed Distributed Neural Network was completed implementing J-Gravity, a Java web-based parallel distribution platform provided under agreement with Titan Lincom, Inc. This platform was used to facilitate artificial-neural-network-based prediction of toxic equivalent factors (TEF) for polychlorinated biphenyls, dioxins, and furans, including values for compounds without established values.

In food safety and counter terrorism research, a new method employing pyrolysis-metastable atom bombardment-time of flight-mass spectrometry (Py-MAB-Tof-MS) demonstrated rapid detection of bacteria in complex matrices such as urine. With collaborators at the University of Montreal, we demonstrated that this method could detect bacteria in a controlled test field release study that simulated an Anthrax outbreak. A novel pattern-recognition approach was established to account for instrumental and biological drift in mass spectra of bacteria generated Py-MAB-Tof-Mass Spectrometry to improve the reliability of this technique. Using a complementary method, matrix-assisted laser desorption ionization (MALDI), protein biomarkers were directly detected from whole cells by Fourier transform MS.

In support of the National Toxicology Program (NTP), analytical methods were developed on key constituents of *Aloe barbadensis* to assure product homogeneity and dose concentrations. Analyses continued on daidzein, ethinylestradiol, genistein, and leucomalachite green. The Mass Spectrometry branch made important contributions to many of these analyses, providing quantitative measurements and assurance of test article purity, which is essential for bioassays. Summary analytical reports on chronic and acute studies were prepared for several of these compounds. Additional studies are envisioned on retinyl palmitate and several therapeutic drugs used for AIDS treatment during 2002.

FY 2002 Plans

Research programs will focus on proteomics, food safety, counter terrorism, metabonomics, biomarkers, and computational chemistry. MALDI-Tof and MALDI-ESI-QTof instruments were acquired for studies in proteomics. The MALDI-Tof will be utilized to characterize intact proteins and post-translational modifications, such as nitration or phosphorylation, which may affect protein function, expression and biological activity. The MALDI-ESI-QTof will be used to sequence peptides for protein identification and quantification of peptides to assess changes in protein expression that may occur in disease states or following a toxic response to a chemical. These Mass Spectrometry techniques may be applied to identify protein biomarkers in

disease states such as cancer, elucidate mechanisms of drug resistance in cells, microbial resistance to antibiotics, and mechanisms of liver toxicity.

Py-MAB-Tof-MS and MALDI Tof-MS will be used to develop rapid methods to identify and differentiate strains of organisms in food safety. Py-MAB-Tof mass spectra of many bacteria will serve as a database for rapid identification of bacteria for issues in food safety and counter terrorism. We will determine whether MS can be used to detect bacteria in complex matrices, such as talc and foods, that may harbor counter terrorism agents. Triple quadrupole mass spectrometry is envisioned for studies in biomarkers of exposure and genetic damage to food toxins, oxidative stress, and identification of contaminants and drugs in complex matrices.

The NMR facilities will be upgraded with a 600 MHz instrument for investigations on macromolecular structures and metabonomic studies. NMR-based metabonomics may identify "spectral biomarkers" in urine and plasma to investigate the effects that drugs may have on metabolism, drug interactions, toxicities and disease states. Metabonomics may address questions on the safety of drugs and in conjunction with proteomics be used to aid the FDA in the approval process of these products.

Artificial neural networks and pattern recognition will be used to identify and detect early markers of breast cancer and brain tumors from proton magnetic resonance scans of rodents and humans and correlate these data to changes in gene expression (in collaboration with the University of Arkansas for Medical Sciences, and the Division of Genetic and Reproductive Toxicology). These approaches will also be used to develop pattern recognition models for lung cancer data and identification of other biomarkers of disease states (in collaboration with the Division of Molecular Epidemiology).

FY 2001 Accomplishments and FY 2002 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Ang, Catharina

- **Development of Analytical Methodologies for Assessing Bioactive Herbal Ingredients in Functional Food Systems** — **E0705601, E0705611** — **CFSAN** — **Method- and Agent-Driven**

Objective(s):

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 teas, drinks, soups, snack, cereals and candies.

FY 2001 Accomplishments:

1) Two manuscripts were completed on the extraction techniques and HPLC determination of major bioactive compounds in St. John's Wort plant leaf/flower mixture and dietary supplements products. Optimum conditions were established.
2) Analytical methods have been developed for the determination of bioactive compounds of St. John's Wort (hypericin, pseudohypericin, hyperforin and adhyperforin) in dietary supplements and functional food systems including tea mix, snack bars, puffs and drinks.
3) Methodologies have also been established for the extraction and determination of phenolic compounds of echinacea components in dietary supplements and tea mixes.
4) Developed an efficient technique for the isolation and purification of hyperforin from St. John's Wort plant for metabolic studies and herb-drug interaction evaluations.

FY 2002 Plans:

1) Investigate factors associated with the instability problems of St. John's Wort bioactive compounds in non-alcoholic, fruit-flavored beverages.
2) Develop analytical methodologies for the determination of ginkgolides in dietary supplements containing *Ginkgo biloba*.

- **Determination of Amoxicillin and Lincomycin Incurred Residues in Salmon and Tilapia for Selection of FDA Method Trial Study Materials** — **P00423** — **CVM** — **Method-Driven**

Objective(s):

Amoxicillin and lincomycin have been identified as having toxicological significance by the CVM, NCTR, and the U.S. Department of Agriculture (USDA) and have been scheduled for incurred-residue FDA method trials. Methods of analysis have been developed and validated for amoxicillin in catfish and salmon and lincomycin in salmon.

Title	Project Number	Collaborator	Strategic Research Goal

1) Validation of the above methods for incurred residues of amoxicillin in salmon and tilapia and lincomycin in salmon are needed.
2) Documentation of incurred-residue confirmation methods by Liquid Chromatography/Mass Spectrometry (LC/MS) are also needed for these regulatory methods.

FY 2001 Accomplishments:

Project has been delayed due to the lack of incurred samples from collaborator, CVM.

FY 2002 Plans:

1) To validate analytical methods using incurred samples and to conduct inter-laboratory validations on the methods as developed. The method trial will be organized by the CVM.
2) To prepare a standard operation procedure of the MS confirmation method for amoxicillin residues in fish tissue.

◆ **Influence of Hyperforin Concentrations on Drug Interactions** — **P00436** — **None** — **Method-Driven**

Objective(s):

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

FY 2001 Accomplishments:

An efficient extraction technique and HPLC method has been developed for the determination of hyperforin content in human plasma samples. Preliminary pharmacokinetic data have been obtained in human objects following a single dose of St. John's Wort dietary supplements.

FY 2002 Plans:

Additional studies will be conducted using different dosages and multi-subjects.

PI: Beger, Richard

◆ **Producing Spectrometric Data Activity Relationship (SDAR) Models for Compounds Binding to Receptors of Toxic Responses** — **E0706801** — **None** — **Predictive Toxicology**

Objective(s):

To produce spectrometric data-activity relationship (SDAR) models using ^{13}C NMR and electron impact (EI)/MS data to predict the potential binding affinity of compounds to specific receptors or produce toxicological response. The major benefit of the experimental SDAR approach is its flexibility since the spectral data can be used for other toxicological systems.

FY 2001 Accomplishments:

1) Three quantitative spectrometric data-activity relationship (QSDAR) modeling publications were accepted for publication.

Title	Project Number	Collaborator	Strategic Research Goal

2) Presented a poster session on SDAR modeling at the Experimental NMR Conference (ENC).
3) Gave five invited oral presentations on SDAR modeling, including the ACD Labs User conference who are very interested in licensing the SDAR patent.
4) Protocol E0708001 was written and granted. Work has begun with Division of Biometry and Risk Assessment and R.O.W. Sciences on developing SDAR-based models that will be trained so it will be able to predict organ-specific carcinogenicity.
5) Protocol E0706811 was granted to examine the potential of the QSAR/SDAR hybrid to predict protein binding.
6) High school mentored by the division won the "first place" award from the Society of Environmental Toxicology and Chemistry at the international science fair for her SDAR modeling of polychlorinated dibenzodioxins and dibenzofurans binding to the Aryl hydrocarbon receptor.
7) Two more SDAR modeling papers are currently being written.

FY 2002 Plans:

1) Expand the SDAR and QSDAR modeling techniques to other toxicological endpoints and publish the results.
2) Develop a new protocol for 3D-QSDAR modeling.
3) Partnerships will be sought to license SDAR modeling patent which will produce royalties for NCTR.
4) Develop a SDAR modeling software product with the licensee(s) of the SDAR modeling patent.
5) Become a beta tester for the SDAR modeling software product that the licensee of the SDAR patent produces.

PI: Billedeau, Stanley

◆ **Development of Methods for Analysis and Confirmation of Erythromycin A Residues in Tissue Samples from Terrestrial and Aquatic Farmed Animals by Liquid Chromatography** — **E0698001** — **CVM** — **Method-Driven**

Objective(s):

Develop determinative and confirmatory analytical chemical procedures using high performance liquid chromatography/electrochemical detection HPLC/ECD and high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometric detection for erythromycin A in biological samples taken from agricultural animals. Specifically, the goal is to develop complete methods for the analysis of erythromycin A in muscle and liver tissue from poultry, non-processed bovine milk, and muscle tissues from salmon, catfish and shrimp. Sensitivity levels for these methods are expected to be at least 100 parts per billion for liver tissue and 50 parts per billion for muscle tissue and milk as requested by the Center for Veterinary Medicine (CVM).

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

1) 14C-Erythromycin work using scintillation counting experiments for evaluation of various extraction systems has been completed with 10 mm phosphate buffer, pH 4.5 in aqueous solution, selected as solution of choice for extraction of the antibiotic from salmon tissue.
2) An HPLC/electrochemical detection (HPLC/ECD) method has been successfully used to analyze erythromycin-spiked salmon tissue extracts at the 100 ppb residue level after cleanup on several types of solid phase extraction (SPE) cartridges including silica-based C-18, PRS, and a number of mixed mode (cation exchange/reversed phase) SPE cartridges.
3) A polymer-based SPE reversed phase cartridge has reported the best HPLC/ECD background trace and preliminary results indicate salmon recovery of about 70% following the developed elution protocol.
4) A confirmatory method has been developed for erythromycin by HPLC/electrospray ionization/MS with linearity and sensitivity as low as 100 confirmatory method pg/µl in standards.

FY 2002 Plans:

1) Validation of the HPLC/ECD method at the 400, 200, and 100 ppb levels in spiked salmon will be completed.
2) CVM has committed to provide NCTR with incurred residues of erythromycin and the dosing protocol has been submitted to the IACUC for approval by Renate Reimschuessel, M.D., CVM.

PI: Buzatu, Dan

- **Comparison of Principal Components Analysis (PCA) and Artificial Neural Networks (ANN) for Prediction of Qualitative and Quantitative Biological End Points from Spectrometric Data** — **E0707701** — **None** — **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.

FY 2001 Accomplishments:

1) Developed a novel method (within the SDAR patent) using an artificial neural network to correlate the ^{13}C NMR spectra of steroid molecules to their binding affinities to the aromatase enzyme.
2) Used a similar approach to train a neural network to learn the relationship between the ^{13}C NMR spectra of dioxin and dioxin-like compounds (PCBs and dibenzofurans) and their toxic equivalence factors (TEFs).
3) The above work has resulted in two manuscripts.

Title	Project Number	Collaborator	Strategic Research Goal

4) Collaborative computational work at the University of Arkansas at Little Rock (UALR) (quantum parameter/ANN based structure activity relationship methods) has resulted in two manuscripts.
5) Conceived the idea of developing a parallel distributed artificial neural network for handling large-scale data sets.
6) Signed license agreement with Titan-Lincom to become a beta test site for a parallel distribution software platform called JGravity.
7) Collaborated on development of a self-optimizing, parallel-distributed artificial neural network using JGravity.
8) Initiated collaboration with UAMS to apply the parallel-distributed artificial neural network to diagnose brain and breast tumors and other tissue anomalies from magnetic resonance (MR) proton and ^{13}C scans.
9) A model was developed with the parallel-distributed artificial neural network (PD-ANN) for the prediction of steroid binding affinities to the corticosteroid binding globulin. This model was developed in support of the patent for the three dimensional quantitative spectral activity relationship (3D-QSDAR) concept.
10) The PD-ANN model performed very well producing a leave-three-out-cross validation variance coefficient (q_3^2) of 0.78, and a leave-ten-out-cross validation variance coefficient (q_{10}^2) of 0.73.
11) Description of the model and the results are included in a manuscript that is currently in the process of internal review and will be submitted to the *Journal of Chemical Informatics and Computational Science*.
12) Several brain tumor diagnostic models were produced from ^{1}H brain magnetic resonance spectra (MRS) using three different pattern recognition methods. These methods included a Principal Components Linear Regression Analysis, the PD-ANN, and Fuzmac (an advanced pattern-recognition software that combines a decision tree with principal components with back-propagation algorithm). Fuzmac was written by Robert Shelton, Ph.D., who is a pattern recognition expert at NASA's Johnson Space Center, and a collaborator.

FY 2002 Plans:

1) The best models for the prediction of brain tumors from ^{1}HMRS had a q_2^2=0.95, a q_4^2=0.88, and a q_{20}^2 of 0.80. This work is currently being drafted into a manuscript that will be submitted to the *Journal of the American Medical Association*.
2) Future work plans include the development of breast cancer diagnostic models from breast proton MRS using the pattern-recognition techniques already described. There are also plans to produce more biological endpoint predictive models using the PD-ANN and Fuzmac in 3D-QSDAR analyses.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Evans, Frederick

- **A New Approach to the NMR Spectroscopy of Drug Purity and the Public Health Implications** — **E0707801** — **None** — **Concept-Driven**

Objective(s):

1) To determine properties and optimize conditions of the NMR spectrometer at the NCTR under high dynamic-range conditions.
2) To develop concepts and methodology for application of NMR spectroscopy to the investigation of very-low-level impurities in drugs using results on genistein as a model.

FY 2001 Accomplishments:

1) Found numerous low-level impurities by high-dynamic-range NMR in a genistein sample that were undetected by conventional chromatographic methods.
2) Proposed that the total of unknown impurities should be considered in evaluating a candidate drug substance. This goes beyond the 0.1% level guideline published in the Federal Register.
3) Detection limit of 0.002% for impurities in a genistein sample under high-dynamic-range conditions. C-13 satellites of genistein detected (0.003%).
4) Developed method to help identify selected resonances that are structurally related to genistein.

FY 2002 Plans:

Further NMR spectroscopy method application and development as it relates to analysis of drug purity under high-dynamic-range conditions.

PI: Gehring, Theresa

- **Development of Multiresidue Methods to Determine and Confirm Sulfonamides in Edible Tissues of Aquacultured Species** — **E0700601** — **CVM** — **Method-Driven**

Objective(s):

To develop analytical chemical methods to determine and confirm sulfonamide (SA) residues at the 1-10 ng/g level in edible tissues of aquacultured species. Technologies used will include liquid chromatography (LC) with postcolumn derivatization and fluorescence detection for the determinative procedure and liquid chromatography with atmospheric pressure chemical ionization mass spectrometry LC-APCI/MS for the confirmatory procedure.

FY 2001 Accomplishments:

Analyses of dosed tissues were assigned to P00415.

FY 2002 Plans:

A final report will be prepared upon receipt and analyses of salmon tissues dosed by the CVM.

Title	Project Number	Collaborator	Strategic Research Goal

- **Determination of Sulfonamide Incurred Residues in Catfish and Shrimp for Selection of FDA Method Trial Study Materials** — **P00415** — **CVM** — **Method-Driven**

Objective(s):

CVM/USDA/NCTR have identified six sulfonamides of toxicological significance to be included in an incurred-residue FDA method trial. Methods of analysis have been developed and validated; all included sulfas in catfish and shrimp.

FY 2001 Accomplishments:

1) Determinative analyses were completed in dosed catfish and shrimp tissues.
2) Analyses of dosed salmon were added to the project, and the project was extended to December 31, 2002.

FY 2002 Plans:

1) Dosed salmon samples will be analyzed when received from the CVM.
2) When all analyses are completed, a manuscript presenting determinative and confirmatory data from incurred tissues from all three species will be prepared.

PI: Lay, Jackson

- **Rapid Identification of Intact Whole Bacteria Based on Spectral Patterns Using Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-Tof MS)** — **E0700501** — **CFSAN** — **Method-Driven**

Objective(s):

1) To evaluate the potential use of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-Tof MS) as a method for the rapid identification of whole bacteria, either by comparison with archived reference spectra or by co-analysis with cultures of known bacteria.
2) To establish a standard set of conditions for the acquisition of MALDI/Tof mass spectra from bacteria suitable for use in bacterial identification.
3) To obtain some measure of the distribution of signals (ions at specific masses) obtained using standard MALDI/Tof MS conditions based on the analysis of a variety of related and unrelated bacteria.
4) To use standard (pattern recognition) as well as newer (artificial intelligence and principal components analysis) mass spectral recognition techniques to evaluate whether or not the standardized mass spectra obtained from bacteria are sufficiently distinct to allow identification of specific bacteria or to select related bacteria from a group.
5) To evaluate the use of mass spectral recognition techniques for the identification of bacteria from mixtures based on MALDI/Tof MS analysis of the mixture.
6) To determine the minimum number of bacteria necessary for obtaining standard mass spectra.

Title	Project Number	Collaborator	Strategic Research Goal

7) To evaluate the effects on the reproducibility of spectra obtained from whole bacteria under different conditions of sample handling, storage, and cell growth.

FY 2001 Accomplishments:

1) Validated of clinical outbreak specificity for *V. parahaemolyticus* with CFSAN.
2) Detected serotype markers (but not toxicity markers) for *Vibrio vulnificus* with CFSAN.
3) The transfer of this technology to CFSAN was completed.
4) Funding for the construction of a state-of-the-art MALDI FTMS for bacterial characterization was successfully obtained through a collaborative project with the University of Arkansas at Fayetteville.
5) Construction of MALDI FTMS started at UAF.
6) Demonstrated that MALDI methodology developed for Tof is not directly applicable to detection using FTMS.
7) One manuscript is in review; and two invited lectures were given on this work.
8) The FDA MS Forum will highlight contributions by FDA and others to this new field.

FY 2002 Plans:

1) Modify experimental approach to detect proteins via high-resolution FTMS.
2) Compare MALDI specificity using Tof and FTMS detection.
3) Identify bacterial marker ions via HRMS using FTMS.
4) Detect over-expressed recombinant proteins directly in cells.
5) With CFSAN, compare FTMS with Tof MS, and Q Tof MS for food safety applications.

PI: Leakey, Julian

◆ **Chronic Bioassay of Chloral Hydrate in Male $B_6C_3F_1$ Mice Using Idealized Body Weight Curves that are Normalized by Modulation of Caloric Intake** — **E0211701, E0211711, E0211722** — **CDER** — **Concept-Driven**

Objective(s):

1) To determine the chronic toxicity and potential carcinogenicity of chloral hydrate administered to male $B_6C_3F_1$ mice by aqueous gavage.
2) To determine the feasibility of utilizing dietary control (i.e., the manipulation of caloric intake) to control body weight gain so that all mice in each experimental group of the bioassay conform to an idealized weight curve.

FY 2001 Accomplishments:

1) The results of E0211701, E0211711, and E0211722 were reported finalized in NTP Technical Report. Briefly, the conclusions of the study were that there was some evidence of hepatocarcinogenicity in the mouse and dietary control effectively controlled variables in the bioassay.
2) Work from this study was presented at the 2001 Society of Toxicology meeting where it received the "Best Presentation in Risk Assessment" award.
3) The NCTR GLP report for all experiments is being finalized for October 2001.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

1) Three manuscripts will be prepared from these studies: a) effectiveness of dietary-control for the cancer bioassay; b) hepatocarcinogenicity of chloral hydrate; and c) chloral hydrate as a peroxisome proliferator.
2) The GLP report has been completed and is currently under evaluation by NCTR Quality Assurance. The initial two manuscripts are currently being completed.

◆ **Effect of Caloric Restriction on Rat Testicular Tumor Formation** — **E0260201, E0260211, E0260221** — **UAMS** — **Concept-Driven**

Objective(s):

All of the aims of this proposal are directed towards understanding the role of dietary components (i.e., caloric restriction) in influencing the ultimate susceptibility of the male reproductive tract to chemical insult.

FY 2001 Accomplishments:

E0260201, E0260211, and E0260221 are the collaborative efforts between the NCTR and the UAMS and represent a graduate thesis. These experiments are essentially complete and we are waiting for Dr. Gandy to provide the final manuscript for the study. The study was not active in 2001.

FY 2002 Plans:

1) Submit a Master of Science thesis as NCTR final report.
2) Prepare two manuscripts.

PI: Miller, Dwight

◆ **Development of Devices/Methods for Determination of Food/Seafood Quality** — **E0687401** — **CFSAN** — **Method-Driven**

Objective(s):

Assist FDA with problems incurred in testing seafood for decomposition by developing an expeditious assay for determining volatile and semivolatile organic compounds in spoiled seafood.

FY 2001 Accomplishments:

Publication drafted for Rank Fish Detector.

FY 2002 Plans:

Final report will be submitted early FY 2002.

◆ **Innovative Methods for Determining Food Quality: Decomposition, Safety and/or Economic Fraud** — **E0699701** — **ORA** — **Method-Driven**

Objective(s):

1) To examine the total volatile bases (TVB) and putrescine (PU), cadaverine (CD) and histamine (HS) methods for potential regulatory use and validation of TVB as an indicator of decomposition.
2) To develop rapid detection methods for the determination of decomposition analytes in seafood.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

1) Canadian testing is being done in two stages; stage one is complete and stage two will be done by late September 2001. Results are very good for Fresh Tag™.
2) National Marine Fisheries have performed field test with Fresh Tag™ in a laboratory in Washington and tests have also been done in Thailand and India.

FY 2002 Plans:

1) Continue support for commercialization of Fresh Tag™, both manufacturing techniques and application to regulatory decision making.
2) Prepare manuscript on TMA and DMA by GC-TI detector.
3) Prepare manuscript on biogenic amine analysis in seafood.
4) Prepare final report.

◆ **Rapid Screening Test for Food Quality** **E0708001** **None** **Predictive Toxicology**

Objective(s):

To develop simple field-compatible methods to test for food quality.

FY 2001 Accomplishments:

1) Developed Ion Chromatograph method for ammonia, trimethylamine and dimethylamine in fish and shrimp to support Fresh Tag™.
2) Completed indole instrumental method and developed colorimetric method for indole with consistent uniform color development.
3) Developed liquid test for aldehydes.
4) Developed a wet chemical test for the determination of carbon monoxide in tuna. This is not a final version.
5) Developed a gas phase test article for lipid peroxidation products but it is not sensitive enough.
6) Began developing a screening system for potential color-reactants for the gas-phase detection of gaseous food decomposition products.

FY 2002 Plans:

1) Redesign commercial version Fresh Tag™ pumping system.
2) Further develop methods of application of Fresh Tag™ ink formulation to plastic substrates.
3) Ink deposition on plastic film using flexographic printing process in progress.
4) Examine ink deposition on plastic film using screen printing process.
5) Perfect ink deposition on plastic film using ultrasonic spray equipment.
6) Apply Ion Chromatographic method for ammonia, trimethylamine and dimethylamine in fish and shrimp to support Fresh Tag™.
7) Examine the potential of a biogenic amine method that does not require derivitization before chromatography or detection.
8) Develop wet chemical test for the determination of carbon monoxide in tuna.
9) Develop optical screening system for potential color-reactants for the gas-phase detection of lipid peroxidation products (aldehydes).
10) Develop aldehyde analytical method to support gas-phase sensor methods and perfect isolation of lipid peroxidation isolation methods.

Title	Project Number	Collaborator	Strategic Research Goal

- **Application of Solid Phase Detection Systems to Explosives in Airplane Cargo** — E0708101 — None — Method-Driven

Objective(s):

1) To detect ammonia (formulation, measurement of ammonia concentrations around container of ammonium nitrate, reformulation of Fresh Tag™ chemistry for label-type detection).
2) To develop polyethylene (PE) or polyvinyl chloride (PVC) film shrink rap detector.
   a) Acid detection.
   b) Detection of oxidizers such as peroxides and NO or NO_2.

FY 2001 Accomplishments:

1) Application/extension of Fresh Tag™ technologies for detection of nitrogen-based explosives made. Fresh Tag™ has been shown to produce color change in the presence of ammonium nitrate, but it is not the typical acid-base color change. This is considered to be an indication that an oxidizer is possibly causing the change.
2) Developed Ion Chromatographic method to detect ammonia in headspace above ammonium nitrate and fish (E0707800). This is to prove that ammonia is or is not present. It was.
3) Tested Fresh Tag™ technology color; did not change to blue but blackish brown. Perhaps indicating an oxidant in the headspace.
4) Developed colorimetric detection system for gas-phase oxidizers in headspace above explosives. Not sensitive enough or fast enough.

FY 2002 Plans:

1) Develop colorimetric detection system for "gas-phase oxidizers" in headspace above explosives. This will be applied to shrink wrap material.
2) Develop colorimetric detection system for solid-phase detection of explosives on surfaces.

PI: Turesky, Robert

- **Risk Assessment of Heterocyclic Aromatic Amines: Development of Novel Biomarkers of *CYP1A2* Activity and DNA Adduct Formation** — E0709101 — None — Agent-Driven

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 1A2, which is believed to be the major P450 involved in the toxication of these chemicals.

Title	Project Number	Collaborator	Strategic Research Goal

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*.
5) Use interspecies metabolism to assess the capacity of human and rat P450A2 orthologues in metabolic activation and detoxication of HAAs to assess human risk.

FY 2001 Accomplishments:

Seven publications were accepted.

FY 2002 Plans:

1) Conduct quantitative analyses of HAAs in Cajun-cooked foods indigenous to southern cooking and meats prepared by Singapore cuisine by HPLC-tandem MS.
2) Establish quantitative LC-tandem MS methods to measure the DNA adducts of the HAAs 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in rat tissues and human hepatocytes.
3) Establish quantitative LC-tandem MS methods to measure the metabolites of MeIQx in human urine. Apply method to collaborative studies with the University of Southern California and NIH, which relates phenotypes and genotypes to specific urinary metabolite markers. Assess the influence of cruciferous vegetables on metabolism of MeIQx in humans, in collaboration with Imperial College, U.K.
4) Determine enzyme kinetic parameters on N-oxidation of HAAs using recombinant human and rat P450 1A2 for interspecies comparisons, in collaboration with the U. of Guelph, Canada.

PI: Wilkes, Jon

◆ **First Phase Development of a Rapid Screening Method for Identification of Complex Mixtures by Pyrolysis-Mass Spectrometry with Computerized Pattern Recognition** | **E0693101** | **CFSAN ORA** | **Knowledge Base**

Objective(s):

To evaluate the feasibility of the application of pyrolysis mass spectrometry (PyMS) with computerized pattern recognition (PattRec) for the rapid identification of a sample: (a) which is a complex chemical mixture; (b) which is member of a set of such mixtures; and (c) for which there is a regulatory need to distinguish the individual members of the set. Typical examples of applications: (a) the rapid identification of culturable pathogenic and non-pathogenic bacteria in food; (b) the distinction of adulterated from unadulterated foods or cosmetics, or generic from brand-name pharmaceutical products; or (c) demonstrating the virginity of plastic materials used in food containers.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

1) Demonstrated using authentic FDA *Vibrio parahaemolyticus* outbreak samples that PyMS has sufficient taxonomic power to distinguish strains not only of different serotypes but also - in the cases shown - from outbreaks in different parts of the U.S. in different years.
2) Patent submitted (U.S. Patent Application number 60/239,549 filed October 10, 2000) which demonstrates how to build and consult a coherent database of microbial PyMS spectra for rapid, strain-level chemotaxonomy of microbial samples. The patent also demonstrates how to accomplish the same kind of coherent assembly and consultation for any other chemo-taxonomic spectral or chromatographic system.
3) Identified three potential licensees for the patent so that FDA Technology Transfer Office agreed to pay for re-writing and fees necessary to turn this Provisional Patent Application into a Full Patent.
4) Arranged for Dephy, Inc. of Montreal, Quebec, Canada, to ship NCTR a novel Pyrolysis MAB/Tof MS (retail value $150,000) optimized for bacterial identification.
5) Arranged for Susan McCarthy of CFSAN, Dauphin Island, AL, to spend two weeks in collaborative research at NCTR.
6) Wrote and received approval for a new protocol to fully investigate Py-MABMS for bacterial identification (ID) applications.

FY 2002 Plans:

1) Present results to FDA Mass Spectrometry Forum (Invited Speaker).
2) Write paper summarizing results.
3) Close this protocol.

FY 2001 Publications

Beger, R., Freeman, J.P., Lay, J.O., Wilkes, J.G. and Miller, D.W., Use of 13C NMR Mass Spectrometric Data to Produce a Predictive Model of Estrogen Receptor Binding Activity, *J. Chem. Inf. Comput. Sci*., 41:219-224. Accepted: 11/1/00 (E0693101)

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Beger, R. and Wilkes, J.G., Models of Polychlorinated Dibenzodioxins, Dibenzofurans, and Biphenyls Binding Affinity to the Aryl Hydrocarbon Receptor Developed Using 13C NMR Data, *J. Chem. Inf. Comput. Sci*., 41(5):1322-1329. Accepted: 12/31/00 (E0706801)

Collins, A.R., Brown, J., Bodganov, M., Cadet, J., Cooke, M., Douki, T., Dunster, C. and Turesky, R., Comparison of Different Methods of Measuring 8-Oxoguanine as a Marker of Oxidative DNA Damage, *Free Radical Research*, 32:333-341. Accepted: 10/1/00 (N/A)

Dean-Ross, D., Moody, J.D., Freeman, J.P., Doerge, D.R. and Cerniglia, C.E., Metabolism of anthracene by a Rhodococcus species, *FEMS Microbiology Letters*, 204:205-211. Accepted: 8/20/01 (N/A)

Duffy, P.H., Feuers, R.J., Seng, J.E., Lewis, S.M., Mayhugh, M.A., Aidoo, A. and Casciano, D.A., The Effects of Different Levels of Dietary Restriction on Aging and Survival in the Sprague-Dawley Rat: Implications for Chronic Bioassay Studies, *Aging*, 13:263-272. Accepted: 12/5/00 (E0692401)

Fenaille, F., Mottier, P., Turesky, R. and Guy, P.A., Quantitation of Malondialdehyde in Milk Powders by Various Analytical Techniques, *Advances in Mass Spectrometry*, 579-580. Accepted: 7/27/01 (N/A)

Fenaille, F., Mottier, P., Ali, S., Hugelshofer, A., Guy, P.A. and Turesky, R., Comparison of Analytical Techniques to Quantify Malondial-dehyde in Milk Powders, *Journal Chromatography A*, 921:237-245. Accepted: 4/19/01 (N/A)

Gangl, E.T., Turesky, R. and Vouros, P., Detection of *In vivo* Formed DNA Adducts at the Part-Per-Billion Level by Capillary Liquid Chromatography/Microelectrospray Mass Spectrometry, *Analytical Chemistry*, 73:2397-2404. Accepted: 4/3/01 (E0709101)

Gautier, J., Holzhaeuser, D., Markovic, J., Gremaud, E., Schilter, B. and Turesky, R., Oxidative Damage and Stress Response from Ochratoxin A Exposure in Rats, *Journal Free Radical Biolog. & Medicine*, 30(10):1089-1098. Accepted: 2/15/01 (E0709401)

Gautier, J., Richoz, J., Welti, D.H., Markovic, J., Gremaud, E., Guengerich, F.P. and Turesky, R., Metabolism of Ochratoxin A: Absence of Formation of Genotoxic Derivatives by Human and Rat Enzymes, *Chemical Research in Toxicology*, 14:34-45. Accepted: 11/8/00 (E0709401)

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Langouet, S., Welti, D., Kerriguy, N., Fay, L.B., Guengerich, F.P., Guillouzo, A. and Turesky, R., Metabolism of the Food Mutagen 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in human Hepatocytes. In: *Advances in Experimental Medicine. Biologically Reactive Intermediates VI: Chemical and Biological Mechanisms in Susceptibility to and Prevention of Environmental Disease*, 459-462. Accepted: 8/28/01 (N/A)

Letarte, S., Wilkes, J.G., and Bertrand, M.J., Detection and Identification of Bacteria in Urine Samples by Py-MAB-MS, *Proceedings of the 49th ASMS Meeting*. Accepted: 6/1/01 (N/A)

Luo, W. and Ang, C.Y., Determination of Formaldehyde in Blood Plasma by High Performance Liquid Chromatography with Fluorescence Detection, *Journal of Chromatography B*, 753:253-257. Accepted: 10/13/00 (N/A)

Moody, J.D., Freeman, J.P., Doerge, D.R. and Cerniglia, C.E., Degradation of phenanthrene and anthracene by cell suspensions of *Mycobacterium* sp. PYR-1, *Appl. Environ. Microbiol*., 67:1476-1483. Accepted: 1/26/01 (E0690101)

Parshikov, I., Freeman, J.P., Lay, J.O., Moody, J.D., Williams, A.J., Beger, R. and Sutherland, J.B., Metabolism of the veterinary fluoroquinolone sarafloxacin by the fungus *Mucor ramannianus*, *J. Ind. Micro. Biotech*., 26:140-144. Accepted: 11/3/00 (E0705201)

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Young, J.F., Tong, W., Fang, H., Beger, R., Chen, J.J., Cheeseman, M.A. and Kodell, R.L., Computational Predictive System for Rodent Organ-Specific Carcinogenicity, *Computational Intelligence: Methods and Applications (CIMA 2001)*, 565-569. Accepted: 5/9/01 (E0708301)

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

The Division of Genetic and Reproductive Toxicology (DGRT) conducts applied basic research to address specific high-priority issues regarding genetic and reproductive 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 conducts research to understand the potential toxicity of specific high-priority drugs, dietary supplements, and/or other agents. Genistein, malachite green and some of the drugs used for AIDS therapy are currently undergoing extensive evaluations in cross-division collaborative research efforts.

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 two disciplinary research areas, the Division conducts research to understand the effects of dietary supplementation. That research primarily focuses on understanding the physiological and genetic consequences of dietary modulation. Recently the Division initiated a new research focus to utilize new molecular approaches to evaluate genomic damage. While administratively a part of DGRT, the new NCTR Functional Genomics Center will be a resource for all NCTR investigators.

Genetic Toxicology

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

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

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 making decisions. As new assays are validated, Division scientists work with international scientists to assure harmonization of protocols and the development of guidelines.

Reproductive/Developmental Toxicology

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 the vitamin 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.

A well-defined database created over the past 20 years led to the initiation of a project to create and validate a computerized knowledge base using quantitative structure-activity relationships to predict which chemicals might affect the normal reproductive function.

Diet and Nutrition

Dietary restriction can increase the length of a rodent's life and decrease the frequency of tumors. Division scientists have also determined that decreasing caloric intake alters many physiological processes in rodents and decreases the frequency of mutations. The group has developed many physiological, biochemical, and morphological procedures that can now evaluate dietary modulation. Because of a unique opportunity to collaborate with medical scientists at the University of Tennessee at Memphis, the research program is able to compare the responses seen in rodents with those seen in calorically restricted humans. These dietary restriction studies are nearing completion. Currently, dietary research focuses on understanding the impact of dietary supplementation such as anti-oxidants, genistein, and herbals.

Genomics

International research efforts are providing the scientific and medical community with increased understanding of the genome 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 in the genome of both rodents and

humans. The Division is developing a new research focus area to use these technologies and to apply them 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. In addition to addressing research questions in the areas of genetic and reproductive toxicology, the new NCTR Functional Genomics Center will be a means by which all NCTR scientists can collaborate and utilize this new technology. It will also provide an opportunity to evaluate the multiple adverse health effects of chemicals and to assist with the harmonization of cancer and non-cancer risk assessment methodologies.

FY 2001 Accomplishments and FY 2002 Plans

Title	Project Number	Collaborator	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 E0697511** — **None** — **Predictive Toxicology**

Objective(s):

In vivo assays are used to evaluate whether chemicals have the potential to induce genetic damage (mutations). In order to understand an assay and to use it for hazard identification, one must first understand the frequency and types of mutations that occur spontaneously (without any chemical exposure). This project is designed to evaluate spontaneous mutation in one gene that is in its normal location (*Hprt*) and one gene (*lacI)* that has been genetically engineered into a strain of rats. This strain of rats, containing the *lacI* transgene is called the Transgenic Big Blue Rat. The specific goals of this project are:

1) To 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.
2) To determine the types of mutations present in the mutants.
3) To determine if rats fed different diets have different spontaneous mutant frequencies and if the types of mutations are different.

FY 2001 Accomplishments:

This project is complete.

FY 2002 Plans:

Prepare the final manuscripts.

- **The Use of Antioxidants in Single and in Mixture to Study the Effects of Dietary Vitamins on Genotoxicity Produced in Rats Treated with the Mammary Carcinogen 7,12-dimethylbenz(a)anthracene and the Radiometic Antitumor Drug Bleomycin** — **E0701401** — **None** — **Predictive Toxicology**

Objective(s):

Antioxidants have been reported to have beneficial health effects including reducing the risk of cancer. They have also been reported to reduce the risk of mutation. In this project, dimethylbenz(a)anthracene (DMBA) and bleomycin (BM), both known carcinogens and mutagens, will be administered to rats. Animals will also receive antioxidants (singly and as a mixture of vitamin C, E, β-carotene and selenium). It is expected that the animals treated with both the carcinogen/mutagen and the antioxidants will have a lower mutant frequency than those animals treated only with the carcinogen/mutagen. Two different genotoxic endpoints, mutation at the *Hprt* gene and chromosomal effects (cytokinesis-block micronucleus) will be used.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

Once the genotoxicity has been determined, experiments will be undertaken to determine the mechanism underlying the inhibitory action of the dietary antioxidants by determining their effects on:

1) Spectra of induced mutations in *Hprt* gene in lymphocytes.
2) Oncogene (H-*ras*, K-*ras*) and tumor suppressor gene, p53 expression.
3) Programmed cell death (apoptosis).
4) The activities of glutathione peroxidase and glutathione S-transferase during DMBA and BM exposures.

FY 2001 Accomplishments:

This project is complete.

FY 2002 Plans:

Write the final report.

◆ **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-phenylimidazo[4,5-f]pyridine (PhIP)** | **E0706311** | **None** | **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. 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.

FY 2001 Accomplishments:

The experiments began; about 64 rats are ready to be sacrificed to determine mutant frequency and types of mutation using RT-PCR and DNA sequencing. Some tissues such as muscle and kidney have been frozen for gene and protein expression, as well as oxidative DNA analysis using flow cytometry.

FY 2002 Plans:

1) The experiments will continue since 46 rats are yet to undergo physical exercise, dietary antioxidant intake and carcinogen treatment.
2) Collection and analysis of data.
3) Results will be presented at scientific meetings and manuscripts will be prepared for publication.

Title	Project Number	Collaborator	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 E0707011**	**None**	**Predictive Toxicology**

Objective(s):

This project is designed to determine if hormone replacement, in this case the phytoestrogens daidzein and genistein, will alter the risk of mammary tumors. Rats will be exposed to a known mammary carcinogen (DMBA) and given daidzein and/or genistein. In order to model the post-menopausal human situation, the rats will be ovariectomized. In addition to the induction of tumors, other endpoints such as the ability of the DMBA to bind to DNA (DNA adduct analysis) and the frequency and types of mutations induced by DMBA will be investigated.

FY 2001 Accomplishments:

The preliminary work involved in this proposal had been completed. The experiments are still ongoing, and most of the animals were sacrificed to conduct the assays.

FY 2002 Plans:

Experiments will continue and data will be collected for manuscript preparation and for scientific meeting presentations.

PI: Branham, William

◆ **Development of a Statistically Robust 3D-QSAR Model to Predict *In Vitro* Rat Uterine Estrogen Receptor Binding Activity**	**E0290001**	**None**	**Knowledge Base**

Objective(s):

There is a Cooperative Research and Development Agreement (CRADA) for this project to develop and validate 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.

FY 2001 Accomplishments:

The QSAR and CoMFA models have been completed and delivered to the EPA. These models are currently being evaluated by the EPA under a contract with Battelle Laboratories to validate the models.

FY 2002 Plans:

Any further refinements to these models will be done via contractor.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	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** — **None** — **Concept-Driven**

Objective(s):

To develop and validate a statistically 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 EPA's screening and testing program for endocrine disruptors.

FY 2001 Accomplishments:

1) Replicate assays have been completed for 204 chemicals assessing binding to the PanVera Recombinant Androgen Receptor (AR).
2) The data are currently being utilized by R.O.W. computational chemists to model the binding of these chemicals to the AR.
3) The data are being processed and manuscripts are in preparation.

FY 2002 Plans:

The AR data will be used to create both QSAR and CoMFA models. These models will be made available for use by the EPA for assessment of AR binding of a large number of environmental chemicals.

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** — **None** — **Predictive Toxicology**

Objective(s):

Because cancer is a disease requiring the induction of mutation and the clonal expansion of mutated cells, one would expect that the developing fetus and young infant would be particularly susceptible to carcinogen exposure. This project will be initiated to evaluate this hypothesis. Experiments will be conducted to:

1) Determine the mutant frequencies in the cII gene of Big Blue mice treated at different ages with direct-acting carcinogens.
2) Determine the mutant frequencies in the cII gene of the target tissues from transgenic mice exposed as neonates and adults to different carcinogens that require metabolic activation.
3) Determine the types of mutations produced in the cII genes of the mutants induced in objectives 1 and 2.

FY 2001 Accomplishments:

Protocol approved.

FY 2002 Plans:

Experiments will be initiated in which transgenic animals are exposed as neonates and adults to different carcinogens.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Dobrovolsky, Vasily

- **Validation of the Mouse Targeted Tk$^{+/-}$ *In Vivo* System for Use in Mutagenicity Studies** — E0701801 — None — **Predictive Toxicology**

Objective(s):

DGRT has developed a new *in vivo* assay for the evaluation of mutant induction. This assay was modeled after the *in vitro* mouse lymphoma assay already used internationally for hazard identification. The mouse lymphoma assay uses the thymidine kinase gene (*tk*) and has been extensively evaluated for its mechanistic basis and shown to detect most, if not all, of the mutational events important to the induction of cancer and other human diseases. This project is designed to further evaluate and validate the *in vivo* assay. Specific goals of this project are:

1) To expand a colony of transgenic *Tk*$^{+/-}$ mice using breeding of *Tk*$^{+/-}$ founders and C57Bl/6 mice, and to transfer the *Tk*$^{+/-}$ genotype to a C57Bl/6 background.
2) To determine spontaneous mutant frequencies at the *Tk* and *Hprt* loci of splenic T-lymphocytes for mice of different ages.
3) To induce mutations in *Tk*$^{+/-}$ transgenic mice using treatment with the point mutagen ENU (ethyl nitrosourea) and the clastogens BLM and y-radiation, and to measure the kinetics of mutant induction at the *Tk* and *Hprt* loci.
4) To breed transgenic *Tk*$^{+/-}$ parents in an attempt to derive *Tk*$^{-/-}$ knockout (KO) mice, and study the biological significance of the *tk* gene in mice.
5) To determine how the *Tk*$^{-/-}$ genotype may affect mutant frequencies at the *Hprt* locus.

FY 2001 Accomplishments:

1) The colony of Tk$^{+/-}$ animals was maintained on the site to provide animals for internal research needs as well as for distribution to five other scientific institutions.
2) Mutation frequencies of Hprt and Tk genes were identified in Tk$^{+/-}$ animals following the treatment with ionizing radiation, etoposide and mitomycin C. A manuscript describing these findings was submitted to *Environmental and Molecular Mutagenesis Journal* (accepted).
3) Age dependent Tk mutant frequencies and types of mutations were identified for Tk$^{+/-}$ males and females.
4) Tk$^{+/-}$ animals were shipped to LRRI for exposure to potential carcinogen butadiene by inhalation, upon the return mutant frequencies were determined for the Hprt and the Tk genes in the exposed animals.
5) Frequencies of Hprt and Tk mutation were determined for butadiene-exposed Tk$^{+/-}$ mice.
6) Tk$^{+/-}$ mice were crossed to Pms2$^{+/-}$ mice to determine Hprt and Tk mutant frequencies in Pms2-deficient mice.
7) Tk$^{+/-}$ animals were submitted to centralized depository, Mutant Mouse Regionall Resource Center (MMRRC), for availability to outside researchers.
8) A chapter, "Analysis of *in vivo* mutation in the Hprt and Tk genes of mouse lymphocytes", was submitted for publishing in the book Molecular Toxicology Protocols.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

1) The colony of $Tk^{+/-}$ animals will continue to be maintained for future treatment with agents of interest to FDA.
2) For simplified distribution of Tk-deficient animals to outside researchers, the breeding duties will be transferred to a centralized service at MMRRC).
3) Cooperation with LRRI will start to determine mutational responces in animals exposed *in utero* to chemicals of interest to FDA.

◆ **ADDEND: Validation of the Mouse Targeted $Tk^{+/-}$ *In Vivo* System for Use in Mutagenicity Studies** — E0701811 — None — **Predictive Toxicology**

Objective(s):

In order to insure the health of the colony and to produce and supply $Tk^{+/-}$ animals to other investigators, it is necessary to conduct routine microbiological surveillance of the colony to assure that it remains pathogen free.

FY 2001 Accomplishments:

Microbiological surveillance of $Tk^{+/-}$ animals was performed for continuous monitoring of the health status of the colony. With health reports generated during this period, our $Tk^{+/-}$ mice were accepted by five other outside researchers for independent purposes.

FY 2002 Plans:

The surveillance program will continue until the $Tk^{+/-}$ animals are accepted by MMRRC. If animals are accepted, then the monitoring program may be discontinued and requests for $Tk^{+/-}$ animals from outside investigators will be redirected to MMRRC.

◆ **ADDEND: Validation of the Mouse Targeted $Tk^{+/-}$ *in vivo* System for use in Mutagenicity Studies** — E0701821 — None — **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.

FY 2001 Accomplishments:

Breeding program for derivation of $Tk^{+/-}$ mice on the Pms2-deficient background has commenced. The first breeding step was done; $Tk^{+/-}$ Pms2+/- mice have been produced in desired quantities.

FY 2002 Plans:

1) $Tk^{+/-}$ animals with $Pms2^{+/-}$, $Pms2^{+/+}$ and $Pms2^{-/-}$ backgrounds will be derived.
2) Spontaneous mutant frequencies will be determined for Hprt and Tk genes in TkPms2 mice.
3) Types of mutations will be identified and the significance of the Pms2 gene in induction of loss of heterozygosity mutation induction will be studied.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

- **Evaluation of the $Tk^{-/-}$ Knockout Mouse as a Model of Systemic Lupus Erythematosus** — E0706901 — None — Predictive Toxicology

Objective(s):

Our initial experiments suggest that mice deficient in the thymidine kinase enzyme (*$Tk^{-/-}$* animals) may be a useful model for studying the human disease lupus. In this project, we will 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 *$Tk^{-/-}$* mice, seeking comparison with published characteristics of Systemic Lupus Erythematosus in mice and humans.

FY 2001 Accomplishments:

1) 120 mice with $Tk^{+/+}$, $Tk^{+/-}$ and $Tk^{-/-}$ genotype were produced.
2) Tissues were collected and preserved from all three types of Tk mice. Blood serum samples were analyzed for the concentrations of thymidine nucleoside.
3) Serum samples were analyzed for the presence of autoimmune abnormalities.
4) Tissues were collected from mice of two different ages (three months old and six months old).

FY 2002 Plans:

1) Tissue samples will be examined for pathology in various organs with the emphasis on kidney and salivary gland.
2) The report on $Tk^{-/-}$ phenotype will be prepared and submitted.

PI: Domon, Olen

- **Evaluation of the Genotoxicity of the Phytoestrogen Coumestrol in Human Lymphoblast Cells that Differ in the Mutational Status of the p53 Tumor Suppressor Gene** — E0705501 — None — Predictive Toxicology

Objective(s):

The phytoestrogens are considered to be potentially beneficial dietary supplements, particularly for peri- and post-menopausal women. The phytoestrogen coumestrol will be evaluated in several *in vitro* gene mutation assays with the following goals:

1) To confirm the ability of coumestrol to break chromosomes. This will be done using the micronucleus assay.
2) To confirm the mutagenicity of coumestrol at the *HPRT* locus.
3) To determine if coumestrol induces large-scale chromosomal damage such as that detected by the *Tk* mutation assay.
4) To determine if the toxicity of coumestrol is due to apoptosis.
5) To determine the effect of coumestrol on the ability of cells to grow and divide normally.

FY 2001 Accomplishments:

1) A manuscript entitled, "Evaluation of the genotoxicity of the phytoestrogen, coumestrol, in AHH-1 *$TK^{+/-}$* human lymphoblastoid cells," was published in *Mutation Research*.
2) Continued the molecular analysis of mutants.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

1) Continue molecular analysis of mutants.
2) Confirm the sequence of the Tk gene in AHH-1 *Tk* $^{+/-}$ cell line.
3) Determine the nature of the mutations in *Tk* mutant clones.

◆ **ADDEND: Evaluation of the Gentoxicity of the Phytoestrogen Coumestrol in Human Lymphoblast Cells that Differ in the Mutational Status of the p53 Tumor Suppressor Gene. I. Molecular Analysis of Coumestrol and Genistein-induced Thymidine Kinase Mutants in AHH-1 Tk$^{+/-}$ and L3 Cells** — **E0705511** — **None** — **Predictive Toxicology**

Objective(s):

In the master project, cells in culture were treated with coumestrol and genistein and clones of cells that were made mutant at the thymidine kinase gene were isolated. In this addendum, these mutants will be evaluated to determine the specific types of mutations that were induced by these two phytoestrogens.

FY 2001 Accomplishments:

A manuscript entitled "Evaluation of the genotoxicity of the phytoestrogen, coumestrol, in AHH-1 TK$^{+/-}$ human lymphoblastoid cells", was published in *Mutation Research.*

FY 2002 Plans:

Experimentation was completed in FY 2001 and a manuscript was published in *Mutation Research*. A final report will be prepared in FY 2002.

PI: Duffy, Peter

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

Objective(s):

1) To determine how various levels and durations of CR affect physiological function, enzymes related to intermediary and drug metabolism, hormonal regulation, blood chemistry, etc.
2) To 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) To validate and automate the use of a new, non-invasive electromagnetic scanning device to measure BF, FFM, and TBW and to compare the results to a conventional chemical fat extraction technique.
4) To 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.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

5) To 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) To determine temporal and environmental factors that modulate the effects of CR.
7) To 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.
8) To develop control data for a reference purified diet that has been formulated to conform to the long-term nutrient requirements of rodent animal models typically utilized in toxicology and nutrition studies.

FY 2001 Accomplishments:

Survival Study

1) Survival profiles were developed for Sprague Dawley rats on different levels of food intake; AIN-93M (purified) and NIH-31 (cereal) diets used.
2) Dietary Restriction (DR) significantly increased 24-month survival in rats raised on the NIH-31 diet; DR didn't increase survival in rats raised on the AIN-93M diet.
3) NIH-31 and AIN-93 survival manuscripts were published in the past year.

Mutation Frequency Study

1) Mutation frequency determined for Sprague Dawley rats on different levels of food intake; AIN-93M and NIH-31 diets used.
2) Mutation frequency assays were conducted and data analysis was completed.
3) 40% DR significantly reduced mutation frequency.
4) Manuscript preparation is nearing completion.

Electron Transport and Mitrochondria Study

1) Effects of DR and age on oxidative damage and electron transport were determined in the mitochondria of muscle tissue; AIN-93M and NIH-31 diets used.
2) Assays were completed and the data were partially analyzed.
3) DR significantly reduced oxidative damage in the mitochondria.

Pathology Study

1) Histopathology completed for Sprague Dawley rats on different levels of food intake; AIN-93M and NIH-31 diets were used.
2) Pathology different for four levels of food consumption (NIH-31 diet); DR decreased proliferation of tumors and renal failure in a linear fashion.
3) Pathology different for cereal and purified diets; NIH-31diet associated with high rate of pituitary and liver tumors and low incidence of chronic renal disease. AIN-93M diet associated with high rate of chronic renal disease and a low incidence of pituitary and liver tumors.
4) Final pathology report to be completed in next few months.

Clinical Blood Chemistry Study

1) Developed an interactive database to store and retrieve clinical chemistry data.
2) Clinical blood chemistries completed for Sprague Dawley rats on various levels of food intake; AIN-93M and NIH-31diets were used.

Title	Project Number	Collaborator	Strategic Research Goal

3) Blood parameters such as triglycerides, alanine aminotransferase, creatinine, total protein, total cholesterol, phospholipids, , lymphocytes (%), and white blood cell count were decreased by DR.
4) Blood parameters such as glucose, alkaline phosphatase, hemoglobin, and neutrophils (%) were increased by DR.
5) Data analysis has been partially completed.

Physiological Study

1) Body temperature, activity, energy metabolism, and respiratory quotient continuously monitored in rat food response study; AIN-93M and NIH-31diets used.
2) Data acquisition stage completed and data analysis partially completed; ORACLE database developed and maintained for physiological studies.
3) DR was associated with a decrease in metabolism and temperature and an increase in activity (NIH-31 and AIN-93M diets).

FY 2002 Plans:

1) Survival, histopathology, physiological, and blood chemistry variables will be monitored with age in Fischer 344 rat at different levels of DR; NIH-31 diet used.
2) Proteomic, genomic, and mutation frequency assays will be conducted in Fischer 344 rats to determine age and DR effects.
3) Data acquisition phase will be completed; data analysis will be partially completed.
4) Data analysis and manuscripts (histopathology, mutation frequency and blood chemistry variables) will be completed for Sprague Dawley rats on NIH-31 and AIN-93M diets.

◆ **ADDEND: Effect of Different Levels of Caloric Restriction on Physiological, Metabolic, Biochemical, Immunological, Molecular and Body Composition Variables in Rats** | **E0692411** | **CFSAN** | **Concept-Driven**

Objective(s):

Requesting 40 additional male Sprague-Dawley rats be added to this protocol to serve as *ad libitum* controls in a dietary restriction (DR) study.

FY 2001 Accomplishments:

1) Additional animals added to E692401 to increase sample size; not separate study.
2) Data was pooled with main study for analysis (see E0692401 for details).

FY 2002 Plans:

See plans for E0692401 for details.

Title	Project Number	Collaborator	Strategic Research Goal

◆ **ADDEND: Task Order #483 & #493 - LIMS Implementation and Review of Heart Rate Variation Analysis Software** | **E0699811** | **Univ of Tenn at Memphis** | **Predictive Toxicology**

Objective(s):

Addendum requested to add ADP resources needed for Task Order #483 - Memphis Study: LIMS Implementation.

FY 2001 Accomplishments:

Physiological Studies

1) Physiological studies (resting and exercise) were conducted to monitor metabolism, energy expenditure, and respiratory quotient in obese human patients before gastric bypass surgery and 3, 6, 12, 24 months after the onset of DR.
2) Developed a non-invasive procedure for continuously monitoring temperature, blood pressure, heart rate, and motor activity in humans.
3) Partially completed physiological studies in 43 patients.
4) Completed the development of interactive Oracle database to store and retrieve physiological data.
5) Data has been partially analyzed.
6) DR significantly altered physiological performance in humans and rats in a similar fashion.
7) Temperature, heart rate, and energy expenditure variables were significantly reduced by DR; DR may reverse or delay the aging process.

H-Scan Physiological and Behavioral Assessment Studies

1) A battery of 12 physiological and behavioral biomarkers was tested in obese patients before and after gastric bypass surgery.
2) Visual reaction time with decision, and muscle movement time with decision increased after the onset of DR (negative impact); visual reaction time, auditory reaction time, muscle movement time, alternate button-tapping time, and muscle movement time with decision decreased after the onset of DR (positive impact).
3) Visual accommodation, memory (length of sequence), forced lung expiratory volume, forced lung vital capacity, vibrotactile sensitivity, and highest audible pitch were not altered by DR.
4) Results suggest that H-Scan physiological and behavioral measures may be excellent biomarkers of aging and DR.
5) DR may reverse or delay the aging process; results are similar to rat studies.
6) Data analysis has been partially completed.

FY 2002 Plans

1) Final analysis of data will be completed in the next few months if statistical support is provided.
2) Manuscripts and final report will be prepared for publication.
3) Results will be used to develop beneficial diets to increase longevity in humans.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Feuers, Ritchie

- **Memphis Study: Evaluation of Calorically Restricted Human Surgical Samples Received from Department of Surgery University of Tennessee, Memphis** — **E0699801** — **Univ of Tenn at Memphis** — **Predictive Toxicology**

Objective(s):

To determine whether rodents and humans behave biologically in the same manner when calorically deprived but nutritionally supplemented.

FY 2001 Accomplishments:

Study sampling completed.

FY 2002 Plans:

To complete the analysis of samples and compilation of data.

PI: Fuscoe, James

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

Objective(s):

1) Establish microarray technology for the NCTR Functional Genomics Center.
2) Develop, print, and establish the methodology for using a "rat gene chip" containing approximately 4,000 genes and a "human gene chip" containing approximately 8,300 genes.

FY 2001 Accomplishments:

1) Purchased gene collections for the rat and human.
2) Identified collaborator to print the gene chips.

FY 2002 Plans:

1) Develop and print rat and human gene chips.
2) Validate the quality of the rat and human gene chips.
3) Prepare a large amount of reference rat RNA.
4) Develop a database for handling the large amounts of data that will be generated.
5) Develop statistical methods for interpreting the experimental results.

PI: Hansen, Deborah

- **Antisense Knockouts of Genes in the Folate Pathway and Effects on Neural Tube Development** — **E0702001** — **None** — **Predictive Toxicology**

Objective(s):

The Division specializes in research to understand how toxicants may induce birth defects such as neural tube defects. This research project addresses the role that the vitamin 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

Title	Project Number	Collaborator	Strategic Research Goal

normal offspring and that interactions between diet and toxicants may be important in producing certain birth defects. The research addresses fundamental mechanisms by which the enzymes that metabolize folic acid may be involved and whether the addition of folic acid or other vitamins would overcome the toxicity of and result in normal neural tube closure and thus in normal offspring. The specific goals of the project are:

1) To determine if knocking out 5,10-methyltetrahydrofolate (MTHFR) enzyme activity in mouse embryos *in vitro* produces neural tube defects.
2) To determine if the addition of exogenous 5-methyltetrahydrofolate is able to overcome the lack of MTHFR activity and produce normal closed neural tubes in mouse embryos treated *in vitro*.
3) To determine if the addition of methionine overcomes the lack of MTHFR activity and results in normal closed neural tubes in mouse embryos treated *in vitro*.
4) To determine if knocking out methionine synthase (MS) activity in mouse embryos *in vitro* produces neural tube defects.
5) To determine if the addition of methionine is able to overcome the lack of MS activity and produce closed neural tubes in mouse embryos treated *in vitro*.
6) To determine if exogenous vitamin B12 is able to overcome the lack of MS activity and produce closed neural tubes in mouse embryos treated *in vitro*.
7) To determine if knocking out methionine adenosyltransferase (MAT) enzyme activity in mouse embryos *in vitro* produces neural tube defects.
8) To determine if the addition of exogenous methionine is able to overcome the lack of MAT activity and produce closed neural tubes in mouse embryos treated *in vitro*.
9) To determine if the addition of exogenous 5-methyltetrahydrofolate is able to overcome the lack of MAT activity and produce closed neural tubes in mouse embryos treated *in vitro*.

FY 2001 Accomplishments:

1) Experimental work was completed on this project during the last year.
2) The manuscript on micro-injection of homocysteine into the amniotic sac to examine the direct effects of excess methionine on embryonic development was published.
3) The manuscript on modulation of folate binding protein-1 and its regulatory element is still undergoing revision and will be submitted for publication soon.

FY 2002 Plans:

1) Submit manuscript on knock-down of folate binding protein-1 and its regulatory sequence.
2) Submit final report.

◆ **Indices of Biotin Nutrition** **E0703401** **None** **Concept-Driven**

Objective(s):

To determine the human requirement for biotin in normal individuals and in individuals in certain circumstances in which biotin status may be impaired. We will determine whether biotin of similar severity to that observed in human pregnancy

Title	Project Number	Collaborator	Strategic Research Goal

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.

FY 2001 Accomplishments:

A draft manuscript was prepared and is currently being revised. A renewal grant was submitted to NIH, and initial indications are that it will be funded.

FY 2002 Plans:

1) Submit manuscript on effects of biotin deficiency during pregnancy in the mouse.
2) Submit final report.

- **Predictability of Animal Data for Human Developmental Toxicity** **E0703501** **CDER Predictive Toxicology**

Objective(s):

To determine if the animal tests currently required for pre-market approval of drugs are adequately predicting possible developmental toxicity risk for humans. This is a collaborative project with CDER in which the already-existing data are abstracted and utilized to help determine whether animal data can be used to predict human health. Both the published literature and data in CDER files will be utilized. The specific strategy for conducting this analysis is as follows:

1) To retrieve reports of human data from published literature and FDA files for therapeutic agents for which there are adequate data to indicate either positive effects or no effect.
2) To retrieve reproductive and developmental toxicity study data in laboratory animals from FDA files or directly from pharmaceutical companies on the same products.
3) To extract specific data elements into a database for qualitative and quantitative comparison.
4) To evaluate data using the expertise of pharmacology/toxicology and clinical/epidemiology project participants.
5) To conduct statistical analyses, initially using multiple-regression analyses and correlation approaches, with more sophisticated analyses as the data permit.
6) To draw conclusions about the predictability of animal testing data and recommend design improvements as appropriate.

FY 2001 Accomplishments:

A manuscript comparing pharmacokinetic aspects of altenolol was published by our collaborators. A draft manuscript comparing the data for human and rodent embryotoxicity of altenolol has been prepared and is currently being revised.

FY 2002 Plans:

A manuscript comparing the embyrotoxicity of altenolol in humans and rodents will be submitted for publication. The final report will be submitted.

Title	Project Number	Collaborator	Strategic Research Goal
◆ **Mechanism(s) of Folate-Responsive Dysgenesis**	**E0707401**	**None**	**Concept-Driven**

Objective(s):

In this project the specific mechanisms by which folate impacts the events that lead to the closure of the neural tube during fetal development will be investigated. The specific aspects and goals of the project are:

1) To 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 (GD) from GD 5 to GD 15, and to determine if the loss of these cohorts of cells during these windows of anti-folate exposure gives rise to recognizable neural tube defects and neurocristopathies in the fetus at term.
2) To characterize the basal expression of FBPI isoforms; and extent and mechanism 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.
3) To determine if sustained quenching of placental cytotrophoblast FBPI by antisense FBPI cDNA over-expression 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.
4) To 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.

FY 2001 Accomplishments:

Experimental work was started on the methotrexate and folate deficiency experiments. Female mice were fed diets with various levels of folic acid added for eight weeks prior to breeding. The animals were then bred and continued to consume the same chow during gestation. Fetuses were examined at the end of gestation for effects on weight and the incidence of various malformations. Few malformations were observed in this study, and samples indicated that plasma folate levels were not significantly altered by the treatments. An additional experiment was started to lower the folate levels even further to examine the effects of these very low folate levels on normal embryonic development. Further analysis of the initial diet experiment is on-going.

A second experiment was conducted in which methotrexate was administered at 12-hour intervals during gestation beginning on day 5 of gestation and continuing until day 15. Twelve (12) hours after the methotrexate injection, an osmotic mini-pump containing folinic acid was implanted into the mice to rescue the animals from the effects of methotrexate. Methotrexate was used for its anti-folic acid effects, so the exposure to the anti-folate was only 12 hours long. The purpose of this experiment was to determine if these short periods of anti-folate exposure would alter particular cell types to produce specific malformations. Different malformations were produced by methotrexate exposure and consisted of exencephaly, cleft palate, open eye,

Title	Project Number	Collaborator	Strategic Research Goal

micrognathia, and various skeletal anomalies. Fetal weight was adversely affected at a number of different exposures. The data were written up in abstract form and submitted for consideration for the annual meeting of the Teratology Society. The data are also being analyzed further.

FY 2002 Plans:

1) Complete analysis of the methotrexate experiment and the first dietary experiment.
2) Complete the second dietary experiment.

◆ **Examination of Embryonic Gene Expression during Neural Tube Closure** — **E0710901** — **None** — **Concept-Driven**

Objective(s):

1) To determine which genes may demonstrate altered expression as a result of valproic acid treatment.
2) To determine if this altered expression is related to teratogenicity in general or to valproic acid specifically.
3) To determine if gene expression is altered in subsets of cells that may be involved in teratogenicity.

FY 2001 Accomplishments:

The protocol was approved in early 2002.

FY 2002 Plans:

Experimental work will begin.

PI: Harris, Angela

◆ **Modulation of Gene Expression in Chemical Carcinogenesis: Analysis of Aflatoxin B_1 Induced Gene Expression in Human Hepatocytes** — **E0704701** — **None** — **Concept-Driven**

Objective(s):

This project is a part of the Division's genomic/proteomics focus area. The goal of this project is to utilize the new gene expression technologies to understand which genes are affected by the exposure of human hepatocytes to a known human carcinogen (aflatoxin B_1). Because this is a new technology, the experimental approach must include research to define the appropriate experimental parameters. Specific goals are to:

1) Verify aflatoxin B_1 effects on steady-state mRNA levels of eight genes previously identified by differential hybridization of a gene filter array to be aflatoxin B_1 (AFB_1)-responsive in human hepatocytes.
2) 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.
3) To 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.

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4) To evaluate selected genes as described for Objective 1.
5) To 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.
6) To compare gene expression of selected genes in human hepatocytes treated with known rat liver chemical carcinogens, including 2-acetylaminofluorene (2-AAF), dimethylnitrosamine (DMN) and methapyrilene.

FY 2001 Accomplishments:

1) Completed the analysis of gene expression data from AFB_1- and APAP-treated primary human hepatocytes from five donors and three donors, respectively.
2) Completed the analysis of gene expression data from AFB_1 and APAP treated HepG2 cells.
3) Evaluated the effects of culturing matrix on basal gene expression levels of primary rat and human hepatocytes using gene filter arrays.
4) Completed analysis of the culturing matrix data.

FY 2002 Plans:

1) Procure human hepatocytes from three additional male donors to analyze the effects of 2-acetylaminofluorene (2-AAF) and dimethylnitrosamine (DMN). Compare to AFB_1 and APAP data.
2) HepG2 cells will be treated as in #1 for comparison.
3) RNA from objectives #1 and #2 will be used to screen approximately 35,000 human genes using filter-array analysis for genes that are differentially expressed after toxicant exposure.
4) RNA from methapyrilene and pyrilamine treated primary rat hepatocytes will be used to screen an additional 10,000 rat genes for differential expression after exposure. Northern blot or RT-PCR analysis will be used to verify differential gene expression.

PI: Heflich, Robert

◆ **Effect of Azathioprine in Somatic Cell and Germline Hprt Mutant Frequencies in the Mouse** — **E0709901** — **None** — **Predictive Toxicology**

Objective(s):

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

FY 2001 Accomplishments:

Protocol prepared and submitted for review.

FY 2002 Plans:

1) Complete review and approval of protocol.
2) Begin Experiment 1: azathioprine range-finding study in male C57Bl/6 mice to establish a treatment protocol that results in a lymphocyte *Hprt* mutant frequency of >5% with retention of reasonable fertility.

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PI: MacGregor, James

◆ **An Efficient Regulatory Method for Evaluating Chromosomal Damage** — **E0714001** — **CDER, CFSAN, CVM, Litron Labs** — **Method-Driven**

Objective(s):

A collaborative project to evaluate a new method for monitoring chromosomal damage, involving NCTR, CDER, CFSAN, CVM and Litron Laboratories has been initiated. Flow cytometric scoring of micronucleated cells in peripheral blood samples is being compared with traditional microscopic scoring, and 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.

FY 2001 Accomplishments:

FY 2002 Plans:

1) Compare flow cytometry method with microscopic scoring in the rat and dog.
2) Determine kinetics of micronucleated cell formation and elimination in the rat and dog.

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** — **None** — **Agent-Driven**

Objective(s):

Malachite and leucomalachite green are currently being tested for carcinogenicity under the National Institute of Environmental Health Sciences Interagency Agreement (NIEHS IAG). 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.

FY 2001 Accomplishments:

Screened 72 control and leucomalachite green-treated rats for *lac*I mutations in the liver tissues. Mutant frequency VS doses were plotted and data were presented at the 2001 TSSRC meeting. *Hprt* mutant frequency was also determined in the spleenic lymphocytes of the treated and control animals. In addition, micronucleus frequency was determined in the bone marrow of treated and control rats. *Lac*I mutational spectrum was developed for 16 week exposure and statistical analysis of mutational data was performed for evaluating any differences between control and treated spectra.

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FY 2002 Plans:

Animals exposed to leucomalachite green for 32 weeks will be evaluated for the *lac*I mutant frequency (MF) in the liver, *Hprt* MF in the lymphocytes and micronucleus frequency in the bone marrow. If there is any significant MF increase, the mutants will be further characterized for types of mutations. The results will be presented at the next TSSRC meeting at NCTR.

◆ **ADDENDUM: Micronucleus and Gene Mutation Analysis in F344 Big Blue Rats Administered Leucomalachite Green in the Diet for 4, 16, and 32 weeks.** | **E0212831** | **None** | **Agent-Driven**

Objective(s):

Malachite and leucomalachite green are currently being tested for carcinogenicity under the NIEHS/NCTR IAG. Recent results from addendum E212821, indicate a two-fold increase in lacI 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 E212821 (32-week dose groups) to include additional indicators of hepatic toxicity.

FY 2001 Accomplishments:

The proposal to evaluate 32 weeks-treated-rats for hepatic toxicity began.

FY 2002 Plans:

Pathology services will evaluate hepatic toxicity of rats exposed to 32 weeks of leucomalachite green in diet.

◆ **ADDEND: Quantitative and Molecular Analysis of 7,12-dimethylbenz[a]anthracene (DMBA) induced Mutations in the Model Blue Rat: Comparison of Mutagenesis in the Trans-gene lacI with the Endogenous Gene Hprt and Cancer Genes H-ras and p53** | **E0690601** | **None** | **Predictive Toxicology**

Objective(s):

1) To determine the mutant frequency and mutation spectrum of the *lacI* transgene of the Blue Rat following exposure to DMBA in surrogate and target tissues and compare these mutant frequencies and mutational spectra to those determined in Objectives 2 and 3.
2) To determine the mutant frequency and mutation spectrum of the endogenous *Hprt* reporter gene in T-lymphocytes from the spleens of Fischer 344 and Blue Rats following exposure to DMBA.
3) To induce mammary tumors in Fischer 344 rats and Blue Rats by exposure to DMBA and screen tumor DNA for mutations in the oncogene, H-*ras* and the tumor suppressor gene, p53.

FY 2001 Accomplishments:

1) This was a long-term project concluded in FY 2000.
2) The technical report will be prepared soon.
3) Several manuscripts generated by this project have been published.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

This project will not continue. A final manuscript derived from this project is under preparation.

◆ **ADDEND: Quantitative and Molecular Analysis of 7,12-dimethylbenz[a] anthracene induced Mutations in the Model Blue Rat: Comparison of Mutagenesis in the Trans-gene lacI with the Endogenous Gene Hprt and Cancer Gene H-ras** — **E0690611** — **None** — **Predictive Toxicology**

Objective(s):

NCTR protocol E0690601 was undertaken in order to validate the use of the Big Blue rat as a model for determining the *in vivo* mutagenicity of potential human toxicants.

FY 2001 Accomplishments:

Results from experiment 1 uncovered unanticipated issues concerning the nature of mutagenic responses in the Big Blue model. These results suggest experiments not included in the original protocol that may resolve these issues. Necessitates using additional animals to complete experiments 1, 2, and 3. However, no work was performed as it was decided not to pursue this project.

FY 2002 Plans:

The technical report will be written.

PI: Mckinzie, Page

◆ **Application of the MutEx/ACB-PCR Method of Genotypic Selection to the Detection of K-ras Mutations** — **E0706601** — **None** — **Predictive Toxicology**

Objective(s):

The majority of methods for quantitating mutation require the use of selective drugs which allow mutants to grow and prevent normal cells from growing. All of these techniques require extensive cell culture and can be time-consuming and expensive. There are techniques that utilize genotypic selection that allow for a molecular amplification of the rare mutant DNA sequences and thus provide for a direct measurement of mutant frequencies. The Division has several projects in which this new technology is being developed. The specific goals of this project are to:

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.

FY 2001 Accomplishments:

1) Determined optimized ACB-PCR conditions for human K-*ras* codon 12 GGT → GAT detection at a sensitivity of 10^{-5}.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

2) Determined optimized ACB-PCR conditions for human K-*ras* codon 12 GGT → GTT detection at a sensitivity of 10^{-5}.

FY 2002 Plans:

1) Make ACB-PCR DNA standards for rat K-ras codon 12 GGT → GAT and GGT → GTT mutations.
2) Optimize ACB-PCR for the rat K-ras DNA standards.
3) Continue with MutEx enrichment for human K-ras codon 12 mutant standards.
4) Begin validation of method with *in vitro* mutagen exposure experiments.

PI: Mittelstaedt, Roberta

◆ **ADDEND: Measurement of H-ras Codon 61 CAA->AAA Mutation in Mouse Liver DNAs using the MutEx/ACB-PCR Genotypic Selection** — **E0704121** — **None** — **Predictive Toxicology**

Objective(s):

Quantify and identify *lac*I 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 tumorigenesis.

FY 2001 Accomplishments:

Tissue collection is complete and *lac*I mutational analysis is nearly complete.

FY 2002 Plans:

Publish the results.

◆ **ADDEND: Measurement of H-ras Condon 61 CAA->AAA Mutation in Mouse Liver DNAs using the MutE/ACB-PCR Genotypic Selection** — **E0704141** — **None** — **Predictive Toxicology**

Objective(s):

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

FY 2001 Accomplishments:

The protocol was written and the animals have been ordered.

FY 2002 Plans:

Treat the animals, collect the tissues, and analyze the mutations.

PI: Morris, Suzanne

◆ **p53 Transgenic Mouse Evaluations of Genistein 28-day and 36-week Studies** — **E0213601** — **None** — **Predictive Toxicology**

Objective(s):

The phytoestrogen genistein is a primary component of a high soy diet. There is currently widespread interest in the impact of a high soy diet on human health. While there is some indication that phytoestrogens may improve health in peri- and post-

Title	Project Number	Collaborator	Strategic Research Goal

menopausal women, there is concern that these compounds may have the potential to be carcinogens. The study is being conducted to investigate this possibility. Specific goals for the project are:

1) To determine the toxicity of genistein in the C57Bl6/J strain and to select doses for the 36-week studies.
2) To identify the potential carcinogenicity of genistein in the p53 transgenic mouse model.
3) To determine if the potential carcinogenicity of genistein relates to changes in the rates of cell death and cell proliferation.
4) To 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.

FY 2001 Accomplishments:

In-life phase, 28-day dose-range-finding study completed; statistical analysis is ongoing with completion expected in early FY 2002.

FY 2002 Plans:

1) Manuscript in preparation from the 28-day dose-range-study.
2) Chronic phase of the experiment cancelled.
3) Technical report to be prepared in late FY 2002.

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

Objective(s):

1) To establish a database for genomics data.
2) To relate the changes in gene expression to *in vitro* genotoxicity measures that are utilized in hazard assessment.

FY 2001 Accomplishments:

1) Gene-tox studies completed with BPDE and gamma radiation; initial studies with cDNA microarrays completed.
2) Data presented at ILSI workshops in March (San Diego) and May (Washington, DC).
3) Seminars presented at CDER (MOD-1) and FDA (Parklawn) for the FDA; poster presented at the 2001 European Environmental Mutagen Society in Ghent, Belgium; seminar presented at Mario Negri Research Institute in Milan, Italy.

FY 2002 Plans:

1) Analysis of expression changes from TK6 cells exposed to BPDE or gamma radiation as detected by cDNA microarrays is currently underway.
2) One manuscript is in preparation and a second manuscript will follow as the data are analyzed.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Parsons, Barbara

- **Measurement of H-ras Codon 61 CAA->AAA Mutation in Mouse Liver DNAs using the MutEx/ACB-PCR Genotypic Selection** — **E0704101** — **None** — **Predictive Toxicology**

Objective(s):

The majority of methods for quantitating mutations require the use of selective drugs that allow mutants to grow and prevent normal cells from growing. All of these techniques require extensive cell culture and can be time-consuming and expensive. There are techniques that utilize genotypic selection that allow for a molecular amplification of the rare mutant DNA sequences and thus provide for a direct measurement of mutant frequencies. The Division has several projects in which this new technology is being developed. The specific goals of this project are:

To 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.

1) To determine whether or not the MutEx/ACB-PCR genotypic selection is sensitive enough to measure the spontaneous frequencies of H-*ras* codon 61 CAA->AAA mutation in three different mouse models: $B_6C_3F_1$, C57BL/6, and the PMS2 mismatch repair-deficient, transgenic mouse.

FY 2001 Accomplishments:

1) The 4-aminobiphenyl newborn mouse assays in three different strains of mice (C57BL/6, $B_6C_3F_1$, and Pms2 mismatch repair-deficient [-/-] mice) have been completed.
2) The ability to measure the background level of spontaneous, oncogenic point mutations in non-tumor tissues was demonstrated.
3) A manuscript entitled, "Prospects for Applying Genotypic Selection of Somatic Oncomutation to Chemical Risk Assessment," was accepted for publication in *Mutation Research*.
4) A presentation entitled, "Measurement of Rare Point Mutation for Use in Risk Assessment," was presented at the Breakfast Meeting of the New Technologies Interest Group, 32nd Annual Meeting of the Environmental Mutagen Society.
5) A poster entitled, "Detection of spontaneous mouse H-*ras* mutation by genotypic selection; PNA enrichment of target sequences and analysis by MutEx/ACB-PCR" was presented at the Breakfast Meeting of the New Technologies Interest Group, 32nd Annual Meeting of the Environmental Mutagen Society.
6) A manuscript entitled, "Developing Methods of Genetic Analysis to Improve Cancer Risk Assessment," was accepted for publication in the FDA's new online journal, *Regulatory Research Perspectives*.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

1) Publish the PNA-based, gene-specific enrichment method, a method that is useful for isolating a population of "target" DNA molecules for mutational analyses.
2) Publish a report on the use of ACB-PCR for identifying secondary mutations that occur during mouse liver tumor progression.
3) Analyze the liver DNAs of 4-aminobiphenyl-treated and untreated C57BL/6, $B_6C_3F_1$ and Pms2 mismatch repair-deficient mice for H-*ras* codon 61 mutation using PNA selection coupled with MutEx/ACB-PCR.

◆ **ADDEND: Measurement of H-ras Codon 61 CAA->AAA Mutation in Mouse Liver DNAs using the MutEx/ACB-PCR Genotypic Selection** — E0704111 — None — 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 destroyed. Additional animals and resources were requested in order to repeat the one-month timepoint of the $B_6C_3F_1$ newborn mouse assay.

FY 2001 Accomplishments:

Newborn $B_6C_3F_1$ mice were treated with 4-aminobiphenyl or the dimethylsulfoxide control and liver tissues were harvested one-month post-treatment, thereby replacing the tissues that were lost.

FY 2002 Plans:

The amount of H-*ras* codon 61 CAA to AAA mutant DNA sequence in these tissues will be measured using a sensitive DNA-based mutation detection method, MutEx/ACB-PCR.

◆ **Pms2 Mismatch Repair-Deficient Mouse Breeding Colony** — S00222 — None — Predictive Toxicology

Objective(s):

The objective is to maintain a breeding colony of Pms2 transgenic mice, which are not available commercially, so this strain can be used in future protocols. Because these animals are mismatch repair-deficient, they accumulate mutation to a greater extent than their mismatch repair-proficient counterparts and, therefore, are a valuable mouse model for mutation research. Heterozygous $Pms2^{+/-}$ animals will be maintained and, as protocols are developed, we will have the capacity to breed the necessary $Pms2^{+/-}$, mismatch repair-deficient animals.

FY 2001 Accomplishments:

A protocol was prepared describing how a mismatch repair-deficient mouse colony (Pms2 mouse) will be established at NCTR because an animal model that is unable to repair chemically induced DNA damage may provide a sensitive system for genetic toxicology studies.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

Pms2, mismatch proficient (+/-) breeders will be maintained so that mismatch repair deficient $Pms2^{-/-}$ animals can be bred as needed for other genetic toxicology studies.

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** — **None** — **Predictive Toxicology**

Objective(s):

Because cancer is a disease requiring the induction of mutation and the clonal expansion of mutated cells, one would expect that the developing fetus and young infant would be particularly susceptible to carcinogen exposure. This project provides information that can be used to evaluate this hypothesis. Experiments will be conducted to 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).

FY 2001 Accomplishments:

1) Collection and analyses of *Hprt* mutant frequencies in 4-ABP-treated and control $Pms2^{+/+}$, $^{+/-}$, and $^{-/-}$ mice was completed.
2) Characterization of *Hprt* mutant clones and mutant sequencing began.
3) Preliminary data were presented in a poster at the Environmental Mutagen Society (EMS) Meeting in March 2001.

FY 2002 Plans:

The characterization of mutant clones and mutant sequencing will continue until complete and the mutational spectra can be compared between treated and control animals, among animals with different Pms2 genotypes, and presumably different DNA repair capacity.

PI: Valentine, Carrie

◆ **The Development of Transgenic Mice Harboring Bacteriophage ϕX174 with Sites Specific for Detecting Mutations at Guanosine: Cytosine Nucleotides, Small Frameshifts, and Extended Deletions** — **E0700201** — **None** — **Predictive Toxicology**

Objective(s):

To find specific mutations in bacteriophage ϕX174 that render the bacteriophage non-infectious and that will revert to plaque-forming ability only when mutation occurs by specific mechanisms: 1) base substitution at a G:C base pair, or 2) frameshift caused by deletion of one or two nucleotides. An additional objective is to determine the feasibility of using ϕX174 to detect the deletion of an extended sequence. Phage-harboring these mutations will be used to construct a transgenic mouse model for measuring mutations *in vivo*.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

1) Manuscript accepted for publication in *Environmental and Molecular Mutagenesis*.
2) Forward mutational assay developed for gene A of bacterial virus ΦX174 carried in mouse cells.
3) Target sites and mutant spectrum identified for the ΦX174 gene A forward mutational assay in untreated cells and cells treated with the mutagen ethyl nitrosourea (ENU).
4) License offered for forward assay in Federal Register.
5) Frameshift assay developed that detects –1 A deletions.
6) Assay begun on ENU- and solvent-treated animal tissues to evaluate sensitivity using single-burst analysis.

FY 2002 Plans:

Continue sensitivity studies until protocol E0711501 is approved.

FY 2001 Publications

Cada, A.M., Hansen, D.K., Laborde, J.B. and Ferguson, S.A., Minimal behavioral or developmental effects from developmental exposure to St. John's Wort (Hypericum perforatum) in Sprague Dawley rats, *Nutritional Neuroscience*, 4:135-141. Accepted: 11/4/00 (N/A)

Chen, T., Mittelstaedt, R.A., Aidoo, A., Hamilton, L.P., Beland, F.A., Casciano, D.A. and Heflich, R.H., Hprt and lacI mutant frequency in relation to DNA adduct formation in N-hydroxy-2-acetylaminofluorene-treated Big Blue Rats, *Environmental and Molecular Mutagenesis*. Accepted: 12/7/00 (E0695801)

Delongchamp, R.R., Valentine, C.R. and Malling, H., Estimation of the average burst size of Phi X174am3, cs70 for use in mutation assays with transgenic mice, *Environmental and Molecular Mutagenesis*, 37:356-360. Accepted: 4/14/01 (E0709501, S00032)

Desai, V.G., Aidoo, A., Casciano, D.A. and Feuers, R.J., Activity profile of glutathione-dependent enzymes and respiratory chain complexes in rats supplemented with antioxidants and treated with carcinogens, *Archives of Biochemistry and Biophysics*, 394(2):255-264. Accepted: 7/26/01 (E0701401)

Djuric, Z., Lewis, S.M., Lu, M., Mayhugh, M.A., Tang, N. and Hart, R.W., Effect of Varying Dietary Fat Levels on Rat Growth and Oxidative DNA Damage, *Nutrition and Cancer*, 39:214-219. Accepted: 2/7/01 (E0260001)

Domon, O.E., McGarrity, L.J., Bishop, M.E., Yoshioka, M., Chen, J.J. and Morris, S.M., Evaluation of the genotoxicity of the phytoestrogen, coumestrol, in AHH-1 $TK^{+/-}$ human lymphoblastoid cells, *Mutation Research*, 474:129-137. Accepted: 3/1/01 (E0705501)

Duffy, P.H., Feuers, R.J., Seng, J.E., Lewis, S.M., Mayhugh, M.A., Aidoo, A. and Casciano, D.A., The Effects of Different Levels of Dietary Restriction on Aging and Survival in the Sprague Dawley Rat: Implications for Chronic Bioassay Studies, *Aging*, 13:263-272. Accepted: 12/5/00 (E0692401)

Duffy, P.H., Lewis, S.M., Mayhugh, M.A., Casciano, D.A. and Feuers, R.J., Effect of the AIN-93M Purified Diet and Dietary Restriction on Survival in the Sprague Dawley Rat: Implications for Chronic Studies, *The Journal of Nutrition*. Accepted: 9/18/01 (E0692401)

Fang, H., Tong, W., Shi, L., Blair, R.M., Perkins, R.G., Branham, W.S., Hass, B.S., Xie, Q., Dial, S.L., Moland, C.L. and Sheehan, D.M., Structure-Activity Relationships for a Large Diverse Set of Natural, Synthetic and Environmental Estrogens, *Chem. Res. Toxicol*., 14(3):280-294. Accepted: 2/8/01 (E0290001)

Gamboa da Costa, G., Hamilton, L.P., Heflich, R.H., Marques, M.M. and Beland, F.A., DNA adduct formation and mutant induction in Sprague Dawley rats treated with tamoxifen and its derivatives, *Carcinogenesis*, 22:1307-1315. Accepted: 11/1/00 (E0701101)

Gamboa da Costa, G., Manjanatha, M., Marques, M.M. and Beland, F.A., Induction of lac1 mutations in Big Blue Rats treated with tamoxifen and alpha-hydroxytamoxifen, *Cancer Letters*, 176:37-45. Accepted: 8/27/01 (E0701101)

Hong, H., Tong, W., Fang, H., Shi, L., Xie, Q., Wu, J., Perkins, R.G., Branham, W.S. and Sheehan, D.M., Prediction of estrogen receptor binding for 58,000 chemicals using an integrated system of a tree-based model with structural alerts, *Environmental Health*. Accepted: 6/5/01 (E0290001)

Khaidakov, M., Bishop, M.E., Manjanatha, M., Lyn-Cook, L.E., Desai, V.G., Chen, J.J. and Aidoo, A., Influence of dietary antioxidants on the mutagenicity of the model mammary carcinogen 7,12-dimethylbenz[a]-anthracene and the antitumor agent bleomycin in female rats, *Mutation Research*, 480:163-170. Accepted: 2/2/01 (E0701401)

Khaidakov, M., Manjanatha, M. and Aidoo, A., Molecular analysis of mitochondrial DNA mutations from bleomycin treated rats, *Mitochondrian*. Accepted: 9/17/01 (E0701401)

Li, J., Feuers, R.J., Buffington, C.K. and Cowan, G.S., Influence of body fat distribution on cardiorespiratory response to exercise testing in bariatric surgical morbidly obese females (Fat distribution and Exercise testing), *Journal of Respirology*, 6(1):9-13. Accepted: 4/17/01 (E0699801)

Mckinzie, P.B., Delongchamp, R.R., Heflich, R.H. and Parsons, B.L., Prospects for Applying Genotypic Selection of Somatic Oncomutation to Chemical Risk Assessment, *Reviews in Mutation Research*, 489:47-78. Accepted: 6/22/01 (E0704101)

Monroe, J.J., Manjanatha, M. and Skopek, T.R., Extent of CpG methylation is not proportional to the *in vivo* spontaneous mutation frequency at transgenic loci in Big Blue rodents, *Mutation Research*, 476:1-11. Accepted: 1/15/01 (E0690601)

Morris, S.M., Pipkin, J.L., Hinson, W.G., Shaddock, J.G., Tolleson, W.H. and Casciano, D.A., Decreased *in vitro* interaction between p53 and nuclear stress proteins in the p53-deficient mouse, *Electrophoresis*, 22:2092-2097. Accepted: 12/2/00 (E0694901)

Parsons, B.L. and Mckinzie, P.B., Developing methods of genetic analysis to improve cancer risk assessment, *Regulatory Research Perspectives*, 1(2):1-11. Accepted: 9/18/01 (E0704101)

Slikker, W., Desai, V.G., Duhart, H.M., Feuers, R.J. and Imam, S.Z., Hypothermia enhances Bcl-2 expression and protects against oxidative stress induced cell death in chinese hamster ovary cells, *Free Radical Biology and Medicine*, 31:405-411. Accepted: 5/1/01 (E0698301)

Young, J.F., Wosilait, W.D. and Luecke, R., Analysis of Methyl Mercury Disposition in Humans Utilizing a PBPK Model and Animal Pharmacokinetic Data, *Journal of Toxicology and Environmental Health*, Part A, 63(1):19-52. Accepted: 10/2/00 (P00393)

Microbiology

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

Executive Summary

The Division of Microbiology serves a multi-purpose function with specialized expertise to perform fundamental and applied research in microbiology. 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 five focal areas: 1) Foodborne pathogens, food safety and methods development; 2) Gastrointestinal microbiology and host interactions; 3) Environmental biotechnology; 4) The use of microorganisms as models to predict the metabolic pathways by which drugs are metabolized in mammals; and 5) Microbiological surveillance and diagnostic support of research.

In FY 2001, Division of Microbiology research scientists provided valuable information to FDA to evaluate key regulatory issues in food safety and environmental biotechnology, with special emphasis on antimicrobial resistance in the food-animal production environment.

Reports of antibiotic-resistant bacteria from farms and animal carcasses are raising concerns that antibiotic use in agriculture may play a role in selecting for antibiotic resistance. This is a very controversial issue, since some contend that the indiscriminate use of antibiotics in agriculture could create a massive reservoir of resistant microorganisms in the environment that could infect humans through the food chain. However, others contend that the abuse of antibiotics in human medicine may instead be largely responsible for the increase in antibiotic resistance. Animal drug industry representatives feel that there is not enough evidence to conclusively demonstrate a link between the use of antibiotics in food animals and the emergence of antibiotic-resistant bacteria. 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.

As part of the Food Safety Initiative, the Division of Microbiology established collaborative research agreements with scientists at the Center for Veterinary

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Medicine (CVM), Arkansas Poultry and Livestock Commission and the Department of Poultry Sciences, University of Arkansas. Researchers in the Division of Microbiology have collected litter, feed and water samples from farms to isolate *Salmonella, Campylobacter*, and *Escherichia coli* to determine if they are fluoroquinolone-resistant. Molecular methods were developed to screen for fluoroquinolone-resistant genes in *Salmonella* spp., *Campylobacter* spp. and *E. coli* isolates from chicken and turkey farms. Our results indicate that poultry products may serve as reservoirs of fluoroquinolone-resistant genes.

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 performing pre-validation studies on an *in vitro* 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 2001, the *in vitro* continuous culture system for the analysis of low levels of antimicrobials on the human intestinal microflora was modified and refined. Recommendations on the methods and protocols for determining the effect of residue 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.

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 get out of place and 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 intrinsically resistant to high concentration of fluoroquinolones. They also determined that these fluoroquinolone-resistant bacteria are also resistant to other classes of antimicrobial agents.

Since there has been concern about the use of antibiotics in agriculture, other approaches are being evaluated to minimize contamination of animal products with foodborne human pathogens. Reducing colonization of animals by pathogenic bacteria by using competitive exclusion treatments, phage therapy, vaccines and farm hygiene 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. 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 antibiotics and the spread of antibiotic-resistance genes. Researchers in the Division of Microbiology have

standardized a quick and accurate *in vitro* assay for determining the efficacy of potential competitive exclusion products. In addition, researchers have characterized vancomycin-resistant isolates from a competitive exclusion product. Our studies indicate that FDA will need to standardize the identification techniques used to characterize the components of competitive exclusion products.

The environmental fate of veterinary drugs and the 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 bio-degradation 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 waste and aquaculture sites.

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. A major initiative in FY 2001 was to develop molecular biology detection procedures for each of the microorganisms in our potential pathogen list and incorporate these methods into our surveillance screening. A major screening of the NCTR animal-breeding colony for *Helicobacter hepaticus* and *Helicobacter bilis* by polymerase chain reaction (PCR) assay was completed.

FY 2001 Accomplishments and FY 2002 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Campbell, Warren

- **SPF Mice Colony** — **E0001000** — **None** — **Center Support**

Objective(s):

FY 2001 Accomplishments:

Analyzed 515 animals, 149 air, 130 environmental swabs and 761 cage water samples for microbiological contamination.

FY 2002 Plans:

Analyze approximately 530 animals, 156 air, 130 environmental swabs and 800 cage water samples for microbiological contamination.

- **SPF Rat Breeding Colony** — **E0011000** — **None** — **Center Support**

Objective(s):

To determine health status of rat breeding colonies maintained under specified pathogen free (SPF) conditions.

FY 2001 Accomplishments:

Analyzed 206 animals, 101 air, 72 environmental swabs and 317 cage water samples for microbiological contamination.

FY 2002 Plans:

Analyze approximately 210 animals, 110 air, 70 environmental swabs and 320 cage water samples for microbiological contamination.

- **Conventional Rat Breeding Colony** — **E0011100** — **None** — **Center Support**

Objective(s):

To determine health status of rat breeding colonies maintained under conventional conditions.

FY 2001 Accomplishments:

Analyzed 46 animals, 89 air, 140 environmental swabs and 140 cage water samples.

FY 2002 Plans:

Analyze approximately 50 animals, 100 air, 170 environmental swabs and 430 cage water samples.

- **Quarantine Animals** — **E0011300** — **None** — **Center Support**

Objective(s):

To determine health status of animals received at NCTR and held under quarantine conditions.

FY 2001 Accomplishments:

Analyzed 61 animals, 115 cage wastes, and 1,798 cage water samples.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

Analyze approximately 70 animals, 120 cage wastes, and 2,000 cage water samples.

- **Diet Prep General Support** **E0014500** **None** **Center Support**

Objective(s):

To 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.

FY 2001 Accomplishments:

Analyzed 103 air; 2,561 environmental swabs; 1,055 BI; 200 feed; and 30 eyewash samples.

FY 2002 Plans:

Analyze approximately 105 air; 2,500 environmental swabs; 1200 BI, 310 feed, and 30 eyewash samples.

- **Animal Husbandry Breeding Support** **E0002200** **None** **Center Support**

Objective(s):

To microbiologically evaluate animals and non-animal samples not specifically designated to an ongoing experiment.

FY 2001 Accomplishments:

Analyzed one (1) mouse, 24 bedding shipments; 5,083 BI; 6,046 environmental swabs; and 294 processed-water samples.

FY 2002 Plans:

Analyze approximately 30 bedding shipments; 6,000 BI; 4,200 environmental swabs; and 300 processed-water samples.

- **Primate Colony Surveillance** **E0023500** **None** **Center Support**

Objective(s):

To determine health status of primate colony.

FY 2001 Accomplishments:

Screened 80 primate fecal waste samples for bacterial and parasitic pathogens.

FY 2002 Plans:

Screen 80 primate fecal waste samples for bacterial and parasitic pathogens.

- **Microbiological Diagnostic Methods: Development, Testing and Evaluation** **E0026200** **None** **Method-Driven**

Objective(s):

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

FY 2001 Accomplishments:

Developed PCR assay for *Helicobacter bilis*.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

1) Develop PCR assay for *Bordetella bronchiseptica* and *Corynebacterium kutscheri.*
2) Develop immunosuppression procedures to be used to monitor for latent bacterial, viral, and protozoan pathogens.

- **General Microbiological Support– Bacteriology, Parasitology, Mycology, and Virology** — S00006 — None — Center Support

Objective(s):

To provide the Center with microbiological surveillance and diagnostic support to determine and maintain the health status of the animal colony.

FY 2001 Accomplishments:

Performed routine microbiological surveillance evaluation on the following: 990 animals; 190 animal wastes; 2,840 cage waters; 425 room airs; 7,275 room swabs; 190 processed feeds; 325 processed waters; 16 feed shipments; 6,520 biological indicators for sterility; and 90 personnel surveillance samples.

FY 2002 Plans:

Plan to complete the following routine microbiological evaluations: 1,400 animals; 200 animal wastes; 425 room airs; 2,900 cage waters; 325 processed waters; 20 bedding shipments; 7,300 room swabs; 190 processed feeds; 16 feed shipments; 6,500 biological indicators for sterility; and 90 personnel surveillance samples.

- **Microbiology Division-Media Preparation** — S00064 — None — Center Support

Objective(s):

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

FY 2001 Accomplishments:

Provided media and supplies to the Divisions of Microbiology, Biochemical Toxicology, Genetic and Reproductive Toxicology, and the animal husbandry and diet preparation contractors.

FY 2002 Plans:

Provide media and supplies to the Divisions of Microbiology, Biochemical Toxicology Division, Genetic and Reproductive Toxicology, and the animal husbandry and diet preparation contractors.

- **Special Epidemiology Investigations of Potential Microbiological Contamination Problems** — S00185 — None — Center Support

Objective(s):

1) To investigate potential microbiological contamination problems.
2) To report non-routine sample time which is not recorded on Sample Collection Report (SCR).

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

Major screening of NCTR animal breeding colony for *Helicobacter hepaticus*.

FY 2002 Plans:

Special monitoring for *Helicobacter* sp. in the NCTR breeding colony during clean-up and re-population procedures.

PI: Erickson, Bruce

◆ **Determining the Effect of Low Levels of Antibiotic Residues on the Human Intestinal Microflora Using an *in vitro* Continuous Culture System** — **E0709201** — **CVM** — **Method-Driven**

Objective(s):

To use an *in vitro* chemostat culture system that mimics the human intestinal tract to determine the concentrations of antibiotic residues in food that produce no adverse effects on the human intestinal microflora. The adverse effects to be evaluated include: 1) changes in numbers of selected organisms; 2) changes in the metabolic activity of the fecal flora relating to metabolism of endogenous and exogenous compounds; 3) development of antimicrobial-resistant strains; and 4) disruption of colonization resistance of pathogenic microorganisms.

FY 2001 Accomplishments:

1) Prepared research protocol E0709201, and guided this proposal through the NCTR protocol review and approval system.
2) Modified and refined the *in vitro* continuous culture system for the analysis of the effect of low levels of antimicrobials on the human intestinal microflora.
3) Completed analysis of the samples collected during exposure of chemostats to ciprofloxacin for volatile fatty acid composition, as an endpoint for system perturbation by low-level antibiotic residues. Initiated chemostat experiments for monitoring the change in the population distribution during the stabilization phase of continuous culture experiments.
4) Prepared a progress report on the use of the *in vitro* continuous culture system for antimicrobial residue testing, and presented the work at the meeting of the Microbial Safety Task Force of the VICH Safety Working Group in Brussels, Belgium.
5) Prepared and presented a topic paper on normal variation in human intestinal microflora for the Microbial Safety Task Force, and led a discussion of how this variation can impact experimental design for *in vitro* or *in vivo* model systems of the human intestinal microflora.

FY 2002 Plans:

1) Improve techniques for monitoring human intestinal microflora cultures using molecular methods for more rapid and accurate analysis of changes in populations of different bacterial strains.
2) Investigate additional *in vitro* methods such as fecal batch cultures or feed-batch cultures to monitor antimicrobial residue effects.

Title	Project Number	Collaborator	Strategic Research Goal

3) Investigate the suitability of the *in vitro* culture methods for testing food additives or pre- and pro-biotic products.
4) Continue participation in the Microbial Safety Task Force, preparing recommendations for methods and protocols for determining the effect of residue levels of antimicrobials on the human intestinal microflora.

PI: Khan, Ashraf

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

Objective(s):

1) To isolate, identify and characterize nalidixic acid and fluoroquinolone-resistant *Salmonella* spp. from chicken farms (animal feed, feces, manure, litters, and animals) by biochemical and polymerase chain reaction.
2) To determine minimum inhibitory concentration for environmental isolates, development of molecular techniques and its comparison with clinical strains.
3) To determine drug-resistance mechanisms in the environmental isolates and their characterization by molecular techniques.
4) To determine influence of seasons and the frequency of isolation of fluoroquinolone-resistant *Salmonella* spp.

FY 2001 Accomplishments:

1) Isolated several ciprofloxacin and nalidixic acid (antibiotic)-resistant *E. coli* from chicken and turkey farms.
2) Characterized ciprofloxacin and nalidixic acid-resistant *E. coli* isolated from chicken and turkey farms.
3) Characterized fluoroquinolones-resistant *Campylobacter* spp. from chicken and turkey farms by pulse-field gel electrophoresis.
4) Developed PCR methods for the rapid detection of multidrug-resistant *Salmonella typhimurium*.

FY 2002 Plans:

1) Continue isolation and molecular characterization of fluoroquinolone-resistant *Salmonella* spp. and *E. coli* from chicken and turkey litters.
2) Determine the influence of climatic conditions on the occurrence of quinolone-resistant *Salmonella* spp. and *E. coli*.
3) A collaborative project with CFSAN (Gulf Coast Seafood Laboratory, Dauphin Island, AL) on "Development of a molecular method to characterize the United States outbreak isolates of *Vibrio parahaemolyticus* O3:K6 isolates" will be initiated.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Khan, Saeed

- **Molecular Screening Methods for the Determination of Vancomycin Resistance in Selective Competitive Exclusion Product CF3 (Preempt™) Bacteria** — E0705301 — None — Method-Driven

Objective(s):

1) To islolate, identify, and biochemically characterize vancomycin-resistant bacteria present in a commercially available competitive exclusion product CF3.
2) To develop a rapid PCR method of the detection of vancomycin-resistance determinant genes, namely, the *VanA0*, *VanB*, *VanC* and *D-ala-D-lac* ligase gene *Ddl*.
3) To characterize plasmid DNA Profile and plasmid-mediated drug resistance transfer.
4) To conduct genetic fingerprinting of the vancomycin-resistant microorganisms present in a Preempt™ culture.
5) To conduct nucleotide sequence analysis of the PCR products of vancomycin-resistant determinant genes showing interesting restriction profiles.

FY 2001 Accomplishments:

1) Characterized vancomycin-resistant isolates from the competitive exclusion product Preempt™ by pulsed-field gel electrophoresis (fingerprint analysis).
2) Identified a 242 kb plasmid that was present in three out of ten isolates.
3) Isolated enterococcal mutator strains after being exposed to several concentrations of different antibiotics.

FY 2002 Plans:

1) Study the drug-resistance transfer using three isolates that harbor a 242 kb plasmid.
2) Restriction, digestion and sequence analysis of the vancomycin resistance markers in vancomycin-resistant isolates from Preempt™.
3) Microarray detection and identification of antibiotic drug-resistant strains of *Mycobacterium tuberculosis* (MTB) and standardization of a procedure as a Co-PI in a collaborative protocol with CDRH.
4) Characterize the enterococcal mutator strains obtained after being exposed to several concentrations of different antibiotics.

PI: Nawaz, Mohamed

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

Objective(s):

1) To isolate and identify fluoroquinolone-resistant *Campylobacter jejuni* and *C. coli* from water, feed, and litter samples in poultry houses.
2) To determine the optimum concentration of nalidixic acid and fluoroquinolone resistance in *C. jejuni* and *C. coli.*

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

3) To determine the influence of various seasons and the frequency of isolation of fluoroquinolone-resistant *C. jejuni* and *C. coli*.
4) To conduct molecular characterization of fluoroquinolone resistance by polymerase chain reaction (PCR), nucleotide sequencing, and single-strand conformation polymorphism (SSCP).

FY 2001 Accomplishments:

1) Isolated 21 fluoroquinolone-resistant *Campylobacters* from chicken samples.
2) Nineteen (19) of the 21 isolates were identified as *C. jejuni* and two were identified as *C*. *coli.* All isolates were resistant to multiple antibiotics.
3) A polymerase chain reaction (PCR) protocol successfully amplified the *flaA* gene from all 21 isolates. This indicated that all isolates were virulent and pathogenic.
4) The isolates were further characterized at the gene level by PCR-RFLP of the *flaA* gene and by pulsed-field gel electrophoresis (PFGE). These results indicated that there were at least 15 different strains of *Campylobacters* in the 21 isolates.
5) Submitted manuscripts for publication on this aspect.

FY 2002 Plans:

1) Morphological, biochemical and genetic characterization of *Campylobacters* isolated from turkey.
2) Characterization of the lipopolysaccharide (LPS) gene in *Campylobacters* that is responsible for triggering the Guilliane Barr Syndrome in humans.
3) Develop a diagnostic method (PCR) to identify the *campylobacter* strains that have this gene.

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

Objective(s):

1) To determine the biodegradation rates and metabolic fate of antimicrobials, oxytetracycline and Sulfadimethoxine-Ormetoprim (Romet-30) (SDO), used in fish farming systems.
2) To isolate, characterize and identify OTC- and SDO-resistant organisms from aquaculture sediment and natural environment samples and conduct molecular characterization of the genes that regulates resistance to the drugs.

FY 2001 Accomplishments:

1) Standardized a isolation technique to select strains of *Aeromonas* spp., *Citrobacter* spp., *Vibrio* spp., and *E. coli*.
2) To date we have isolated 12 strains of *Aeromonas* spp. from aquaculture sediments. These isolates were identified as *A. veronii*, *A. hydrophilia* and *A*. *caviae.*

All strains were resistant to multiple antibiotics. However, none of the strains were resistant to oxytetracycline, trimethoprim and sulfamides.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

1) Isolation and characterization of oxytetracycline (OTC) and sulfadimethoxine-ormetoprim resistant bacteria from aquaculture sediments.
2) Molecular characterization of the genes that regulate resistance to the drugs.

PI: Rafii, Fatemeh

- **Importance of Human Intestinal Microflora in Conversion of Phytoestrogens to Estrogenic Compounds** — **E0700701** — **None** — **Concept-Driven**

Objective(s):

1) To 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) To assess the estrogenic effect of each phytoestrogen metabolite produced by intestinal bacteria.
3) To determine the bacterial species producing estrogenic metabolites from phytoestrogens and elucidation of enzymes involved in various steps of these metabolic processes.
4) To determine the effects of phytoestrogens and their metabolites on the population, composition, metabolic activity, and enzyme production of bacteria from the human gastrointestinal tract.

FY 2001 Accomplishments:

1) An anaerobic intestinal bacterium, capable of cleaving the C-ring of the isoflavonoid phytoestrogen daidzein to produce a nonestrogenic metabolite, *O*-demethylangolensin, was isolated and characterized as a *Clostridium* sp.
2) A constitutive lipoamide dehydrogenase enzyme from *Mycobacterium* sp. was purified and shown to reduce aromatic nitro compounds to aromatic amines.
3) The effect of fluoroquinolones on resistance development in anaerobic bacteria from the human intestinal tract was evaluated.
4) The role of reductive enzymes in resistance to nitro antimicrobial drugs among bacteria from the human intestinal tract was demonstrated.

FY 2002 Plans:

1) Continue investigation of the metabolism of phytoestrogens by bacteria from the human gastrointestinal tract.
2) Screen the intestinal microfloras of individuals consuming soy diets for the presence of phytoestrogen-metabolizing bacteria.

Title	Project Number	Collaborator	Strategic Research Goal

- **Elucidation of the Mechanism of Resistance Development in Anaerobic Bacteria from the Human Intestinal Tract** — **E0709301** — **CFSAN** — **Knowledge Base**

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.

FY 2001 Accomplishments:

1) Detected anaerobic bacteria from the human intestinal tract that are intrinsically resistant to high concentrations of fluoroquinolones.
2) Determined that these fluoroquinolone-resistant bacteria are also resistant to other classes of antimicrobial agents.

FY 2002 Plans:

1) Develop fluoroquinolone-resistant mutants of *Clostridium* species from the human intestinal tract.
2) Detect multi-drug-resistance pumps in those anaerobic bacteria that are resistant to fluoroquinolones.
3) Detect factors contributing to resistance to fluoroquinolones in anaerobic bacteria from the human gastrointestinal tract.

PI: Sutherland, John

- **Biotransformation of Fluoroquinolones by Fungi** — **E0705201** — **None** — **Method-Driven**

Objective(s):

1) To measure the kinetics of biodegradation of veterinary fluoroquinolone drugs in natural matrices.
2) To identify the potential metabolites produced by fungi from fluoroquinolones.
3) To assess the residual antibacterial activity and potential risks of the metabolites formed from these drugs.

FY 2001 Accomplishments:

1) The fungus *Pestalotiopsis guepini* was shown to transform the fluoroquinolones ciprofloxacin and norfloxacin to the *N*-acetyl, desethylene-*N*-acetyl, and *N*-formyl derivatives of each, as well as to metabolites lacking the piperazine ring.
2) The soil fungus *Mucor ramannianus* was shown to transform the veterinary fluoroquinolone sarafloxacin to *N*-acetylsarafloxacin and desethylene-*N*-acetylsarafloxacin.
3) When grown on rice hulls (used as poultry litter), the fungus *Trichoderma viride* transformed sarafloxacin to a 4-hydroxy-3-oxo-4-vinylcyclopent-1-enyl derivative and norfloxacin to *N*-acetyl and 4-hydroxy-3-oxo-4-vinylcyclopent-1-enyl derivatives.
4) The soil fungus *Rhizoctonia* sp. was shown to oxidize the fluoroquinolones enrofloxacin and ofloxacin to microbiologically inactive *N*-oxides.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

1) Determine the potential of microorganisms to play a role in the detoxification and removal of veterinary fluoroquinolone residues from animal wastes and contaminated sites.
2) Determine the metabolic pathway used by the soil fungus *Beauveria bassiana* to transform cinoxacin, a drug used to treat urinary tract infections.
3) Identify the metabolites produced by fungi from oxolinic acid, an antimicrobial quinolone used in aquaculture.
4) Isolate cultures of microorganisms from soil and chicken litter that have the ability to degrade fluoroquinolones to inactive products; purify the metabolites for chemical analysis and identification.

PI: Wagner, Robert

- ***In vitro* Model and Molecular Analysis of Competitive Exclusion Products** — **E0704901** — **CVM Method-Driven**

Objective(s):

1) To 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) To define the antimicrobial susceptibility patterns of the component bacteria using Minimal Inhibitory concentration measurements.
3) To sequence analysis of 16s rRNA polymerase chain reaction (PCR) products from defined culture component bacteria and develop a database containing the sequences for use in subsequent identification of the organisms in undefined CE products.
4) To apply the 16s rRNA sequence analysis procedure to detect and identify effective CE component bacteria in undefined CE products.

FY 2001 Accomplishments:

1) Completed objectives 1 and 2 and most of objectives 3 and 4. Obtained an extension for completion of objectives 3 and 4.
2) Completed objective of developing a defined CE culture to test the *in vitro* assay and the CE product isolation and identification protocol.
3) Developed the *in vitro* CE assay.
4) Completed and submitted the manuscript entitled, "An *in vitro* assay to evaluate competitive exclusion products for poultry" to *The Journal of Food Protection.*

FY 2002 Plans:

Complete (objectives 3 and 4) evaluation of the CE product bacteria isolation and identification protocol and publish a manuscript on the results.

Title	Project Number	Collaborator	Strategic Research Goal

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

  Objective(s):

  Adapt an enterocyte culture model of colonization resistance by enteric microbial flora against *Salmonella* sp. colonization/invasion to measure concentrations of antimicrobial drugs as food residues that would inhibit the barrier effect of the consumers' intestinal flora.

  FY 2001 Accomplishments:

  Submitted a concept paper, and it was approved, "Measurement of antimicrobial drug concentrations that inhibit colonization resistance."

  FY 2002 Plans:

  1) Prepare a standardized model colonization resistance bacterial mixture that protects Caco-2 cells from invasion by *Salmonella enterica* serotype *typhimurium* and *Campylobacter jejuni*.
  2) Set up experimental parameters for the colonization resistance perturbation assay.
  3) Test antibiotics listed in the protocol for concentrations that perturb colonization resistance.

- **Probiotic Effects on Host Defense Against Enteric Pathogens** — E0709701 — CFSAN — Knowledge Base

  Objective(s):

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

  FY 2001 Accomplishments:

  1) Developed a concept paper entitled, "Probiotic effects on host defenses against enteric pathogens" into a complete protocol E0709701.
  2) Ordered gnotobiotic isolators for use in the protocol.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

Upon approval of the protocol, isolators will be assembled, mice will be acquired, and objectives 1-3 from the protocol, the first phase of the experimental timeline, will be conducted.

PI: Wang, Rong-Fu

- **Proteomic Approaches to Elucidate Bio-degradative Pathways** — E0711801 — None — **Method-Driven**

Objective(s):

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

FY 2001 Accomplishments:

1) Concept paper approved; protocol written, submitted for review, and approved.
2) Developed a protein isolation and separation method from *Mycobacterium* PYR-1 using IPGphor isoelectric focusing system.
3) Found several proteins that were significantly altered in their expression levels when the strain is exposed to pyrene or other PAHs, such as pyrene, phenanthrene, anthracene, and fluoranthene during their metabolism. The protein patterns from PAHs-induced samples showed altered expression levels when compared with uninduced sample as well as compared with each other.

FY 2002 Plans:

1) To use proteomic approach to isolate putative catabolic proteins that are over-expressed when microorganisms are grown in the presence of polycyclic aromatic hydrocarbons.
2) To develop software to analyze 2D-gels.
3) To identify those differentially expressed proteins by N-terminal sequencing and mass spectrometry.
4) To identify condition-specific marker proteins, which seem to be part of the adaptive response of the organism under different conditions, such as to the presence or changes of metabolizable PAHs in their environment.
5) To clone and express those genes that appear related to PAH degradation.

- **Novel Molecular Approach for the Detection and Analysis of the Predominant Bacterial Species in the Human Gastrointestinal Tract** — E0711901 — None — **Method-Driven**

Objective(s):

1) To develop rapid molecular methods for quantification of human intestinal bacteria.
2) To analyze qualitatively analysis of the microbial community for the major concerned genera that comprise the human intestinal microflora and discover the species which are difficult to cultivate.
3) To develop microarray for the detection of intestinal bacteria in fecal samples.
4) To develop microarray for the detection of foodborne pathogens in foods.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

1) Concept paper approved; protocol written, submitted for review, and approved.
2) Developed an oligo-microarray method for the detection of intestinal bacteria in fecal samples.
3) Developed a DNA-chip method for the detection of intestinal bacteria in fecal samples that will be used to replace microarray method without using any expensive equipment.

FY 2002 Plans:

1) To analyze qualitatively analysis of the microbial community for the major concerned genera that comprise the human intestinal microflora and discover the species which are difficult to cultivate.
2) To develop microarray for the detection of intestinal bacteria in fecal samples.
3) To develop microarray for the detection of foodborne pathogens in foods.
4) To continue using and modifying the two methods developed for the detection of human intestinal bacteria in different samples.

FY 2001 Publications

Balish, E. and Wagner, R.D., *Oroesophageal candidiasis* is lethal for transgenic mice with combined natural killer and T-cell defects, *Medical Mycology*, 39:261-268. Accepted: 10/17/00 (N/A)

Cerniglia, C.E. and Sutherland, J.B., Bioremediation of polycyclic aromatic hydrocarbons by ligninolytic and non-ligninolytic fungi*, British Mycological Society Symposium Series, 23* :136-187. Accepted: 5/31/01 (N/A)

Cha, C., Coles, B.F. and Cerniglia, C.E., Purification and characterization of a glutathione S-transferase from *Cunninghamella elegans*, *FEMS Microbiology Letters*, 203:257-261. Accepted: 7/25/01 (N/A)

Cha, C., Doerge, D.R. and Cerniglia, C.E., Biotransformation of malachite green by the fungus *Cunninghamella elegans*, *Applied and Environmental Microbiology*, 67:4358-4360. Accepted: 6/28/01 (N/A)

Dean-Ross, D., Moody, J.D., Freeman, J.P., Doerge, D.R. and Cerniglia, C.E., Metabolism of anthracene by a *Rhodococcus* species*, FEMS Microbiology Letters*, 204:205-211. Accepted: 8/20/01 (N/A)

Erickson, B.D., Campbell, W.L. and Cerniglia, C.E., A rapid method for determining the tuberculocidal activity of liquid chemical germicides, *Current Microbiology*, 43:79-82. Accepted: 11/28/00 (E0696501)

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Khan, A.A., Wang, R., Cao, W., Doerge, D.R., Wennerstrom, D.E. and Cerniglia, C.E., Molecular cloning, nucleotide sequence and expression of genes encoding a polycyclic aromatic ring dioxygenase from pyrene dioxygenase from *Mycobacterium* sp. strain PYR-1*, Appl. Environ Microbiol*., 67:3577-3585. Accepted: 6/1/01 (E0704801)

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Myers, M.J., Campbell, W.L., Wang, R., Paine, D.D. and Cerniglia, C.E., Validation of a PCR-based method for the detection of rendered bovine-derived materials in feedstuffs, *J. Food Protection*, 64:564-566. Accepted: 11/3/00 (P00408)

Nawaz, M.S., Erickson, B.D., Khan, A.A., Khan, S.A., Pothuluri, J.V., Rafii, F., Sutherland, J.B., Wagner, R.D. and Cerniglia, C.E., Human health impact and regulatory issues involving antimicrobial resistance in the food animal production environment, *Regulatory Research Perspectives*, 1(1):1-10. Accepted: 7/2/01 (N/A)

Parshikov, I., Freeman, J.P., Lay, J.O., Moody, J.D., Williams, A.J., Beger, R. and Sutherland, J.B., Metabolism of the veterinary fluoroquinolone sarafloxacin by the fungus *Mucor ramannianus*, *J. Ind. Micro. Biotech*. 26:140-144. Accepted: 11/3/00 (E0705201)

Parshikov, I., Heinze, T.M., Moody, J.D., Freeman, J.P., Williams, A.J. and Sutherland, J.B., The fungus *Pestalotiopsis guepini* as a model for biotransformation of ciprofloxacin and norfloxacin, *Appl. Micro. Biotech*., 56:474-477. Accepted: 3/23/01 (E0705201)

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Rafii, F., Hehman, G.L. and Lunsford, P.E., Purification and characterization of an enzyme from *Mycobacterium* sp. Pyr-1, with nitroreductase activity and an N-terminal sequence similar to lipoamide dehydrogenase, *Archives of Microbiology*, 176:381-385. Accepted: 7/31/01 (E0695901)

Sietmann, R. and Cerniglia, C.E., Novel ring cleavage products in the biotransformation of biphenyl by the yeast *Trichosporon mucoides, Appl. Environ. Microbiol*., 67:4158-4165. Accepted: 6/26/01 (N/A)

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Molecular Epidemiology

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Researchers utilizing Transgenomics Wave System for SNP Discovery

Executive Summary

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 development of a "Structural Genomics Center" for discovery of single nucleotide polymorphisms (SNPs) and its application to human diagnostics.

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 inter-individual 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 food borne heterocyclic amines, environmental aromatic amines and polycyclic aromatic hydrocarbons, on widely used, as well as on tobacco usage. Projects on the etiology of human cancers of the colon/rectum, pancreas, larynx, esophagus, breast, ovary, prostate, lung, urinary bladder, and bone marrow are ongoing. These are outlined as follows:

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

1) Metabolic polymorphisms, DNA repair, and individual cancer susceptibility.
   a) Genetic and epigenetic regulation of cytochrome P450 1A2.
   b) Polymorphisms of cytochrome P450 1B1 and tissue-dependent expression.
   c) Polymorphisms of sulfotransferases.
   d) Polymorphisms of glutathione S-transferases.
   e) Inter-individual variation in DNA repair capacity.
   f) Characterization of peroxidases toward metabolic activation.

g) Gender-specific variation in drug metabolism.

2) Chemoprevention.
   a) Modulation of expression of multi-drug resistance genes.
   b) Coffee and tea effects on *N*-acetyltransferases.
   c) Effects of tea constituents on expression of genes cytochrome P450 1A2, H-*ras*, and the normal epithelial cell specific gene (NES1).
   d) DNA methylation, DNA methyltransferases, and homocysteine toxicity.

Epidemiology and post-market surveillance for chemical toxicants found in foods, drugs, cosmetics, and medical devices:

1) Etiology of human colorectal cancer: role of dietary heterocyclic amines.
2) Etiology of human breast and prostate cancers in African-Americans and Caucasians.
3) Etiology of human pancreatic cancer: role of carcinogen and drug exposures, chronic pancreatitis, and dietary imbalance.

Human exposure biomonitoring and DNA adduct detection:

Biomarkers of exposure and susceptibility to breast, prostate, laryngeal, esophageal, lung, colon, and urinary bladder cancers.

FY 2001 Accomplishments and FY 2002 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Coles, Brian

- **Dietary Isothiocyanates, Glutathione S-Transferases, and Colorectal Neoplasia** — **E0320001** — **None** — **Concept-Driven**

  Objective(s):

  To explore the relationship between dietary isothiocyanates, glutathione *S*-transferase (GST) induction, and colon polyp recurrence. NCTR's direct objective is to quantitate glutathione *S*-transferase in human plasma.

  FY 2001 Accomplishments:

  1) Prepared approved protocol/grant and completed Institutional Review Board (IRB) reviews.
  2) Prepared CRADA between NCTR and Arizona Cancer Center to allow transfer of designated funds to NCTR. Protocol now in FDA with appropriate committee.
  3) Validated methodology to be used: i.e., use of hGSTA1 antibody assay as the appropriate methodology to measure glutathione S-transferase (GST) alpha in plasma; the finding that GSH affinity matrices and HPLC are not quantitative when using plasma.
  4) Received seminar invitation to Arizona Cancer Center to discuss plans in detail.
  5) Quantitated 600 human plasma samples to GST alpha content showing considerable variability. This variability demonstrates that statistical power will be available for examination of GST in plasma with respect to recurrence of colorectal adenomas.

  FY 2002 Plans:

  Preparation of report for Arizona Cancer Center, with discussion and preparation of manuscripts as appropriate. Include more patients in study of plasma GST after an initial analysis of current data.

- **ADDEND: The Role of Glutathione S-transferase genetic Polymorphisms in Breast Cancer Sensitivity to Radio- and Chemotherapy** — **E0701511** — **None** — **Predictive Toxicology**

  Objective(s):

  1) To 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 to evaluate associations between phenotypes in tumor tissue and risk of breast cancer recurrence.
  2) To determine inherited GSTM1, GSTT1 and GSTP1 genotypes in normal tissue from these same women, and to determine associations of GSTM1, GSTT1 and GSTP1 genotype with phenotype in tumor tissue.
  3) To evaluate if GST genotypes predict breast cancer recurrence following treatment, controlling for other factors that may relate to prognosis.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

Using epidemiological methods, individuals were examined via the tumor registry for survival following adjuvant chemotherapy for which cyclophosphamide was a constant component.

1) Systemic lack of GSTM1 or GSTT1 (GSTM1 and GSTT1 null genotypes) is associated with increased survival following chemotherapy.
2) Low GSTP1 in tumor is associated with increased survival. The GSTP1 "lle" genotype is also associated with increased survival.
3) The GSTA1 genotype (GSTA1*B) that indicates low GSTA1 in liver, showed the greatest association with increased survival. Low GST in tumor did not appear to influence survival.
4) The mechanism may act via decreased detoxification of cyclophosphamide metabolites, notably its therapeutic metabolite that is detoxified by GSTA1.

The results suggest that in the future genotyping may be used to advantage for design of the chemotherapeutic regimens most applicable to an individual.

This project addressed the role of genetic polymorphisms in enzymes involved in the metabolism of chemotherapeutic agents, as well as reactive oxidant products created by radiation and chemotherapy, in survival after treatment for breast cancer. Normal archived tissue was genotyped for GSTM1, P1, and T1, and relationships between genotype and survival evaluated. We were also interested in the relationship between enzyme expression in tumor tissue and survival, as well as association with genotype. Tumor tissue was stained for GSTP1, GST alpha, and GST mu. For 250 women treated for breast cancer, subject eligibility was determined, follow-up data compiled, archived pathology blocks retrieved, and genotyping assays conducted. Data on concordance of genotypes from paired tumor and normal tissue samples were compiled. For 103 subjects, immunohistochemical staining for GSTP1, GST alpha, and GST mu expression was completed and statistical analysis for survival according to GSTP1, GSTM1, and GSTT1 genotypes was carried out. Two papers reporting the findings for GSTP1 and another reporting results for GSTM1 and GSTT1 were published in *Cancer Research*. Data were also obtained for polymorphisms in CYP3A4 and MnSOD and the results are being prepared for publication.

FY 2002 Plans:

1) Continue investigation of the apparent effects of GST genotypes on chemo-therapy using different study populations.
2) Investigate polymorphisms in cytochrome P450 that could affect cyclophos-phamide metabolism with respect to chemotherapy.
3) Investigate polymorphism in enzymes associated with protection from oxidative stress with respect to chemotherapy.
4) Explore the hypothesis that plasma levels of the therapeutic metabolite of cyclophosphamide (phosphoramide mustard) varies according to GST (and other) genotypes and that this is responsible for the apparent effect on survival of breast cancer.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Hammons, George

- **Methylation Profile, Gene Expression, and Enzyme Activity of *CYP1A2* in Human Livers** — **E0696201** — **None** — **Predictive Toxicology**

Objective(s):

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.

FY 2001 Accomplishments:

Mechanistic analysis examining the methylation status of the CpG site adjacent to an AP-1 binding site in the 5'-flanking region of *CYP1A2* has provided important insight into its regulation. The methylation status of this site varied among individuals; hypermethylation of the site was associated with reduced *CYP1A2* expression. Methylation status analysis at four other identified CpG sites in the 5'-flanking regions has been initiated. DNA methyltransferase expression also varied among individuals; significantly higher levels were found in smokers. This may be one of the important factors contributing to the variation in methylation status of *CYP1A2* susceptibility.

FY 2002 Plans:

Mechanistic analysis in *CYP1A2* regulation continues to complete examination of the four additional specific CpG sites in *CYP1A2* (these are located in binding sites identified in the 5'-flanking region). Factors controlling smoking-induced increased DNA methyltransferase expression will also be determined. Increased understanding of the underlying mechanisms controlling the large interindividual differences in the expression of *CYP1A2* has important implication for drug efficacy and cancer.

PI: Kadlubar, Fred

- **Rapid, Population-based, Screening Methodology for Genetic Polymorphisms in Adverse Drug Metabolizing and/or Cancer-Related Risk Alleles** — **E0300001** — **None** — **Predictive Toxicology**

Objective(s):

1) To develop and fabricate a SNP microarray chip or "risk-tox chip" for the analysis of genetic polymorphisms that affect individual cancer or adverse drug risk.
2) To validate the "risk-tox chip" by comparative analyses with standardized methodologies.
3) To automate the methodologies for large population risk assessment using the "risk-tox chip" in a robotic work station.
4) To establish NCTR as an alpha test site to introduce "risk tox chip" screening analysis as rapid and reliable frontline screening methodology for clinical and population-based molecular epidemiological studies.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

During this period, we established and validated conventional genotyping methods for 28 gene targets and their associated polymorphisms. Our first attempts at DNA microarray chips have proven to be very reliable method for profiling genotypes as demonstrated by a more than 99% concordance between the microarrays and conventional genotyping assays (PCR-RFLP), based on a mini-chip made so far (*ras, NAT2*, and *COMT*).

In a colon polyp prevention trial involving 1,429 subjects, questionnaire information was used to assess potential exposure to heterocyclic amines. Using our high-throughput DNA microarrays, 686 individuals who had undergone colonoscopy by year 3 were genotyped for all common *NAT2* alleles in one work-day. Only those in the highest tertile of red meat consumption who were rapid acetylators showed a significant increased risk and the odds ratios indicated gene-dose dependence. These data suggest that a one-third reduction in red meat consumption is in itself a sufficient preventive measure for colon polyp recurrence and thus should appreciably lower colorectal cancer risk. Conventional genotyping methods were developed for 33 gene targets and their associated polymorphisms for the purposes of validating our DNA microarray chip. A mini-chip with six genes showed >99% concordance and was successfully applied to a colon polyp recurrence trial. However, our CRADA partner, Genometrix®, went bankrupt and all these efforts were lost.

FY 2002 Plans:

We will continue our process of SNP discovery associated with cancer susceptibility using pharmacogenomics. These will include NAT1, NAT2, COMT, CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, GSTA1, GSTA2, GSTM1, GSTM3, GSTT1, GSTP1, GSTZ1, SULT1A1, MTHFR, NQO1, MPO, SOD, FMO3, HYL, UGT1A1, UGT1A6, UGT2B7, and UGT2B15. High-throughput genotyping will be achieved using a Sequenom-MS system being validated in collaboration with an NCI contract with Bioserve Technologies at a cost $1.50/genotype/sample.

To achieve our goals, we are developing a DNA microarray platform to genotype patients for all the major enzyme variants that would enable us to predict carcinogen susceptibility, adverse drug reactions, and perhaps chemotherapeutic drug efficacy.

◆ **Chemoprotection of DNA Adducts of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in the Rat**	**E0689401**	**None**	**Predictive Toxicology**

Objective(s):

To examine the effect of the glutathione S-transferase inducers, phenethylisothiocyanate, diallyl sulfide (DAS), 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (Oltipraz), garlic powder, cabbage powder, 2(3)-tert-butyl-4-hydroxyanisole (BHA), kahweol palmitate, cafestol palmitate, quercetin, tannic acid, α-angelicalactone, Green tea, and ethoxyquin on the metabolism and DNA adduct formation of the food borne carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, in the Fischer 344 rat.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

Preliminary studies with human hepatocytes were carried out with the coffee lipids, kahweol and cafestol, which had been shown to down regulate N-acetyltransferase in rat hepatocytes. However, the experiments were unsuccessful due to apparent toxicity of the compounds.

FY 2002 Plans:

This experiment needs to be repeated before the project is completed. Then, a manuscript can be submitted for publication and final report prepared.

- **A Case-Control Study of Pancreatic Cancer and Aromatic Amines** — **E0694601** — **None** — **Predictive Toxicology**

Objective(s):

To 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.

FY 2001 Accomplishments:

Analysis of molecular epidemiological data from our completed case-control study on pancreatic cancer has been ongoing and our data indicate that the slow *NAT1*4* allele is a significant risk factor. Laboratory studies on chronic pancreatitis, which is the strongest predisposing factor for the development of pancreatic cancer, has shown a five-to-15 fold increase in the levels of CYP1A1, CYP1B1, CYP2C9, and CYP3A4, with the latter present at the highest levels, comparable to about 5-10% of that found in human liver. Variation in expression of GST phenotype has been assessed, with hGSTA2, known to be critical for carcinogen detoxification and protection from lipid peroxidation, being the major isoform in normal pancreas and found to be strongly down-regulated in pancreatitis. A novel GST polymorphism in the coding region of hGSTA2, which we discovered, was cloned into an expression vector and found to modify activity 2.5-fold. Three publications were submitted and accepted during this period.

FY 2002 Plans:

The case-control studies are being further analyzed and, together with the laboratory studies, will be prepared for publication in FY 2002.

- **Role of Acetylation and N-Oxidation in Colorectal Cancer** — **E0694701** — **None** — **Predictive Toxicology**

Objective(s):

To 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.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

Analysis of molecular epidemiological data from our completed case-control study on colorectal cancer at UAMS showed that low-activity GSTA1 genotype, which we discovered, (together with preference for well-done red meat) and high-activity CYP2A6 phenotype, together with high preserved meat intake resulted in a four-to-5-fold increased cancer risk, thereby implicating dietary heterocyclic amines and nitrosamines as etiologic agents.

FY 2002 Plans:

Start a new case-series study by collecting normal and tumor tissue from all case subjects and examine differential gene expression (and possibly protein expression) as a function of stage at diagnosis, response to therapy, time to recurrence, influence of smoking, and specific metabolic polymorphisms.

- **Chemical Carcinogenesis: Epithelial Cells in Breast Milk** — **E0697801** — **None** — **Predictive Toxicology**

Objective(s):

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

FY 2001 Accomplishments:

Laboratory and animal data indicate that several classes of carcinogens, including aromatic and heterocyclic amines and PAHs, could be etiologic factors in breast cancer. However, epidemiologic studies do not support an association between cigarette smoking, a vehicle for delivery of those carcinogens, and breast cancer risk. This project was designed to develop methodology to separate exfoliated ductal epithelial cells from human breast milk. Ductal epithelial cells are those from which most breast cancers arise, thus, they are the ideal target tissue for evaluation of carcinogen-DNA adducts. In this study, exfoliated cells were isolated and evaluated for DNA adducts. In collaboration with the EPA, milk from the same samples were evaluated for mutagenicity in *Salmonella* strain YG1024, which is a frameshift strain derived of TA98 that over expresses the acetyltransferase gene and is highly specific for detecting aromatic and heterocyclic amines. For samples analyzed in this fashion (n=25), 88% (22/25) were positive for mutagenic activity. In these studies, DNA adducts were identified in 66% of the specimens. Bulky adducts by 32P-postlabelling-TLC appeared to run in a pattern similar to aromatic amine standards. These data: 1) corroborated previous findings of mutagenicity of human milk; 2) demonstrated the utility of breast fluids, such as milk, as non-invasive sources for identifying the source of mutagen activity; and 3) illustrated the need to develop methods to quantify human exposures to potential carcinogens. A manuscript

Title	Project Number	Collaborator	Strategic Research Goal

detailing our results was accepted for publication. This study was then followed up to specifically identify the carcinogen-DNA adducts present using 32P-postlabelling-HPLC and synthetic standards of the C8-dG adducts of PhIP and ABP and N2-dG adduct of BP, which were added to each reaction as UV markers. Of the 47 samples analyzed, adducts were found in 30 samples. Twenty-nine (29) samples contained detectable levels of PhIP adducts, with a mean value of 4.8 adducts/107 nucleotides; 18 were positive for ABP adducts with a mean level of 4.7 adducts/107 nucleotides; and 13 were found to contain BP adducts with mean level of 1.9 adducts/107 nucleotides. These data indicate that women are exposed to several classes of dietary and environmental carcinogens.

FY 2002 Plans:

Because we are collecting extensive exposure data from women who are providing milk specimens, our ultimate aim is to evaluate associations between adducts, mutagenicity, and exposures to chemical carcinogens, as well as modification of relationships by genetic polymorphisms in genes related to carcinogen metabolism.

◆ **ADDEND: The Role of Human Cytochrome CYP1B1 in Drug Metabolism and Carcinogenesis** — **E0699011** — **None** — **Predictive Toxicology**

Objective(s):

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

FY 2001 Accomplishments:

1) Project completed.
2) Manuscript published.

FY 2002 Plans:

Final Report to be submitted.

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** — **None** — **Predictive Toxicology**

Objective(s):

1) To determine the effects of nicotine and other cigarette components on exocrine and endocrine human pancreatic cells *in vitro*.
2) To 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.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

1) *CYP1A2* is highly expressed in human pancreatic tumors, but not in normal pancreatic tissue. *CYP1A1* was not expressed in pancreatic tumor cells.
2) DT-diaphorase is an important enzyme in the metabolism of xenobiotics. It may act as either a detoxification or activation enzyme. DT-diaphorase can also catalyze bioactivation of antitumor quinones and this feature has been considered as a therapeutic strategy to exploit the elevated activity of this enzyme in some tumors. Our study demonstrated that DT-diaphorase is highly expressed in human pancreatic tumor cell lines and in human pancreatic tissue from heavy smokers. High expression was also noted in some human pancreatic adenocarcinomas, but not all tumors. Recent studies have shown that a C- to T-base transition mutation at position 609 of the DT-diaphorase cDNA is associated with reduced activity. Sequencing and polymorphism analysis of this region in some of the pancreatic tumors have shown association with lack of DT-diaphorase expression.
3) The heterogeneous nuclear ribonucleoprotein (HnRNP) was up-regulated in pancreatic tissue from smokers. It was significantly expressed in pancreatic tissue from female smokers. All of the above up-regulated genes are potential biomarkers for early detection and chemotherapeutic intervention.
4) Nicotine exerts gender differences on expression of critical genes involved in carcinogenesis, metabolism, and toxicity in normal and tumorigenic pancreatic cells. Nicotine up-regulated *NF-kB*, *K-ras, Nes-1*, and *MnSOD* in pancreatic cells. Nicotine addictivity in cigarettes has been proposed as a regulatory issue for FDA. FDA regulates transdermal nicotine patches used in smoking cessation therapy. This study seeks to determine if FDA should be concerned about nicotine long-term effects on critical genes involved in carcinogenesis and as a potential risk factor for the development of pancreatic cancer.

FY 2002 Plans:

1) Complete *in vitro* studies on zinc effects in nicotine-treated cells. Conduct study on zinc effects on expression and activity of *CYP1A2* and DT-diaphorase.
2) Increase the number of human pancreatic tissues from cancer patients and from smokers, particularly samples from African-Americans to complete specific aim 5 of the master protocol.

◆ **ADDEND: Colorectal Adenoma Study - H-ras and K-ras Methylation (Task 4)** | **E0707131** | **None** | **Predictive Toxicology**

Objective(s):

To provide analytical support for the analysis of *H-ras* and *K-ras* methylation in red blood cell specimens for an intramural study being conducted by the Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI).

FY 2001 Accomplishments:

Started methylation profile analysis of *H-ras* on the colorectal adenoma study. Global methylation analysis is currently being conducted on these samples.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

Complete the *H-* and *K-ras* global methylation study on the colorectal adenomas from NCI. This protocol seeks to develop better biological assays to predict human genetic damage and potential mechanisms of action of chemopreventive agents in colon cancer.

- **ADDEND: Colorectal Adenoma Study - IGF-1 Hypermethylation (Task 5)** — **E0707141** — **None** — **Predictive Toxicology**

Objective(s):

To provide analytical support for the analysis of insulin-like growth factor (IGF)-1 hypermethylation in red blood cell specimens for an intramural study being conducted by the Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI).

FY 2001 Accomplishments:

Not yet started.

FY 2002 Plans:

1) Initiate studies on site-specific methylation studies on the promoter region of the IGF-1 gene in lymphocytes from a case-control study of colon adenomas. Insulin-like growth factor-1 is a low molecular weight peptide that mediates cell proliferation action of growth hormones. Alterations in regulatory feedback loops for growth factors can shift cells from paracrine to autocrine control. Hypermethylation is known to regulate a number of genes through inactivation of expression.
2) Specific primers will be designed to detect the wild-type, methylated and unmethylated CpG sites in the promoter region of the IGF-1 gene.

- **Mechanistic Actions of Chemo-preventive Agents in Pancreatic Cancer** — **E0707601** — **None** — **Predictive Toxicology**

Objective(s):

To screen a number of agents found in natural products and establish mechanistic data on their potential as anti-cancer agents against pancreatic cancer.

FY 2001 Accomplishments:

Protocol approved.

FY 2002 Plans:

1) Screen 15 agents, of which some are currently in clinical trials, for their potential anti-cancer effects in pancreatic cancer by examining whether they modulate the expression or activity of one or more of the biochemical or molecular processes associated with carcinogenesis, mutagenesis, proliferation, apoptosis, invasion and metastasis, and angiogenesis.
2) Identify combination of these agents with approved cancer drugs for adverse or synergistic effects.
3) Use Clontech Human Cancer microarrays (expression) to determine if these agents affect other biological or biochemical pathways in carcinogenesis.

Title	Project Number	Collaborator	Strategic Research Goal

PI: McClure, Gail

- ***In vivo* Modeling of Steroid-mediated Gender Effects in Drug Metabolism** — E0704301 — None — **Predictive Toxicology**

Objective(s):

1) To characterize the activity of *CYP1A2* in female subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive (oc) usage, menopause, and hormone replacement therapy (HRT).
2) To characterize the activity of *CYP1A2* in male subjects with regard to age.
3) To measure estradiol, progesterone, testosterone, cortisol, IL-1, IL-6, and IL-10 levels in female and male subjects studied for *CYP1A2* activity.
4) To correlate the activity of *CYP1A2* with circulating levels of cytokines and/or circulating levels of steroid hormones.
5) To statistically assess the impact of each of the measured variables on the *CYP1A2* phenotype.

FY 2001 Accomplishments:

Phase I (E0704301) of the initial study required the recruitment of 160 participants divided into eight subgroups for assessment of hormone and cytokine levels and *CYP1A2* activity. Phase II (E0704311), that received further funding in 1999, added recruitment of a ninth subgroup, another 20 pregnant volunteers, to the overall set of participants and added technical support to assess hormone and cytokine interactions with CYPs 3A and 2D6. Because two probe drugs were being administered simultaneously, validation of lack of interaction of the two drugs was done by repeating a portion of the initial volunteers. Before initiation of Phase II of the study, four-repeat series from 25 volunteers had been completed in Phase I (E0704301 used caffeine only as probe drug). Of these 25, 11 individuals reproduced the full four-series three times, taking the probe drug caffeine only (original Phase I data), dextromethorphan only (added probe of Phase II), and the combination drug regime to validate lack of drug-interaction with the two probe drugs. A slow-down of volunteer recruitment occurred because of increasingly stringent requirements in consent forms allowing DNA analyses requiring major consent revisions. IRB requirements for DNA analysis have now been satisfied for Phase II. Original volunteers are currently being re-consented to allow DNA analyses. Training of UAMS General Clinical Research Center (GCRC) personnel is complete and GCRC is now assisting in the collection/initial pre-processing procedures used in this protocol. Currently, recruitment of new volunteers is occurring at a rate that equals our lab's maximum capacity. Great progress has been made in recruitment of our 55-70 subgroups and, with present enrollment rates, these subgroups should be complete by summer of 2002. Concern still exists over recruitment of pregnant females. These continue to be the hardest subgroups to recruit.

Samples in Biorepository before slow-down due to IRB revisions:

Assays completed to date:
23 Phase I participants x 4 weeks = 92
81 Phase II participants x 4 weeks = 320

Title	Project Number	Collaborator	Strategic Research Goal

Total samples as of 9/01/01 = 412 samples

Analysis of samples is progressing at a steady rate. All but seven of the accrued samples from this past year's work have been extracted for analysis of caffeine metabolites. All samples are analyzed in triplicate for *CYP1A2* metabolic ratios. Currently, all samples have been analyzed at least once and most of the total at least twice.

FY 2002 Plans:

IL-6, IL-1 and IL-10 assays and *CYP1A2* analysis will be completed for this sample set as recruitment continues. To date 80% of recently accrued specimens have been analyzed for IL-6, hormones, and *CYP1A2*. IL-1 and IL-10 assays will begin in 2002 as well as *CYP2D6* and 3A analysis. Stratification of *CYP1A2* activity showed interesting differences in activity when data were stratified by activity level. A Phase III proposal will be submitted to increase accrual number to 60 each in three subgroups (normal cycling women, OC users, and young men) to supply sufficient power when data is stratified-based on *CYP1A2* activity.

◆ **ADDEND: Part II of *In vivo* Modeling of Steroid-mediated Gender-effects in Drug Metabolism** | **E0704311** | **None** | **Predictive Toxicology**

Objective(s):

1) To 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) To measure estradiol, progesterone, testosterone, cortisol, IL-1, IL-6 and IL-10 levels in female and male subjects studied for *CYP* activity.
3) To correlate the activity of *CYP2D6* and 3A4 with circulating levels of cytokines and/or circulating levels of steroid hormones.
4) To statistically assess the impact of each of the measured variables on the *CYP2D6* phenotype and *CYP3A4* activity level.

FY 2001 Accomplishments:

Sample processing and recruitment is proceeding at a successful rate in most subgroups. Ten (10) individuals are registered in the database waiting to start the study. In one year under this study we have successfully recruited 34% of the targeted study subjects for analysis, and completed sample processing on 80% of those samples collected to date.

We are currently seeking a new consultant in the UAMS Department of Obstetrics and Gynecology. Efforts have also been made to improve recruitment of individuals in older subgroups through the Center on Aging, University of Arkansas for Medical Sciences, which is lending support in the recruitment effort.

A total of 223 samples have been collected from 62 individuals. IL-6 assays are complete on all female samples collected to date and will soon be completed on all males as well. *CYPlA2* activity is currently being determined on all samples.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

IL-6, IL-1 and IL-I0 assays and *CYPlA2* analysis are planned for this sample set as recruitment continues. To date, 80% of the specimens collected have been analyzed for IL-6, hormones, and *CYPlA2*. IL-l and IL-I0 assays will begin in 2001. Recruitment for E0704311 will continue with additional efforts to increase participation of individuals over 55 years of age.

PI: Poirier, Lionel

◆ **Methylation Status and Cancer Risk** **E0704601** **None** **Predictive Toxicology**

Objective(s):

To learn whether methylation status, determined by non-invasive procedures, may be a biomarker of cancer risk in humans. The methylation status will be assessed by measurement of S-adenosylmethionine (SAM), S-adenosylhomocysteine (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 the National Cancer Institute (NCI).

FY 2001 Accomplishments:

The major scientific findings made in this study are the following:

1) Dietary homocystine increases blood homocysteine and produces a hypo-methylating environment and increased hyperplasia *in vivo*.
2) The elevated activity of DNA methyltransferase in pre-neoplastic liver is partly the result of the presence of endogenous, competing DNA in the nuclear preparation of the enzyme.
3) Alterations in methyl metabolism previously seen in cancer have been extended to diabetes and clinical depression in humans and to atherosclerosis in rats.

FY 2002 Plans:

1) Bring to a stage of reasonable evaluation the blood analyses of SAM, SAH and HCys in two large clinical collaborative studies: one on COMT and breast cancer and the other on the effects of rapid weight loss on several biomarkers of health.
2) Submit for publication the responses to enzyme inhibitors by the DNA methyltransferases in normal and neoplastic colon.
3) Publish the above studies on diabetes, depression and atherosclerosis.
4) Examine changes in specific site methylation in pre-neoplastic livers in a collaborative study with the NCCRI of Japan.

◆ **Colorectal Adenoma Study - Task 1** **E0707101** **None** **Predictive Toxicology**

Objective(s):

To provide analytical support for the analysis of SAM and SAH in red blood cell specimens for an intramural study being conducted by the Nutrition Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

FY 2001 Accomplishments:

Finalized IAG and shipping of tissue samples.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

The FDA/NCTR will measure SAM and SAH concentrations on 521 (467 study subjects plus 54 quality control samples) blood specimens from the previous colorectal adenoma study undertaken by NCI.

- **ADDEND: Colorectal Adenoma Study - SAM and SAH (Additional samples for Task 1)** **E0707111** **None** **Predictive Toxicology**

Objective(s):

To provide analytical support for the analysis of SAM and SAH in red blood cell specimens for an intramural study being conducted by the Nutrition Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

FY 2001 Accomplishments:

IAG was approved.

FY 2002 Plans:

The NCTR/FDA will measure the plasma and red blood cells SAM and SAH concentrations in 165 subjects and controls from a previous colorectal adenoma study undertaken by NCI.

- **ADDEND: Colorectal Adenoma Study (Task 3)** **E0707121** **None** **Predictive Toxicology**

Objective(s):

To provide analytical support for the analysis of MTHFR-specific activity in red blood cell specimans for an intramural study being conducted by the Nutrition Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

FY 2001 Accomplishments:

Finalized IAG and collection of tissue samples.

FY 2002 Plans:

The MTHFR levels in lymphocytes from the same 165 subjects and patients as in E707111, E707131, and E707141 will be determined. The results from the controls and the patients will be compared with each other, with all other methyl-related parameters in the NCI study, as well as with dietary intakes.

PI: Ratnasinghe, Luke

- **Breast Cancer in African-American Women: Metabolic Modification of Dietary and Hormonal Risk Factors** **E0701501** **None** **Method-Driven**

Objective(s):

To examine the role of inter-individual 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.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

The above project is a case-control study of genetic and environmental risk factors for breast cancer in African-American and Caucasian women. The purpose of this pilot study was to develop a novel method of recruitment, focused primarily on minority women, and investigate previously unexplored risk factors in breast cancer epidemiology. Eligible cases and controls are being contacted by women who are breast cancer survivors and asked to participate in the study. To date, interviews have been completed for 322 women with breast cancer, aged 29-75, and 141 community controls. The participation rate (the proportion of women who complete the study) is 76% for Caucasian women and 61% for African-Americans. The infrastructure for case-control epidemiologic studies has been built, and a specimen bank was established to enable exploration of future hypotheses.

FY 2002 Plans:

We will continue data collection through 2002. Methodologies are in place and we are currently identifying patients from several hospitals and records from physicians with large breast surgery practices in Little Rock. Interviews are ongoing. When data collection is complete, genotyping for polymorphisms in a number of genes involved in the metabolism of carcinogens and hormones will be performed, and evaluated in relation to case/control status and exposure information derived from questionnaire data.

◆ **Prostate Cancer: Exposure, Susceptibility and DNA Adducts** **E0702101** **None** **Method-Driven**

Objective(s):

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

FY 2001 Accomplishments:

Little is known regarding etiologic factors in prostate cancer. Furthermore, it is unclear why African-American men have the highest prostate cancer rates in the world. This project is a case-control study in Arkansas that consists of two subprojects. The first is a nested study; men who are undergoing a needle biopsy for a high PSA or palpable mass are asked to consent to withdrawal of an additional core. Specimens have been stored for >1000 men, 300 of whom have been diagnosed with prostate cancer. If patients are diagnosed with prostate cancer and agree to participate in the case-control study, their tissue will be evaluated for carcinogen-DNA adducts. The larger case-control study ascertains patients from a number of hospitals. To date, 450 cases and 150 controls have been interviewed.

Title	Project Number	Collaborator	Strategic Research Goal

Specimens are being banked for genotyping at the completion of data collection.

FY 2002 Plans:

Data collection will continue for the final year of this study, with cases ascertained and interviewed, and specimens collected. Data entry is being performed on an on-going basis. During this year, we will begin genotyping specimens and will conduct postlabelling assays to identify DNA adducts in biopsy specimens from men with prostate cancer. We will also conduct a complete biochemical characterization of 30 radical prostatectomy samples.

During FY 2002, we will develop a study to utilize the sample set of >1000 subjects with prostate biopsies who consented to the study and were free of cancer. These will include laboratory methods for measurement of additional markers of DNA damage, variation in gene expression using cDNA microarrays, and protein pattern recognition using MALDI-TOF in normal tissue from men free of cancer and ascertainment of cancer incidence during two-to-five years of follow-up among biopsy (cancer-) negative men.

FY 2001 Publications

Cha, C., Coles, B.F. and Cerniglia, C.E., Purification and characterization of a glutathione S-transferase from *Cunninghamella elegans, FEMS Microbiology Letters*, 203:257-261. Accepted: 7/25/01 (N/A)

Coles, B.F., Huber, W., Yang, M., Teitel, C.H., Lang, N.P. and Kadlubar, F.F., Effect of polymorphism in the human glutathione S-transferase A1 promoter on hepatic GSTA1 and GSTA2 expression, *Pharmacogenetics*, 11:663-669. Accepted: 5/30/01 (E0689401)

Hammons, G.J., Yan, Y., Jin, B., Blann, E., Kadlubar, F.F. and Lyn-Cook, B.A., Specific Site Methylation in the 5'-Flanking Regions of *CYP1A2*: Interindividual Differences in Human Livers, *Life Sciences*, 69:839-845. Accepted: 1/31/01 (E0696201)

Kadlubar, F.F., Concluding remarks: Symposium on Genetic Susceptibility to Environmental Toxicants, *Mutation Research*, 482:111-113. Accepted: 8/21/01 (NA)

MacGregor, J., Collins, J. and Kadlubar, F.F., *In vitro* Human Tissue Models in Risk Assessment: Report of a Consensus-Building Workshop, *Toxicological Sciences*, 59(1):17-36. Accepted: 10/15/00 (N/A)

Muskhelishvili, L., Thompson-Carino, P., Kusewitt, D.F., Wang, C. and Kadlubar, F.F., *In Situ* Hybridization and Immunohistochemical Analysis of Cytochrome P450 1B1 Expression in Human Normal Tissues, *Journal of Histochemistry and Cytochemistry*, 49:229-236. Accepted: 11/15/00 (E0699011)

Poirier, L.A., Fink, L.M., Delongchamp, R.R. and Wise, C.K., Blood S-Adenosyl-methionine Concentrations and Lymphocyte Methylenetetrahydrofolate Reductase Activity in Diabetes Mellitus and Diabetic Nephropathy, *Metabolism*, 50:1014. Accepted: 3/27/01 (NA)

Poirier, L.A., Wise, C.K., Delongchamp, R.R. and Sinha, R., Blood Determinations of S-adenosylmethioine, S-Adenosylhomocysteine, and Homocysteine: Correlations with Diet, *Cancer Epidemiology Biomarkers and Prevention*, 10(6):649-55. Accepted: 4/16/01 (E0707101)

Poirier, L.A., Nutrition and DNA Methylation, *Proceedings of the 31st International Symposium of the Princess Takamatsu Cancer Research Fund*, 40-43. Accepted: 12/12/00 (NA)

Southern, F.N., Eidt, J.F., Poirier, L.A. and Moursi, M.M., Increasing levels of dietary homocysteine with carotid endarterectomy produced proportionate increases in plasma homocysteine and intimal hyperplasia, *Atherosclerosis*, 158:129-38. Accepted: 12/22/00 (N/A)

Wiese, F.W., Thompson-Carino, P. and Kadlubar, F.F., Carcinogen Substrate Specificity of Human COX-1 and COX-2, *Carcinogenesis*, 22/1:5-10. Accepted: 11/10/00 (E0689401)

Yang, M., Coles, B.F., Kadlubar, F.F. and Delongchamp, R.R., Individual Differences in Urinary Cotinine Levels in Japanese Smokers: Relation to Genetic Polymorphism of Drug-metabolizing Enzymes, *Cancer Epidemiology Biomarkers and Prevention*, 10:589-593. Accepted: 3/29/01 (E0689431)

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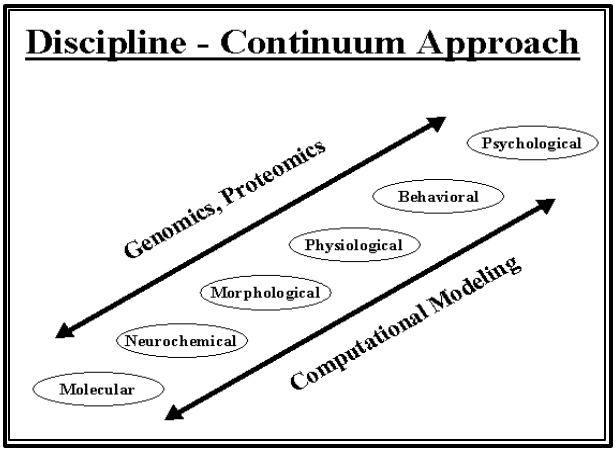
Executive Summary



The Division's approach to research integrates various disciplines to solve neurotoxicological problems with the aid of genomics, proteomics and computational modeling.

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

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 in the thousands and yet guidelines for neurotoxicity risk assessment remain vague and underdeveloped compared to 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.

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 neurotoxicology research group 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 problems.

Significant progress has been accomplished in the understanding of the role of body temperature and substituted amphetamine or other chemical (e.g., fenfluramine, ephedrine, MDMA, ibogaine) exposure in neuronal cell death, seizure activity, biomarkers of oxidative stress, and expression of critical regulatory proteins

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

controlling apoptosis (e.g., bcl-2, p53 and Bax). Amelioration of neurotoxicity was observed with hypothermia or cellular energy stabilizing agents such as L-carnitine.

Developmental exposure to estrogenic agents such as genistein and nonylphenol were shown to increase salt intake in both sexes and decrease the volume of selected sexually dimorphic nuclei in the male rat. The time courses for neuronal cell death, astrogliosis, seizure activity and other behavioral alterations were determined in the rodent for the excitotoxicant, kainic acid, the convulsant, pilocarpine and the free-radical-generating organometal, aurothioglucose.

The ability to distinguish populations of normal and Attention Deficit Hyperactivity Disorder (ADHD) children and to demonstrate the 'normalizing' effects of methylphenidate in ADHD children were accomplished with the use of the NCTR Operant Test Battery. This same behavioral assessment tool was used to demonstrate the dose-dependent retardation of learning in developing monkeys exposed to selected anticonvulsants.

The overall goals of the Division of Neurotoxicology are to develop and validate quantitative biomarkers and immediate precursor events of neurotoxicity and to use these to elucidate toxic 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), neuro-pathological, neurophysiological, and behavioral assessments to determine effects and modes of neurotoxicity. Unique features of the NCTR's neurotoxicology research efforts are the capabilities to determine target-tissue concentrations and cellular interactions of neurotoxicants and to reduce the uncertainty of extrapolating findings across species by effectively using rodent and nonhuman primate animal models as well as humans whenever possible.

Over the last decade, expertise, equipment and facilities have been woven together to pursue the overall goals of neurotoxicology research through six 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 for 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 proteomics tools will be further developed. Key personnel will be recruited and extensive training will be provided for existing staff so that new technologies can be incorporated into our research approach.

FY 2001 Accomplishments and FY 2002 Plans

Title	Project Number	Collaborator	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** — **CDER** — **Agent-Driven**

  Objective(s):

  1) To determine the effects of ibogaine on dopamine, serotonin, and their metabolite concentrations in different regions of mouse and rat brain.
  2) To determine the effects of ibogaine on reactive oxygen species (ROS) and lipid peroxidation in different regions of mouse and rat brain.
  3) To 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) To evaluate the effects of ibogaine on the activity of nitric oxide synthase (NOS) in different regions of mouse and rat brain.
  5) To determine the levels of ibogaine, noribogaine and neurohormone prolactin and corticosterone in plasma of mouse and rat.
  6) To evaluate the neurohistological effects of ibogaine in different brain regions in the mouse and the rat and to correlate them with any neurochemical alterations.

  FY 2001 Accomplishments:

  1) All the animal treatment and tissue harvest work has been finished. Tissues are in the freezer to be analyzed.
  2) Four manuscripts have been published and two more have been submitted for publication.

  FY 2002 Plans:

  The technical report will be completed.

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

  Objective(s):

  To investigate if direct infusion of compounds into the brain produces similar changes in the neurotransmitter system in rats, we will inject ibogaine, noribogaine and the structurally related compound harmaline directly into the brain and will evaluate the changes in neurotransmitter levels. Requesting additional 24 male adult Sprague Dawley rats.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

Experimental part has been completed.

FY 2002 Plans:

Two manuscripts have been submitted and the technical report will be submitted soon.

- **ADDEND: The Effects of Ibogaine on Neurotransmitter Systems: Correlation with Body Temperature and Electroencephalogram (EEG)** — E0698321 — None — Agent-Driven

Objective(s):

To investigate what effect ibogaine might have on the electroencephalogram profile along with the time course of temperature changes in rats exposed to this compound. We would like to inject ibogaine 50 mg/kg, i.p. in five male adult Sprague Dawley rats instrumented for the EEG and temperature recording.

FY 2001 Accomplishments:

One manuscript has been published and one has been submitted for publication.

FY 2002 Plans:

The technical report will be submitted.

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

Objective(s):

1) To compare acute toxicity in young animals using two forms of iron commonly used in iron supplements and one form to be used in fortification.
2) To 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 the brain and liver of young mice and rats.
3) To determine the effects of high doses of iron compounds on various blood cells and the distribution of iron and iron-binding proteins in different regions of the brain and other visceral organs in young animals.
4) To determine if high doses of iron compounds produce significant changes in neurotransmitter concentrations and the activity of nitric oxide synthase in different regions of the brain in young mice and rats.
5) To determine if high doses of iron compounds produce pathological alteration in the brain and other visceral organs in young mice and rats.

FY 2001 Accomplishments:

Experiments are in progress with this CFSAN collaborative project. Some data have been analyzed and is in preparation for a manuscript.

FY 2002 Plans:

Experiments will be performed according to the protocol.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Binienda, Zbigniew

- **Metabolic Correlates of the Neurotoxicity Associated with Exposure to the Mitochondrial Inhibitor 3-nitropropionic Acid (3-NPA) in the Rat: The Role of Free Fatty Acids (FFA)** — **E0701001** — **CFSAN** — **Concept-Driven**

Objective(s):

1) To evaluate the acute effects of the mitochondrial inhibitor 3-NPA on brain metabolic activity using electrophysiological, neurochemical, and neurohistological endpoints:
   a) Spontaneous electrical brain activity and averaged visual-evoked potentials.
   b) FFA concentration in different brain regions.
   c) Brain regional monoamine neurotransmitter concentrations: dopamine, serotonin, and their metabolites.
   d) Microscopically detectable neuronal damage.
2) To assess the possible neuroprotective effect of L-carnitine in the rat model of 3-NPA-induced histotoxic hypoxia.

FY 2001 Accomplishments:

1) All experiments designed to evaluate the effect of L-carnitine on the 3-NPA inhibition of SDH were conducted successfully.
2) Initial set of surgical preparations and experiments to collect the EEG signals from rats administered with 3-NPA has been performed.
3) The manuscript dealing with the effect of L-carnitine in 3-NPA toxicity has been published in *Toxicology Letters*.
4) Data on SDH inhibition and the effect of L-carnitine were presented at the Lake Tahoe Conference and published in the *Annals of the New York Academy of Science.*

FY 2002 Plans:

1) Perform set of electrophysiological experiments to evaluate the effect of 3-NPA combined with L-carnitine pre-treatment on the EEG and brain temperature.
2) Analyze collected data.
3) Prepare the manuscript on the effect of L-carnitine agonist the 3-NPA toxicity using the EEG and brain temperature as endpoints.

PI: Bowyer, John

- **Evaluation of the Neurotoxic Effects and Determination of the Mechanisms of Induction of Limbic Seizures Produced by Amphetamine and Related Compounds** — **E0702401** — **None** — **Concept-Driven**

Objective(s):

1) To measure the effects of dose and age on the susceptibility of amphetamine-induced limbic-type seizures in three different strains of rat and mouse, and

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

identify areas in the brain, in particular the limbic system, where cell death and neuroplastic changes occur after amphetamine-induced seizures.

2) To determine the seizure-genic capabilities of amphetamine, phentermine, methylphenidate, and ephedrine in rat and mouse; the extracellular brain levels of these compounds necessary to induce seizures; and whether hyperthermia plays a role in the seizure induction.
3) To determine via brain microdialysis if extracellular glutamate levels are elevated in the limbic system (hippocampal rudiments and piriform cortex) prior to and during seizures induced by amphetamines.
4) To elucidate the role the noradrenergic, as well as the glutamatergic, system plays in seizures generated by amphetamines. Furthermore, begin to determine how agonists and antagonists of these two neurotransmitter systems can potentiate the seizure genesis of amphetamine.

FY 2001 Accomplishments:

This work has resulted in the publication of three papers this year. Excellent progress has been made in the further determination of the brain regions and neuronal populations damaged by ephedrine and amphetamine. Also, a new sub-chronic dosing paradigm has been developed for amphetamine that does not require hyperthermia to cause neurodegeneration and damage. This paradigm is more applicable to the most common type of potential abuse that occurs in humans.

FY 2002 Plans:

Working on the further characterization of the types of neurons damaged in the parietal cortex, piriform cortex, and amygdala by amphetamine. This will be necessary for identifying the mRNA and protein changes in neurons that both survive and degenerate following amphetamine exposure. It will also aid in the ability to extract total RNA from primarily affected neurons within the various regions where neurotoxicity occurs.

- **Multiple cDNA Array Analysis of the Temporal Changes in mRNA Species after Neurotoxic Events** — E0707301 — None — **Predictive Toxicology**

Objective(s):

1) To develop the use of cDNA arrays as a means of detecting changes that are potential indicators of subtle and severe neurodegeneration at time points of several days up to months after neurotoxic insult.
2) To 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) To 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.
4) To compare differences in mRNA changes in specific brain regions of adult versus neonatal rats.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

The work on accomplishing the goals of this protocol has progressed significantly over the last year. The Clontech 1.2K array now gives excellent results and can be used at least two or three times (cutting the costs of the experiments by 50%). The results indicate that although there are significant differences in the mRNA profiles there are few changes greater than two-fold in any mRNA species in the three brain regions at any of the time points we have analyzed. To get larger and statistically significant increases in the mRNA changes, the PI will have to overcome several technical problems (listed in the FY 2002 Plans sections). The manuscript on this project is to be submitted. Also, work is ongoing with the Division of Biometry and Risk Assessment to determine the best methods of evaluating significant changes between mRNA samples using the arrays. This type of analysis is somewhat similar to what DGRT is doing with cDNA arrays from liver RNA.

FY 2002 Plans:

The PI has three main technical problems that have to be overcome to really address how mRNA species are altered after neurotoxic exposures to amphetamine: 1) the mRNAs from the affected neurons are diluted by the mRNAs from glia and unaffected neurons; 2) the 1.2K array may not contain many of the mRNA species that are being changed; and 3) the threshold for detecting low-level mRNA species (those expressed at low levels) is difficult with the arrays, in general, at present. A solution for the first problem is to use the laser capture microscope, or some other type of microdissection technique, to localize and isolate affected neurons. The second problem can be overcome by using the larger 4K Clontech array that became available in October 2001. The third problem will be more difficult to overcome, but PI is working on ways to selectively amplify the "weakly" expressed versus "strongly" expressed mRNA species.

PI: Chelonis, John

◆ **Effects of Prenatal Cocaine on Behavioral Plasticity** | **E0663307** | **None** | **Agent-Driven**

Objective(s):

To 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.

FY 2001 Accomplishments:

1) Finalized CRADA with UALR and continued with study as defined in the grant proposal.
2) Presented preliminary findings at the University of Missouri at Columbia at a conference on learning and memory and at the annual meeting of the Association for Behavioral Analysis in New Orleans.
3) Manuscript published.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

Continue with study as defined in the grant proposal and present findings at regional and national scienitific meetings as data accumulate.

- **Decision Making in Children with Attention Deficit Disorder** — **E0703101** — **None** — **Agent-Driven**

Objective(s):

1) To determine if children diagnosed with Attention Deficit Hyperactivity Disorder (ADHD) inattentive subtype, ADHD hyperactive/impulsive subtype, and ADHD combined subtype differ from each other and children without any psychiatric problems in their ability to delay gratification.
2) To determine if children diagnosed with ADHD inattentive subtype, ADHD hyperactive/impulsive subtype, and ADHD combined subtype, and controls differ in the degree that they discount delayed rewards using a delay of gratification procedure in which choices are made for hypothetical amounts of money.
3) To determine for each ADHD subtype, the relationshp between severity of ADHD symptoms and delay of gratification in both of the tasks mentioned above.
4) To obtain preliminary data for determining relationships between measures of delay of gratification and other commonly used measures for assessing impulsivity in children with ADHD.

FY 2001 Accomplishments:

1) Student assistants have been available to the PI through UALR to assist in moving this project forward.
2) Two publications.

FY 2001 Plans:

Continue patient accrual to determine if children diagnosed with Attention Deficit Hyperactivity Disorder (ADHD) inattentive subtype, ADHD hyper active/impulsive subtype, and ADHD combined subtype differ from each other and from children without any behavioral problems in their performance of the NCTR Operant Test Battery (OTB) and in their ability to delay gratification.

- **Complex Brain Function in Children as Measured by Performance in the NCTR Operant Test Battery** — **E0703301** — **None** — **Agent-Driven**

Objective(s):

To administer a battery of automated tests (games) to measure aspects of learning, short-term memory and attention, motivation, time perception, and color and position discrimination.

FY 2001 Accomplishments:

1) Several presentations were made in a variety of settings in which the findings from this study were presented. These include: 1) annual meeting of the Argentine Society for Pharmacology and Experimental Therapeutics, Mendoza, Argentina, November 2000, Plenary Lecture; 2) Center for Drug Evaluation and Research, FDA, Rockville, Maryland, December 2000; 3) National Institute for Child Health and Development, NIA, Bethesda, Maryland, April 2001, Workshop

Title	Project Number	Collaborator	Strategic Research Goal

on Adverse Drug Events in Pediatrics; and 4) European Teratology Society Annual Meeting, September 2001, Lake Balaton, Hungary. Speaker for symposium entitled "Human and animal postnatal behavioural studies: Are animal models predictive for the human situation?"

2) Three publications.

FY 2002 Plans:

Continue patient accrual and assessment using the NCTR Operant Test Battery and submit an additional two to three manuscripts.

PI: Ferguson, Sherry

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Genistein Exposure on Sexually Dimorphic Behaviors** — **E0212213** — **None** — **Agent-Driven**

  Objective(s):

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

  FY 2001 Accomplishments:

  The draft technical report has been written for the master protocol (E0212311).

  FY 2002 Plans:

  The protocol will be closed out after final acceptance of the technical report.

- **ADDEND: A Pilot Study to Identify Cost-Effective Sexually Dimorphic Behaviors Sensitive to the Effects of Developmental Exposure to Estrogenic Compounds (Methoxychlor)** — **E0212313** — **None** — **Agent-Driven**

  Objective(s):

  To identify easily-automated, cost-effective behavioral assays which are sexually dimorphic and sensitive to developmental exposure to environmental estrogens.

  FY 2001 Accomplishments:

  The manuscript is in preparation.

  FY 2002 Plans:

  The manuscript will be submitted and the technical report will be written to be included as part of the complete technical report for the master protocol (E0212311).

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Nonylphenol Exposure on Sexually Dimorphic Behaviors** — **E0212513** — **None** — **Agent-Driven**

  Objective(s):

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

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

A manuscript has been published describing the neurochemical effects of dietary nonylphenol exposure on dopamine release in the striatum of male and female rats.

FY 2002 Plans:

A draft of the technical report will be completed.

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Vinclozolin Exposure on Sexually Dimorphic Behavior** — E0212613 — None — Agent-Driven

Objective(s):

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

FY 2001 Accomplishments:

A manuscript has been published describing the neurobehavioral alterations resulting from developmental and chronic dietary exposure to vinclozolin.

FY 2002 Plans:

A draft of the technical report will be completed and the protocol will be closed out.

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Ethinyl Estradiol Exposure on Sexually Dimorphic Behaviors** — E0212913 — None — Agent-Driven

Objective(s):

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

FY 2001 Accomplishments:

The manuscript is in preparation.

FY 2002 Plans:

The manuscript will be submitted and the technical report will be written to be included as part of the complete technical report for the master protocol (E0212911).

- **ADDEND: The Effects of Developmental/Chronic Genistein Exposure over Multiple Generations on Maternal, Play, Mating/Reproductive Behaviors and Neurochemical Measures** — E0213213 — None — Agent-Driven

Objective(s):

To 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.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

Four manuscripts have been published or are in press from this study. One describes the neurochemical effects of dietary genistein amphetamine-stimulated dopamine release in the striatum of male and female rats. Another describes maternal behavior in genistein-treated rats.

FY 2002 Plans:

Additional manuscripts will be written and submitted.

- **ADDEND: The Effects of Developmental/Chronic Nonylphenol Exposure over Multiple Generations on Sexually Dimorphic Behaviors, and Neurochemical Measures** — E0213513 — None — Agent-Driven

Objective(s):

To 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.

FY 2001 Accomplishments:

One manuscript has been published describing the neurochemical effects of dietary nonylphenol exposure. Another manuscript is in press describing the effects of dietary nonylphenol exposure on learning and memory.

FY 2002 Plans:

Additional manuscripts will be written and submitted.

- **Validity of Developmental Cerebellar Stunting in the Rat as a Model for Attention Deficit Hyperactivity Disorder: Behavior and Neurochemistry** — E0704001 — None — Concept-Driven

Objective(s):

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

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

1) Five manuscripts have been published.
2) An initial study of strain differences in the Spontaneously Hypertensive Rat, its normotensive control (the Wistar-Kyoto) and the NCTR Strain 23 has been completed.
3) Two manuscripts submitted from this study.

FY 2002 Plans:

Additional manuscripts will be written and submitted. Results from the SHR study will be presented at scientific meetings.

PI: Patterson, Tucker

◆ **Neurotoxicological and Behavioral Assessment of the Human Immunodeficiency Virus (HIV) Suppressors 2',3'-dideoxycytidine (ddC) and Thalidomide in Rhesus Monkeys** — **E0250201 E0250211** — **None** — **Predictive Toxicology**

Objective(s):

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

FY 2001 Accomplishments:

All data were compiled and a manuscript is in preparation.

FY 2002 Plans:

Submit manuscript and final technical report.

PI: Paule, Merle

◆ **ADDEND: T.O. #682 - Statistical Analysis of ddC/Thalidomide Behavior Tasks: Neurotoxicological and Behavioral Assessment of the HIV Suppressors ddC and Thalidomide in Rhesus Monkeys** — **E0250221** — **None** — **Predictive Toxicology**

Objective(s):

To implement task order initiated for the statistical analysis of the behavioral task endpoints of monkeys exposed to ddC and thalidomide versus a control group.

FY 2001 Accomplishments:

Statistical analyses in progress.

FY 2002 Plans:

Complete final analyses and provide assessment for publication with other study data.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

- **Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions (97032) - ASTRA CRADA** — **E0280001, E0280011, E0280021** — **None** — **Predictive Toxicology**

  Objective(s):

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

  FY 2001 Accomplishments:

  1) Presentation to the annual meeting of the Argentine Society for Pharmacology and Experimental Therapeutics in a symposium on Neurotoxicology and Behavior, Mendoza, Argentina, November 2000. "Developmental effects of chronic NMDA receptor and fast sodium channel blockade on complex brain function in monkeys."
  2) Presentation to the Arkansas Chapter Society for Neuroscience, Jefferson, Arkansas, October 2000. "Chronic blockade of fast sodium channels and NMDA receptors during development: Effects on the acquisition of several cognitive functions in rhesus monkeys."
  3) Two manuscripts published.

  FY 2002 Plans:

  Submit final manuscript for publication.

- **Effects of Chronic Methylphenidate (Ritalin) Administration on 'cognitive' Functions in the Rhesus Monkey** — **E0683700** — **None** — **Agent-Driven**

  Objective(s):

  To 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.

  FY 2001 Accomplishments:

  1) Awaiting behavioral data analyses for interpretation and write-up for publication.
  2) Awaiting consultation with administration concerning disposition of pathology data.

  FY 2002 Plans:

  1) Await behavioral data analyses for interpretation and write-up for publication.
  2) Await consultation with administration concerning disposition of pathology data.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

- **Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity** — **E0691401, E0691411, E0691421, E0691431** — **None** — **Predictive Toxicology**

Objective(s):

1) To determine the acute effects of a variety of prototypic psychotropic agents on rodent performance in an Operant Test Battery (OTB) containing tasks designed to model several complex brain functions.
2) To determine the relative sensitivities of the behavioral endpoints monitored in the rodent OTB to pharmacological disruption.
3) To compare and contrast the acute effects of these psychotropic agents on rodent and primate OTB performance to determine the degree to which behavioral findings in rodents can be extrapolated to primates.
4) To validate the use of rodent operant performance as useful predictors of neurobehavioral toxicity.
5) To add to existing knowledge of the neurochemical and neurophysiological basis of complex brain functions.

FY 2001 Accomplishments:

Awaiting data analyses.

FY 2002 Plans:

Interpret data analyses and prepare manuscripts.

- **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** — **None** — **Predictive Toxicology**

Objective(s):

1) To 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) To determine the acute dose-effect relationships of several drugs believed to act primarily at subtypes of specific neurotransmitter receptors using rhesus monkey OTB performance.
3) To 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) To 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.
5) To determine if the acute behavioral effects of the exogenous compounds of interest differ with regard to gender in the rhesus monkey.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

Data interpretation pending completion of requested analyses.

FY 2002 Plans:

Interpret and publish data as analyses are completed.

- **ADDEND: Task Order's #746 - MBS: Develop and Deploy DROID on Linux; and #747 - MBS: Diagnostic Tools for DROID Implemented on Linux** — **E0703311** — **None** — **Agent-Driven**

Objective(s):

To set up addendum to cover task order requirements dealing with MBS and development/deployment for DROID on Linux.

FY 2001 Accomplishments:

Conversion of MBS system to Linux complete; first system deployed.

FY 2002 Plans:

Replicate Linux systems to replace all existing MBS systems.

PI: Scallet, Andrew

- **ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: II. Genistein** — **E0212215** — **None** — **Agent-Driven**

Objective(s):

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

FY 2001 Accomplishments:

1) Developed a description of findings on genistein for publication.
2) Prepared a paper on dose-response modeling for publication.

FY 2002 Plans:

Determine reproducibility of findings and complete final report.

- **ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: I. Methoxychlor** — **E0212315** — **None** — **Agent-Driven**

Objective(s):

1) To determine whether developmental exposure to methoxychlor may modify the sexually dimorphic areas of the adult rodent brain.
2) To compare neurochemical and neurohistological biomarkers of methoxychlor exposure for their relative sensitivity and concordance.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

Relative dose-exposure parameters for methoxychlor were determined, as well as estimates of its effects on the sexual differentiation of the rat brain. No effects on sexual differentiation were observed, perhaps because of the relatively low amounts of effective estrogenic activity that were achieved by the dosing regime.

FY 2002 Plans:

A final report including these findings will be submitted to the study sponsors (NTP).

- **ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: III. Nonylphenol** — E0212515 — None — Agent-Driven

Objective(s):

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

FY 2001 Accomplishments:

1) Developed a description of our findings on nonylphenol for publication.
2) Prepared a paper on dose-response modeling for publication.

FY 2002 Plans:

Determine reproducibility of findings and complete final report.

- **ADDEND: Neurotoxicological Effects of Exposure to an Anti-Androgenic Compound during Development: Vinclozolin** — E0212615 — None — Agent-Driven

Objective(s):

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

FY 2001 Accomplishments:

Include vinclozolin data in final report.

FY 2002 Plans:

- **ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds During Development: V. Ethinyl Estradiol** — E0212915 — None — Agent-Driven

Objective(s):

1) To determine whether developmental exposure to ethinyl estradiol may modify the sexually dimorphic areas of the adult rodent brain.
2) To compare neurochemical and neurohistological biomarkers of ethinyl estradiol exposure for their relative sensitivity and concordance.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Accomplishments:

1) Developed a description of our findings on ethinyl estradiol for publication.
2) Prepared a paper on dose-response modeling for publication.

FY 2002 Plans:

Determine reproducibility of findings and complete final report.

- **ADDEND: Multigenerational Exposure to Estrogenic Compounds: I. Genistein Effects on Volume of the Sexually Dimorphic Nucleus** — E0213215 — None — Agent-Driven

Objective(s):

To 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.

FY 2001 Accomplishments:

Determine reproducibility of findings.

FY 2002 Plans:

Complete final report.

- **ADDEND: Multigenerational Exposure to Estrogenic Compounds: II. Nonylphenol Effects on Volume of the Sexually Dimorphic Nucleus** — E0213515 — None — Agent-Driven

Objective(s):

To 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.

FY 2001 Accomplishments:

FY 2002 Plans:

Complete the dissection, processing, sectioning, staining and measurement of the brains, through the stages of computer-image analyses and data presentation and evaluation.

- **Estimating Quantitative Neurotoxicity Risk from Domoic Acid Exposure** — E0693001 — CFSAN — Agent-Driven

Objective(s):

1) To correlate pharmacokinetic profiles of single and multiple doses of domoic acid with associated quantitative neurohistological and behavioral effects in non-human primates.
2) To identify genetic factors modulating domoic acid sensitivity in Wistar rats.
3) To identify neurochemical biomarkers of domoic acid exposure and damage.

FY 2001 Accomplishments:

Prepared an invited chapter on domoic acid in *Neurotoxicity*.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2002 Plans:

Submit final report.

PI: Schmued, Laurence

- **Development and Validation of a Neuro-histochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanisms of Action of Neurotoxicants** — **E0701301, E0701311, E0701321, E0701331** — **None** — **Predictive Toxicology**

Objective(s):

1) To 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) To localize throughout the central nervous system, histochemical and pathological changes resulting from exposure to different classes of neurotoxicants.
3) To 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.

FY 2001 Accomplishments:

1) All of the data for the histochemical test battery have been collected. The following specific agents and their respective mechanistic classes have been studied, and their pattern of brain pathology characterized: kainic acid/domoic acid (excitotoxin); 3-NPA/MPTP (mitochondrial inhibitors; aurothioglucose (free radical generator); methamphetamine (dopamine agonists); d-fenfluramine/ MDMA/ibogaine (serotonin agonists); MK-801/ketamine (glutamate agonists); and pilocarpine (muscarinic agonist).
2) In 2001, a manuscript on the time course of kainic-acid-induced neuropathology was published, and manuscripts characterizing the distribution of brain lesions resulting from the respective exposure to aurothioglucose and pilocarpine were both accepted for publication.

FY 2002 Plans:

1) Virtually all of the data have been collected to finish this protocol and most of the related manuscripts have already been published. All that remains to complete this protocol is to finish several publications. Revisions will have to be made on the two recently accepted manuscripts.
2) Two additional manuscripts should be written. One will characterize MDMA-induced neuropathology. The other manuscript will be a review comparing the different patterns of brain pathology generated by compounds of diverse neurotoxic mechanisms.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

PI: Slikker, William

- **Quantitative Procedures for Neurotoxicity Risk Assessment** — E0310001 — None — **Predictive Toxicology**

  Objective(s):

  To 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.

  FY 2001 Accomplishments:

  The combined use of conformational analysis and three dimensional (3-D), quantitative structure-activity relationship (QSAR) methods were used to rationalize the inhibitory potencies of a series of organophoshorus pesticides against the acetylcholinesterase enzyme. The 3-D pharmacophore models were characterized by at least one hydrogen bond acceptor site and two-to-three hydrophobic sites and demonstrated good correlation (r^2=0.994) between the predicted and experimental IC_{50} values. This approach can be used to screen databases of organophosphorous and other chemicals for their neurotoxicity potential via the inhibition of acetylcholinesterase.

  FY 2002 Plans:

  Develop a preliminary biologically based dose-response model for food borne pesticides.

- **Validation Study of the Physiologically Based Pharmacokinetic (PBPK) Model for Description of Low-dose, Long-term Exposure of 2,4-dichlorophenoxyacetic Acid (2,4-D) Dosimetry in the Central Nervous System (CNS)** — E0699201 — CFSAN — **Predictive Toxicology**

  Objective(s):

  To obtain central nervous system (CNS) pharmacokinetic profiles of 2,4-D transport in the rat after low-dose, chronic dosing (28 days). The data will be used to validate the previously developed PBPK model which simulates the uptake, distribution, and clearance of 2,4-D.

  FY 2001 Accomplishments:

  First manuscript describing the PBPK model development was published. This PBPK model should be an effective tool for evaluating the target-tissue doses that may produce the neurotoxicity of organic acid toxicants after low-dose exposures to contaminated foods or the environment. Neurochemical analyses were completed to generate data testing the hypothesis that long-term, low-dose 2,4-D produces changes in neurochemical parameters.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

Second manuscript describing the biological effects of low-dose, chronic 2,4-D exposure has been written and will be submitted. This CFSAN collaborative project served as a partial basis for the EPA/NCTR/FDA IAG on dose-response modeling of food borne pesticides.

- **Preliminary Studies for the Effects of Chronic Dexfenfluramine Administration in the Rhesus Monkey** — **E0702601, E0702611, E0702621** — **CDER** — **Predictive Toxicology**

Objective(s):

1) To determine if the rhesus monkey demonstrates cardiac valve changes due to chronically administered dexfenfluramine.
2) To determine if the rhesus monkey demonstrates neurobiological changes due to chronically administered dexfenfluramine.

FY 2001 Accomplishments:

Ultrasound, preliminary pathology, and pharmacokinetic analyses completed. A poster (abstract) describing the effect of chronic dexfenfluramine administration on monkey weight gain, behavioral parameters, regional brain serotonin concentrations, serotonin transporter concentrations, and plasma levels of dexfenfluramine and major metabolite has been presented and published.

FY 2002 Plans:

Complete data analysis and submit final report including heart and ultrasound and pathology data.

FY 2001 Publications

Anderson, V., Carneiro, M., Bulterys, M., Douglas, G., Polliotti, B. and Slikker, W., HIV in pregnancy - Perinatal infections: HIV and co-infections in the placenta and therapeutic interventions - A workshop report, *Placenta Trophoblast Research*, 15:S34-S37. Accepted: 1/5/01 (N/A)

Baumann, M.H., Pablo, J., Ali, S.F., Rothman, N. and Mash, D.C., Noribogaine (12-hydroxyibogamine): A biologically active metabolite of the antiaddictive drug ibogaine, *Annals of the New York Academy of Sciences*. Accepted: 8/15/01 (E0698301)

Binienda, Z.K. and Ali, S.F., Neuroprotective role of L-carnitine in the 3-nitropropionic acid induced neurotoxicity, *Toxicology Letters*, 125:67-73. Accepted: 7/16/01 (E0701001)

Binienda, Z.K., Sadovova, N.V., Rountree, R.L., Scallet, A.C. and Ali, S.F., Effect of L-carnitine pretreatment on 3-nitropropionic acid-induced inhibition of rat brain succinate dehydrogenase activity, *Annals of the New York Academy of Sciences*, 939:359-365. Accepted: 4/15/01 (E0701001)

Bowyer, J.F., Holson, R.R., Miller, D.B. and O'Callaghan, J.P., Phenobarbital and dizocilpine can block methamphetamine-induced neurotoxicity in mice by mechanisms that are independent of thermoregulation, *Brain Research*, 919:179-183. Accepted: 8/21/01 (E0690301)

Bowyer, J.F., Hopkins, K.J., Jakab, R.L. and Ferguson, S.A., l-ephedrine-induced neurodegeneration in the parietal cortex and thalamus of the rat is dependent on hyperthermia and can be altered by the process of *in vivo* brain microdialysis, *Toxicological Letters*, 125:151-166. Accepted: 9/18/01 (E0702401)

Cada, A.M., Hansen, D.K., Laborde, J.B. and Ferguson, S.A., Minimal behavioral or developmental effects from developmental exposure to St. John's Wort (Hypericum perforatum) in Sprague-Dawley rats, *Nutritional Neuroscience*, 4:135-141. Accepted: 11/4/00 (N/A)

Desaiah, D., Reddy, S.L., Imam, S.Z. and Ali, S.F., Role of neuronal nitric oxide in methamphetamine neurotoxicity and protection by nNOS inhibitor, *Pure Applied Chemistry*, 72:1001-1006. Accepted: 10/15/00 (N/A)

El yazal, J., Rao, S.N., Mehl, A. and Slikker, W., Prediction of organophosphorus acetylcholinesterase inhibition using three-dimensional quantitative structure-activity relationship (QSAR) methods, *Toxicological Sciences*, 63:223-232. Accepted: 7/20/01 (E0310001)

Ferguson, S.A., Cada, A.M., Gray, E.P. and Paule, M.G., No alterations in performance of two interval timing operant tasks after a-difluoromethylornithine (DFMO)-induced cerebellar stunting, *Behavioural Brain Research,* 126:135-146. Accepted: 5/4/01 (E0704001)

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Ferguson, S.A., Flynn, K.M., Delclos, K.B., Newbold, R. and Gough, B.J., Effects of lifelong dietary exposure to genistein or nonylphenol on amphetamine-stimulated striatal dopamine release in male and female rats, *Neurotoxicology and Teratology*. Accepted: 9/19/01 (E0213213)

Ferguson, S.A., Paule, M.G. and Holson, R.R., Neonatal dexamethasone on day 7 in rats causes behavioral alterations reflective of hippocampal, but not cerebellar, deficits, *Neurotoxicology and Teratology*, 23:57-69. Accepted: 10/2/00 (E0704001)

Ferguson, S.A., A review of rodent models of attention deficit hyperactivity disorder, *The Neuropharmacology of Psychostimulant Drugs: Implications for ADHD*, 209-220. Accepted: 10/5/00 (NA)

Flynn, K.M., Delclos, K.B., Newbold, R. and Ferguson, S.A., Behavioral responses of rats exposed to long term dietary vinclozolin, *Journal of Agricultural and Food Chemistry*, 49:1658-1665. Accepted: 1/19/01 (E0212613)

Imam, S.Z. and Ali, S.F., Aging increases the susceptibility to methamphetamine-induced dopaminergic neurotoxicity and hyperthermia in rats by increasing the production of peroxynitrite, *Journal of Neurochemistry*, 78:952-959. Accepted: 6/1/01 (E0703801)

Imam, S.Z., El yazal, J., Newport, G.D., Itzhak, Y., Cadet, J., Slikker, W. and Ali, S.F., Methamphetamine-induced dopaminergic neurotoxicity: Role of peroxynitrite and neuroprotective role of antioxidants and peroxynitrite decomposition catalysts, *Annals of New York Academy of Sciences*, 939:366-380. Accepted: 3/5/01 (E0703801)

Imam, S.Z., Itzhak, Y., Cadet, J., Islam, F., Slikker, W. and Ali, S.F., Methamphetamine-induced alteration in striatal p53 and bcl-2 expressions in mice, *Molecular Brain Research*, 91:174-178. Accepted: 5/8/01 (E0698301)

Imam, S.Z., Newport, G.D., Itzhak, Y., Cadet, J., Islam, F., Slikker, W. and Ali, S.F., Peroxynitrite plays a role in methamphetamine-induced neurotoxicity dopaminergic neurotoxicity: evidence from mice lacking neuronal nitric oxide synthase gene or overexpressing copper zinc superoxide dismutase, *Journal of Neurochemistry*, 76:745-749. Accepted: 10/2/00 (E0703801)

Itzhak, Y., Martin, J.L. and Ali, S.F., Comparison between the role of the neuronal and inducible nitric oxide synthase in methamphetamine-induced neurotoxicity and sensitization, *Annals of the New York Academy of Sciences*, 914:104-111. Accepted: 10/2/00 (N/A)

Kim, C., Sandberg, J.A., Slikker, W., Binienda, Z.K., Schlosser, P.M. and Patterson, T.A., Quantitative exposure assessment: application of physiologically-based pharmacokinetic (PBPK) modeling of low-dose, long-term exposures of organic acid toxicant in the brain, *Environmental Toxicology and Pharmacology*, 9:153-160. Accepted: 12/30/00 (E0699201)

Paule, M.G., Using identical behavioral task in children, monkeys and rats to study the effects of drugs, *Current Therapeutic Research*, 62:820-833. Accepted: 9/18/01 (E0703301)

Popke, J., Allen, R.R., Pearson, E., Hammond, T. and Paule, M.G., Differential effects of two NMDA receptor antagonists on cognitive-behavioral development in nonhuman primates II, *Neurotoxicology and Teratology*, 23:333-347. Accepted: 3/5/01 (E0280001)

Ray, S.K., Wilford, G.G., Ali, S.F. and Banik, N.L., Calpain upregulation in spinal cords of mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's disease, *Annals of the New York Academy of Sciences*. Accepted: 8/1/01 (E0698301)

Scallet, A.C. and Meredith, J.M., Quantitative three-dimensional reconstruction: Feasibility for studies of sexually dimorphic hypothalamic development in rats, *Neurotoxicology and Teratology*, 24:1-5. Accepted: 9/19/01 (E0213201)

Scallet, A.C., Nony, P.A., Rountree, R.L. and Binienda, Z.K., Biomarkers of 3-nitropropionic acid (3-NPA)-induced mitochondrial dysfunction as indicators of neuroprotection, *Annals of the New York Academy of Sciences*, 939:381-392. Accepted: 3/20/01 (E0701001)

Schmued, L.C. and Hopkins, K.J., The progression of neuronal, myelin, astrocytic, and immunological changes in the rat brain following exposure to goldthioglucose, *Brain Research*. Accepted: 9/6/01 (E0701301)

Slikker, W. and Sobotka, T., Neurotoxicology: Molecular Biomarkers, Transgenics and Imaging Technologies, *Proceedings of the Alternative Toxicological Methods Meeting*. Accepted: 8/16/01 (N/A)

Slikker, W., Desai, V.G., Duhart, H.M., Feuers, R.J. and Imam, S.Z., Hypothermia enhances Bcl-2 expression and protects against oxidative stress induced cell death in chinese hamster ovary cells, *Free Radical Biology and Medicine*, 31:405-411. Accepted: 5/1/01 (E0698301)

Slikker, W., Jonas, S., Auer, R.N., Palmer, G.C., Narahashi, T., Youdim, M., Maynard, K.I., Carbone, K.M. and Trembly, B., Neuroprotection: Past successes and future challenges, *Annals of New York Academy of Sciences*, 939:465-477. Accepted: 4/1/01 (N/A)

Slikker, W., Scallet, A.C., Doerge, D.R. and Ferguson, S.A., Gender-based differences in rats after chronic dietary exposure to genistein, International *Journal of Toxicology*, 20:1-5. Accepted: 3/5/01 (E0213213)

Thiriet, N., Zwiller, J. and Ali, S.F., Induction of the immediate early genes egr-1 and c-fos by methamphetamine in mouse brain, *Brain Research*, 919:31-40. Accepted: 8/14/01 (E0698301)

Tor-Agbidye, J., Yamamoto, B. and Bowyer, J.F., Seizure activity and hyperthermia potentiate the increases in dopamine and serotonin extracellular levels in the amygdala during exposure to d-amphetamine, *Toxicological Sciences*, 60:103-111. Accepted: 12/11/00 (E0702401)

Walker, L.M., York, L., Imam, S.Z., Ali, S.F. and Mayeux, P.R., Oxidative stress and reactive nitrogen species generation during renal ischemia, *Toxicological Sciences*, 63:143-148. Accepted: 6/4/01 (N/A)

Xu, Z., Seidler, F.J., Ali, S.F., Slikker, W. and Slotkin, T.A., Fetal and adolescent nicotine administration: Effects on the CNS serotonergic systems, *Brain Research*, 914:166-178. Accepted: 7/12/01 (E0709801)

Ye, X., Carp, R.I., Schmued, L.C. and Scallet, A.C., Fluoro-jade and silver methods: Application to the neuropathology of scrapie, a transmissible spongiform encephalopathy (TSE), *Brain Research Protocols*. Accepted: 10/10/00 (P00409)

Ye, X., Meeker, H.C., Scallet, A.C. and Carp, R.I., Comparison of NADPH diaphorase activity in the brains of hamster infected with scrapie strains 139H or 263K or with normal hamster brain homogenate, *Histology and Histopathology*, 16:997-1004. Accepted: 6/25/01 (N/A)

Ye, X., Rountree, R.L., Scallet, A.C., Meeker, H.C. and Carp, R.I., Evaluation of neurodegeneration in scrapie-infected animals by selective methods that detect cellular degeneration, *Brain Research*, 910:175-178. Accepted: 5/1/01 (N/A)

Ye, X., Scallet, A.C. and Carp, R.I., Changes in immune and endocrine systems in scrapie-infected animals, *Cur. Med. Chem.-Imm., Endoc. & Metab. Agents*. Accepted: 5/25/01 (N/A)

Veterinary Services

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Division of Veterinary Services provides research support through on-site contractor for histopathology.

Executive Summary

The Division of Veterinary Services provides professional and technical support to the various NCTR research divisions 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 (AAALAC International). 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 2001 Accomplishments

Immediate Office

During 2001, the Division provided oversight and veterinary management of all laboratory animals and housing facilities at NCTR. Divisional personnel completed and submitted annual reports assuring compliance with federal regulations and NIH guidelines relative to the Animal Care and Use Program. Personnel participated in semi-annual program reviews, facility inspections, and experimental protocol reviews as part of the NCTR Institutional Animal Care and Use Committee proceedings. The director serves as a member of the FDA Research Animal Committee and its AAALAC International accreditation subcommittee. Divisional personnel serve as government project officers for the pathology services, animal care and diet preparation, and rodent bedding contracts. Divisional personnel were responsible for breeding, rearing, and/or acquiring all experimental animals used on-site including two new transgenic mouse breeding colonies for use in NCTR's Phototoxicity Center. Divisional personnel participated as instructors in the on-site technician certification program authorized by the American Association for Laboratory Animal Science (AALAS).

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Animal Care/Diet Preparation Services

All experiments at NCTR utilizing animals were supported by contract animal care personnel. These experiments entailed as a minimum the daily animal care support of an average of 5,595 rodents, 16 rabbits, and 88 rhesus monkeys. Technical manipulations for these studies included one or more of the following procedures: tattooing, vaginal lavages, tumor palpations, injections (SQ, IM or IV), oral gavage, behavioral testing, and blood collection. The animal care/diet preparation program manager, a veterinarian, also provided clinical veterinary care and oversight for the environmental enrichment program for the NCTR nonhuman primates. Contract diet preparation personnel provided consultation and nutritional support and diet preparation for several carcinogenicity studies including malachite green, leucomalachite green, and urethane, funded through the Interagency Agreement with the National Institute for Environmental Health Sciences' National Toxicology Program. In addition to supplying adequate diet to the many rodents, rabbits and nonhuman primates maintained at the Center, diet preparation personnel produced 21,240 kg of dosed-diet for those studies requiring dosing by ingestion. Quality Assurance personnel performed more than 2,500 quality control audits of contractor-performed procedures. One contract employee contributed to the new NRC Nonhuman Primate Nutrition Handbook due to be published in early 2002.

Pathology and Pathology-related Services

During 2001, eight new employees entered the Laboratory Technician apprenticeship training program sponsored by NCTR's pathology contractor, Pathology Associates, International. In addition to routine histological examination of tissues, contract personnel also performed many special morphologic techniques. Utilizing immunohistochemistry techniques, the following assays were performed in support of NCTR research: cell proliferation assays, apoptosis assays, markers for hormones, markers for oncogenes, and markers for other proteins as requested. Utilizing non-radioactive *in situ* hybridization techniques, contract personnel have also performed assays with fluorescein-labeled oligonucleotide cocktail for detection of histone, H2b, H3 and H4 mRNA sequences, RNA labeling with digoxigenin-UTP by *in vitro* transcription with RNA polymerases, and oligonucleotide tailing with digoxigenin-dUTP. Image analysis, including microdensitometry, planar morphometry, particle analysis, and cell proliferation and apoptosis indices have been accomplished in support of NCTR research projects.

During 2001, contract employees worked with on-site and off-site personnel to implement the NTP LDASII system at NCTR for pathology data collection on NTP-sponsored studies. Also during 2001, contract personnel authored or co-authored 21 publications.

FY 2002 Plans

- Continue to support the research mission of NCTR but seeking ways to become more efficient in doing so.
- Continue to supply methods development where needed to support the NIEHS IAG work at NCTR.
- Implement a paper-less pathology data system that will bring together NCTR's In-life (Multigen) system for access to In-life data, clinical observations, gross pathology data, tracking information, clinical pathology, and sperm analysis. The system is being designed on-site by R.O.W. Sciences with the assistance of Pathology Associates, International.
- Implement a core facility in pathology for Laser Capture Microdissection services.
- Continue a quality laboratory animal care program that is consistent with state and federal laws, regulations, and guidelines.

FY 2001 Publications

Delclos, K.B., Bucci, T.J., Lomax, L.G., Latendresse, J.R., Warbritton, A.R., Weis, C.C. and Newbold, R., Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats, *Reproductive Toxicology*, 15(6). Accepted: 8/25/01 (E0212201)

Hard, G., Howard, P., Kovatch, R. and Bucci, T.J., Rat kidney pathology induced by chronic exposure to fumonisin B_1 includes rare variants of renal tubule tumor, *Toxicological Pathology*, 29:379-386. Accepted: 7/15/01 (E0210801)

Khan, S.A., Khan, A.A., Nawaz, M.S., Depaola, A., Andrews, A.M. and Cerniglia, C.E., DNA packaging and developmental intermediates of a broad host range *Vibrio vulnificus* bacteriophage 71A-6, *Mol. Cell. Probes*, 15:61-69. Accepted: 12/8/00 (E0700101)

Muskhelishvili, L., Thompson-Carino, P., Kusewitt, D.F., Wang, C. and Kadlubar, F.F., *In Situ* Hybridization and Immunohistochemical Analysis of Cytochrome P450 1B1 Expression in Human Normal Tissues, *Journal of Histochemistry and Cytochemistry*, 49:229-236. Accepted: 11/15/00 (E0699011)

Sams, R.L., Couch, L.H., Miller, B.J., Okerberg, C.V., Warbritton, A.R., Wamer, W., Beer, J. and Howard, P., Basal cell proliferation rates in female SKH-1 mice treated with alpha- and beta-hydroxy acids, *Toxicology Applied Pharmacol*., 175:76-82. Accepted: 5/30/01 (E0213101)

PI: Akerman, Greg

- **Effect of Mutation in the p53 Tumor Suppressor Gene on Gene Expression Profiles in Young and Aged Mice** **E0712901**

Objective(s):

The most commonly mutated gene in human cancers is the p53 tumor suppressor gene. One model that has been developed to probe p53 function is the development of the p53 knock-out and p53-deficient mice in which a deletion of protons of exon 4 and intron 5 of p53 was introduced by molecular genetic techniques. The results of these studies will be of direct benefit to the FDA in that the increased knowledge and understanding of this model will aid in the evaluation of regulatory submissions of data obtained with the p53-deficient mouse model.

PI: Ali, Syed

- **Neurotoxicity Assessment of Substituted Amphetamines** **E0708801**

Objective(s):

1) To evaluate the neurotoxicity of the eight most popular ephedra-containing dietary supplements sold in the market place and consumed by the public.
2) To determine the IC-50 of these dietary supplements using PC-12 cultured cells.
3) To determine if the *in vitro* exposure to these dietary supplements selectively induces a specific genomic change in PC-12 cultured cells using cDNA arrays.
4) To determine if multiple doses of these dietary supplements selectively induces specific genomic changes in different regions of mouse brain using cDNA arrays.
5) To determine if multiple doses of these compounds produce significant changes in neurotransmitter concentrations in different regions of brain in mice.
6) To 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) To 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.
8) To determine if multiple doses of these dietary supplements produce pathological alteration in brain and other visceral organs in the mouse.

- **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 Subject** E0712101

  Objective(s):

  1) To determine the post-translational protein modifications in the protein extracts of nigral and striatal tissues in substituted amphetamines and MPTP-treated mice.
  2) To 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) To 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) To determine the effect of various nNos inhibitors on substituted amphetamine and MPTP-induced free radical production and monoamines concentration in mice brains.
  5) To study the correlation of dopaminergic protection of nNos inhibitors to be free radical scavenging effects of nNos inhibitors.

PI: Beger, Richard

- **Methods for Predicting Toxicological Properties of Molecules from their NMR Chemical Shifts Through-bond and Through-space Distance Connectivity Patterns** E0712601

  Objective(s):

  Produce models that use nuclear magnetic resonance (NMR) data and infuse three-dimensional atom-to-atom through-bond connectivity and atom-to-atom through-space intra-molecular 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, E0707701 and E0708301.

PI: Bendre, Sachin

- **DNA Damage and Somatic Cell Mutation in Children Born to Mal-nourished Mothers** E0710701

  Objective(s):

  1) To establish populations of well-nourished and malnourished mothers and their offspring by clinical examination and establishing a Body Mass Index for pregnant women and by anthropometry on their newborns.
  2) To identify subpopulations among the well-nourished and malnourished pregnant women whose diets have not been supplemented or have been supplemented through clinical intervention of varying periods and/or doses with iron and folate.

3) To measure the frequency of 6-thioguanine-resistent lymphocytes as a marker of somatic cell mutant frequency in the women and their offspring.
4) To explore possible mechanisms for any alterations in the frequency of 6-thio guanine-resistant lymphocytes in the offspring of both supplemented and unsupplemented malnourished mothers.

- **Effect of Azathioprine on Somatic Cell and Germline *HPRT* Mutant Frequencies in Humans.** **E0713701**

Objective(s):

We propose to test the hypothesis that *in vivo* selection by azathioprine affects both somatic cell and germline *HPRT* mutant frequencies in humans. The first experiment will use the patient database maintained by the 'Scientific Registry of Transplant Recipients' (SRTR), Ann Arbor, MI. Using this database, an estimate will be made of the number of children born to patients treated with azathioprine so that we can make an estimate of the size of the population at risk. After an Institutional Review Board (IRB) approval and informed consent of the patient, data regarding the dose and duration of drug treatment, and reproductive and sexual indices will be obtained from all the patients who have had children while being treated with azathioprine. The number and sex of the children and grandchildren, if any, will be recorded. Incidences of miscarriages, infertility and fetal malformations or disease will also be calculated using this data. This additional information may indicate whether or not azathioprine toxicity is affecting the first generation offspring.

In another set of experiments, blood samples will be obtained from patients and their children who were conceived while the patient was taking azathioprine. This will involve a collaborative effort with the Renal Transplant Unit and the Departments of Neurology, Gastroenterology and Rheumatology at the University of Arkansas for Medical Sciences (UAMS). *HPRT* lymphocyte mutant frequencies (MFs) will be calculated for such parent child pairs and an effort will be made to look for any sex biases in children. Similarly, blood will be obtained from the children (if any) of the first generation female children to screen for lymphocyte *HPRT* mutant frequency.

In addition, to test the hypothesis that toxicity to germ cells may affect 'X-bearing' sperm in males, a semen sample will be obtained from male patients currently receiving azathioprine treatment who show high lymphocyte *HPRT* mutant frequencies. Semen analysis for sperm abnormalities, *HPRT* mutant frequency in the sperm, and the percentage of 'X-bearing' and 'Y-bearing' sperm will be calculated.

PI: Binienda, Zbigniew

- **The Role of Mitochondrial Energy Disruption in the Mechanism of Neurotoxicity: Neurophysiological, Neurochemical, and cDNA Micro-array Approach.** **E0711001**

Objective(s):

1) To define neurophysiological and neurochemical phenotypes associated with brain exposure to 3-nitropropionic Acid (3-NPA) and L-carnitine.
2) To define changes in patterns of gene expression induced by 3-NPA and L-carnitine in the rat brain.

3) To 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) To establish the relationship between 3-NPA-induced physiological, neuro-chemical phenotypes and transcriptome profiling in the rat brain model.

PI: Bishop, Michelle

- **Fluorescent-based Detection of Oxidative DNA Damage in Cells Treated *In vitro* Using Flow Cytometry and Fluorescence Microscope** **E0712701**

Objective(s):

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

PI: Buzatu, Dan

- **High Speed Parallel Distributed Artificial Neural Network Project** **E0713101**

Objective(s):

To develop a high-speed, parallel-distributed, self-optimizing artificial neural network.

- **^{13}C Magnetic Resonance Breast Cancer Diagnostic Models** **E0713601**

Objective(s):

Develop the high-field magnet ^{13}C Magnetic Resonance (MR) pulse sequences necessary to obtain single voxel MR scans of rat breast tumor tissue. Data from the tumor MR scans and in combination with gene array data will be used in conjunction with advanced pattern-recognition software to develop breast cancer diagnostic models.

PI: Chen, James

- **Experimental Design and Analysis of GeneArray Expression Data** **E0711201**

Objective(s):

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

PI: Chen, Junjian

- **Somatic Alterations in Prostate Cancer and Its Precursor Lesions** **E0711301**

Objective(s):

1) To test the hypothesis that homoplasmic mutations in mitochondrial genome are elevated in human prostate carcinomas as a consequence of increased oxidative stress.
2) To test the hypothesis that at least some of the homoplasmic mtDNA mutations detected in prostate carcinomas are also detectable in evolutionary related precursor lesions identified in the same prostate biopsies.

3) To test the hypothesis that the incidence and type of homoplasmic mtDNA mutations in benign prostatic hyperplasia differ from those in prostate carcinomas.
4) To test the hypothesis that homoplasmic mtDNA mutations are more sensitive than nuclear markers in delineation of clonal evolution of prostate cancers.

- **Molecular Profiling of Metabolic and Repair Capabilities of Breast Cancer Patients by Pathway-based Tissue cDNA Macroarray** **E0712301**

Objective(s):

To develop a pathway-based cDNA macroarray approach to study tissue-specific inter-individual variability of drug metabolism and DNA repair capacity of breast cancer patients.

PI: Chen, Tao

- **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**

Objective(s):

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

- **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**

Objective(s):

The MLA is currently used internationally for regulatory decision making and it is the mammalian *in vitro* genetic toxicology assay preferred by both the FDA and the US EPA. The debate continues as to the underlying mechanistic difference between the small and large colony mutant phenotypes and the significance of such analysis in hazard characterization and mechanistically-based risk assessment. In this study, we will explore the fundamental mechanistic difference(s) between the small and large colony mutant phenotypes by analysis of mitotic recombination and/or chromosome deletion, DNA microarray analysis of gene expression, analysis of the recombinase and telomerase activities.

PI: Chou, Ming

- **A Study of Genotoxic Mechanisms of Carcinogenic Pyrrolizidine Alkaloids and Pyrrolizidine Alkaloid N-Oxides** **E0710401**

Objective(s):

1) To characterize the structures of the eight DHP-derived DNA adducts.
2) To study metabolism of retronecine-based pyrrolizidine alkaloids (riddelliine, retrorsine and monocrotaline), heliotridine-based pyrrolizidine alkaloids (lasiocarpine and heliotrine), otonecine-based pyrrolizidine alkaloids (senkirkine and clivorine), and pyrrolizidine alkaloid N-oxides (isatidine, riddelliine N-oxide, monocrotaline N-oxide and lasiocarpine N-oxide) by liver microsomes of F344 rats, B6C3F mice, and humans of both sexes, and compare metabolism profiles.
3) To 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) To 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) To 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) To determine whether or not some dietary supplements (including comfiey, soltsfoot, and liquorice) sold in the United States contain genotoxic pyrrolizidine alkaloids determined by DHP-derived DNA adduct formation.
7) To 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) To 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.
9) To determine the effect of Chinese herbs, such as liquorice, and their active components, such as glycyrrhizin and glycyrrhetinic.

PI: Delclos, K. Barry

- **Protective Effects of Soy-containing Diets Against Renal Toxicity: Implications for Animal Toxicity Assessments** **E0714201**

Objective(s):

Examine the effects of diet and timing of exposure on renal toxicity induced by nonylphenol, DEHP, and MEHP.

PI: Dobrovolsky, Vasily

- **Transgenic Mouse Model for Detecting *in vivo* Mutation Using a Green Fluorescent Protein Reporter** **E0713801**

  Objective(s):

  To make new transgenic mice in which the mutation can be determined using high throughput method of flow cytometry and fluorescent microscopy. Current methods to select for rare mutations in a population of normal cells require extensive culturing of cells and the use of drugs that allow the mutant, but not the normal cells, to grow. These methods are subject to technical difficulties and are time-consuming and expensive. This project is directed toward developing a new approach to mutation detection using fluorescent markers.

PI: Feuers, Ritchie

- **Development of Techniques in Proteomics for Uses in Genetic Toxicology Studies** **E0713501**

  Objective(s):

  To develop techniques in proteomics with the goal of utilization of such techniques.

PI: Fuscoe, James

- **Assessment of the Expression of Disease-Associated Susceptibility Genes during the Life Cycle of Rats** **E0712201**

  Objective(s):

  Use a "rat chip" to quantitate the expression of 4,000 genes in the liver of rats throughout their life cycle. Verify the expression levels by quantitative polymerase chain reaction (PCR) or Northern Analysis.

PI: Hass, Bruce

- **Identification of Target Sites for UVB Irradiation in Gene A of ÔX174 contained as a Transgene in Mouse Embryonic Cell PX-2** **E0710101**

  Objective(s):

  To determine whether an increase in mutant frequency of a mouse cell line containing the ÔX174 transgene results from exposure of the cells to UVB light, the mutagenic component of sunlight.

PI: Jakab, Robert

- **Genetic Profiling of Regenerating and Degenerating Neurons after Amphetamine-Neurotoxicity** **E0713401**

  Objective(s):

  1) To determine the gene expression profile of three cell groups uniquely susceptible to amphetamine exposure.
  2) To determine the first appearance of the three effected cell groups and the pattern and timetable of their density changes.
  3) To compare the genetic profiles of adjacent cortical neurons susceptible and resistant to amphetamine.

4) To compare the genetic profiles of *de novo* striatal TH* neurons, adjacent TH* neurons, and midbrain TH* neurons.
5) To search for genes, mRNA species, and proteins - candidates for potential pharmacological manipulations that can aid the recruitment of striatal neurons to produce and release DA.

PI: James-Gaylor, Sandra

◆ **Mechanisms of Methylation Dysregulation and DNA Damage in a Rat Model of Nutritional Hepatocarcinogenesis** **E0712801**

Objective(s):

1) To confirm that the presence of uracil and abasic sites in preneoplastic DNA from folate-/methyl-deficient rats create nonproductive high affinity binding sites for the DNA methyltransferase that compromise normal DNA methylation at the replication fork resulting in genome-wide hypomethylation.
2) To determine (a) whether the double-stranded loss of cytosine methylation is maintained in folate-/methyl-deficient rats after nutritional repletion of methyl donors; or (b) whether the original methylation pattern and chromatin structure can be reestablished; (c) whether the increase in dnmt1 expression is stimulated by global loss of methyl groups; and (d) whether dmnt1 expression is decreased by methyl repletion.
3) To 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.
4) To use microarray slides printed with the rat cDNA library in collaboration with the Division of Genetic and Reproductive Toxicology 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.

PI: Leakey, Julian

◆ **Impact of Dietary Supplements on Women's Health Issues** **E0708401**

Objective(s):

1) To set up and validate a comprehensive *in vitro* assay system capable of determining whether constituents of dietary supplement preparations inhibit any major isoform of the drug metabolizing enzymes.
2) To set up and validate a comprehensive *in vitro* assay system capable of determining whether constituents of dietary supplement preparations induce any major isoform of the drug metabolizing enzymes.
3) To utilize these assay systems to comprehensively identify and determine the occurrence and variability of potentially toxic constituents in different crude preparations of commonly used dietary supplements, such as St. John's Wort and echinacea.

PI: Montgomery, Beverly

- **Development of Rodent Kidney Cell Culturing Methods for Use in Evaluating *In vivo* Mutation** **E0712501**

  Objective(s):

  The specific goal of this methods development protocol is to gain experience in and adapt kidney cell-culturing techniques that will be used for measuring endogenous reporter gene mutant frequencies in rats and mice.

PI: Patterson, Tucker

- **Analyses of the Rat Hippocampus via DNA Microarrays and a Novel Antibody Array, Coupled with Laser Capture Micro-dissection (LCM)** **E0713901**

  Objective(s):

  1) To measure gene and protein expression in regions (CA1, dentate gyrus, etc.) of the hippocampus to determine regional distribution.
  2) To determine the effect of aging on regional distribution of hippocampal proteins in three strains of rats.
  3) To determine if aging, behavioral performance and alterations in gene and protein expression in the hippocampus are related.
  4) To 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.

PI: Paule, Merle

- **Pharmacological Counter-Measures for Space Motion Sickness** **E0712401**

  Objective(s):

  To establish effectiveness and quantify side effects for potential drug counter-measures for Space Motion Sickness (SMS).

PI: Pearce, Bruce

- **Bio-Preg to Windows 2000 Upgrade** **E0713001**

  Objective(s):

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

PI: Ratnasinghe, Luke

- **SNP Discovery using Denaturing HPLC (Phase 1) and Mutation Analysis – Center for Structural Genomics** **E0714301**

  Objective(s):

  To develop methods for the detection, and identification of single nucleotide polymorphisms (SNPs) in epidemiological studies, using denaturing high performance liquid chromatography (HPLC). We will be able to detect variants in PCR fragments of specific candidate genes, and identify SNPs that are associated with cancer in pooled breast cancer, prostate cancer and control DNAs from the epidemiological studies currently underway in collaboration with UAMS (E0701501, E0701701, E0702101).

PI: Roberts, Dean

- **Two-Dimensional micro-LC Proteomics Using Stable-isotope Affinity Tags for Differential Display of Toxicity-induced Biomarkers** **E0710301**

  Objective(s):

  To investigate changes in protein-expression profiles using two-dimensional liquid chromatograph (LC), stable-isotope-coded affinity tags, and mass spectrometry for differential display of biomarkers associated with doxorubicin-induced mitochondrial toxicity.

PI: Schmued, Laurence

- **Proteomics of Toxicant-Induced Neuronal Degeneration** **E0711101**

  Objective(s):

  1) To resolve the chemical identity of the endogenous protein(s) associated with neuronal cell death as identified by Fluoro Jade B binding.
  2) To determine if the same proteins are expressed regardless of the mechanism of neurodegeneration.
  3) To resolve the metabolic pathway by which the "degeneration protein" is generated.
  4) To resolve the chemical identity of the fluorescent component in Fluoro-Jade B responsible for the high-affinity labeling of degenerating neurons.

PI: Shvartsburg, Alexander

- **Development of Novel Methods for Peptide Sequencing and Characterization of Organic Molecules Using the MS/MS of Multiple Charged Metalated Ions** **E0712001**

  Objective(s):

  To investigate new methods for characterization of organic and biological molecules using tandem mass spectrometry, with a special focus on peptide sequencing. The overwhelming majority of work to date has involved protonated species. Here it is proposed to systematically research the addition of multiple charged metal ions to peptides and amino acids, and the MS/MS fragmentation pathways of resulting complexes.

PI: Turesky, Robert

- **Toxicological Effects of Ochratoxin A** **E0709401**

  Objective(s):

PI: Valentine, Carrie

- **Evaluation of the Potential of the Gene A Forward Mutational Assay of PhiX174 for Improving Sensitivity of Transgenic Mutation Assays** **E0711501**

  Objective(s):

  1) To determine the appropriate experimental conditions to identify single bursts of mutations fixed *in vivo*.
  2) To develop a microplate scoring method that will identify *in vivo* bursts within numerous aliquots.

3) To determine the spontaneous mutant frequency and ethyl nitrosourea-induced mutant frequency by single burst analysis for mouse splenic lymphocytes.
4) To continue development of a frameshift assay for phiX174 in gene J by the collaborator.

PI: Velasco-Gonzalez, Cruz

- **An Investigation of the Effects of Adjusting Intensities from cDNA Arrays on the Assessment of Differential Gene Expression** **E0709601**

Objective(s):

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

PI: Xu, Zengjun

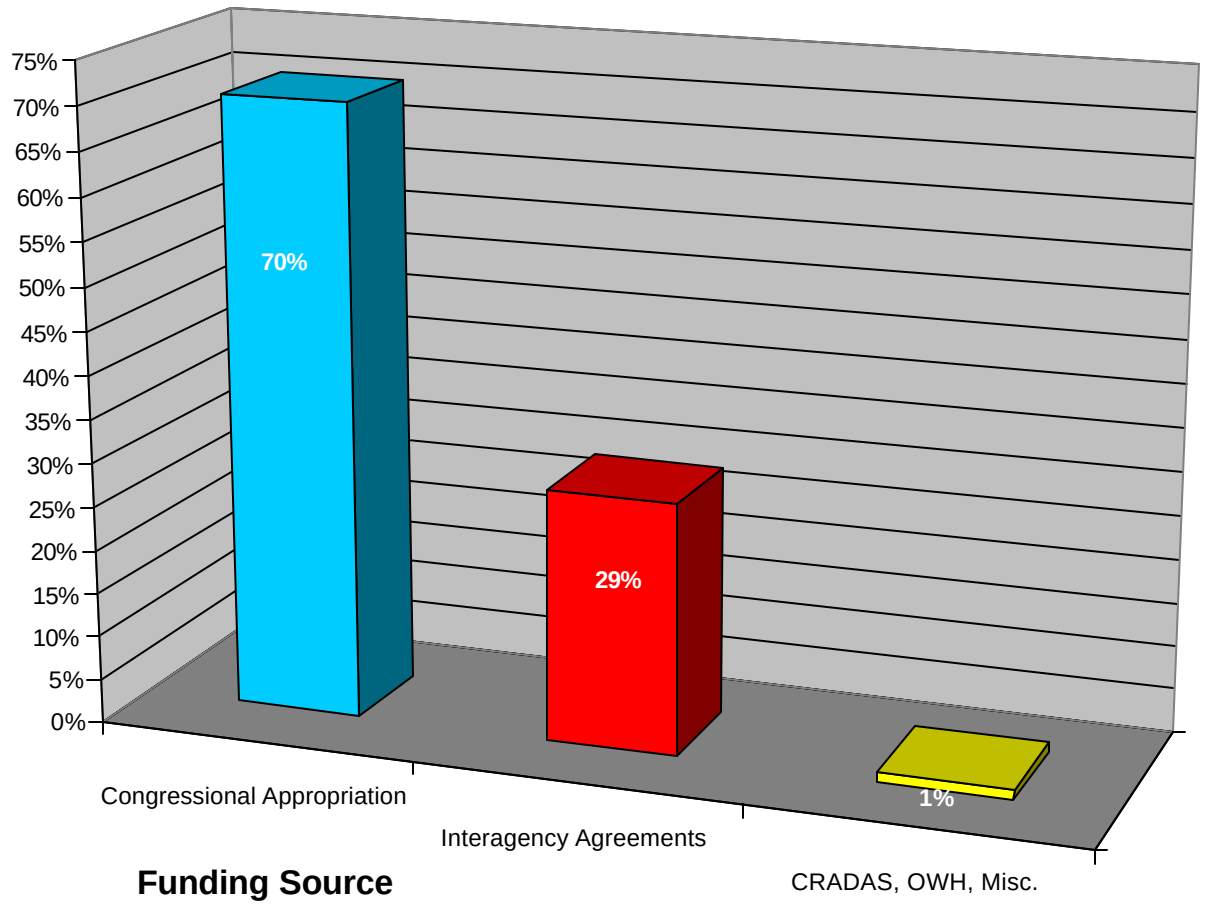
- **Adolescent Nicotine Administration Effects on CNS Serotonergic Systems** **E0709801**

Objective(s):

1) To determine whether adolescent nicotine administration elicits axonal/terminal damage in 5HT systems.
2) To determine if adolescent nicotine administration alters 5HT presynaptic activity.
3) To determine 5HT receptor and signaling activity and functions induced by adolescent nicotine exposure.
4) To determine if adolescent nicotine administration produces changes in cAMP-mediated signal transduction, 5HT metabolic enzymes and/or 5HT receptors.
5) To evaluate behaviors linked to 5HT function that have also been associated with aspects of depression.

SUMMARY OF
EXTERNALLY FUNDED PROJECTS*

RELATIVE PROPORTIONS OF NCTR BUDGET



*Details of projects presented under individual research division reports.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

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).

With financial support from the National Toxicology Program (NTP), which is conducted under the auspices of the NIEHS, the NCTR has agreed to conduct animal bioassays, mechanistic studies, and risk assessments on a number of compounds of regulatory interest to both the NIEHS and the FDA. This IAG has allowed NCTR to conduct studies including: a mycotoxin, fumonisin B_1, a study nominated by the FDA Center for Food Safety and Applied Nutrition (CFSAN); the pediatric sedative, chloral hydrate, nominated by FDA's Center for Drug Evaluation and Research (CDER); malachite green, a therapeutic agent used in aquaculture, nominated by FDA's Center for Veterinary Medicine (CVM); and the interaction of ethanol and urethane, nominated by CFSAN. Also, a mechanistic study on riddelliine, a compound of interest to CFSAN, is being supported by the FDA NIEHS IAG. Studies are also beginning on the risk associated with *Aloe vera* exposure in dietary supplements.

Additional research funded via the FDA NIEHS IAG includes a series of studies on several endocrine-active compounds including genistein, ethinyl estradiol, and nonylphenol. The studies will determine the endocrine-disrupting effects of these compounds on reproduction, behavior, and carcinogenesis over multiple generations.

As a result of CFSAN's concern about the potential interaction of ultraviolet (UV) light and over-the-counter cosmetics containing alpha- or beta-hydroxy acids, support for development of a unique Phototoxicology Research and Testing Laboratory at the NCTR was received from NIEHS/NTP. Risk assessments on a number of FDA-regulated products suspected of interaction with sunlight or fluorescent tube-generated light began in FY 1999.

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.

As an offshoot of a patent and licensing agreement with Cox Recorders dealing with the detection of decomposed food, the Federal Aviation Administration (FAA) has entered into an agreement with scientists at the Center to explore methods of detecting explosives in airline baggage.

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: 1) the development of methodologies to assay hydroxylation of endogenous estrogens as that process relates to the risk of developing breast cancer; 2) research on the effects of dietary supplements on women's health issues; and 3) research to 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)

Cox Recorders of Belmont, NC, continues their efforts to market a consumer-based, low-tech indicator of food freshness patented as Fresh Tag™ which was developed in collaboration with scientists in the Division of Chemistry at NCTR. These scientists are currently making modifications to Fresh Tag™ technologies based on interactions with the Canadian Centre for Fisheries Innovation.

NCTR has received support via a CRADA with Genometrix® to develop a "Risk-Tox DNA micro-array" for rapid, high-throughput genotyping. The results of this CRADA will provide the foundation for FDA to genotype patients for all the major enzyme variants that would predict susceptibility to carcinogens, adverse drug reactions, chemotherapeutic drug efficacy, and individualized dosing of therapeutics.

Both the American Chemistry Council (ACC), formerly the Chemical Manufacturers Association (CMA), and the Environmental Protection Agency (EPA) have provided NCTR support for the development of a computerized predictive Estrogen Knowledge Base (EKB). Using Quantitative Structure-Activity Relationships (QSAR), the EKB will be able to screen chemical structures for estrogen activity. The EKB will also serve as a prototype for predicting activity of chemical classes such as androgens and thyroid hormones, and may be applied to other toxic endpoints such as neurotoxicity and carcinogenesis.

NCTR's Division of Neurotoxicology has 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.

A CRADA with the Arizona Cancer Research Center, University of Arizona, is supporting research into the relationship of dietary habits and colon cancer. Specifically, the objectives of this research are to explore the relationship between dietary isothiocyanates, glutathione S-transferase induction, and colon polyp recurrence.

It has been determined that animals exposed to cocaine during gestation fail to alter their behavior in response to important changes in their environment (i.e., to adapt). Researchers at the University of Arkansas at Little Rock and in the Division of Neurotoxicology at NCTR are expanding upon these findings by examining additional aspects of behavioral plasticity/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 staff 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.

Another example of leveraging with local institutions is that NCTR staff in the Division of Neurotoxicology have access to a behavioral testing laboratory at the Arkansas Children's Hospital (ACH) and at the University of Arkansas at Little Rock, where results of behavioral studies obtained in animals at NCTR are verified in humans at ACH.

Collaborations by NCTR scientists 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, in FY 2001-2002, 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.

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Glossary of Acronyms and Abbreviations

This glossary is provided to assist you in interpreting acronyms, abbreviations, and phrases you encounter while reading this publication. This is not meant to take the place of standard language or Scientific dictionaries, which should be referred to if any short form of scientific terms do not appear in this glossary. Also, you may refer to the Chemical Index, located at the end of this publication as a quick reference to locate other occurrences of a specific term.

1D or 1-D	one dimensional
^{1}HNMR	Proton Nuclear Magnetic Resonance
2-AAF	2-acetylaminofluorene
2D or 2-D	two dimensional
3D or 3-D	three dimensional
3-HIA	3-hydroxyisovaleric acid
3TC	lamivudine
4-ABP	4-aminobiphenyl
8-MOP	8-methoxypsoralen
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care, International
AACR	American Association for Cancer Research
AALAS	American Association for Laboratory Animal Science
ACB	allele-specific competitive blocker
ACC	American Chemistry Council (ACC), formerly Chemical Manufacturers Association (CMA)
ACD	Advanced Chemistry Development
ACH	Arkansas Children's Hospital
ACPS	Advisory Committee for Pharmaceutical Science
ADHD	attention deficit hyperactivity disorder
ADP	automatic data processing
AIDS	acquired immunodeficiency syndrome
AIN	American Institute of Nutrition
ANN	artificial neural networks
AP-1	apurinic-1
APAP	acetaminophen
APCI/MS	atomospheric pressure chemical ionization/mass spectrometry
AR	androgen receptor
Ars	accumulation rates
ASMS	American Society of Mass Spectrometry
AZT	Zidovudine
BD IX	rat strain
BELLE	biological effects of low level exposures
BF	body fat

BHA	2(3)-tert-butyl-4-hydroxyanisole
BM	bleomycin
BSE	bovine spongiform encephalopathy
cAMP	cyclic adenosine monophosphate
CBER	Center for Biologics Evaluation and Research, FDA
CD	Sprague-Dawley
CD	cadaverine
CDER	Center for Drug Evaluation and Research, FDA
cDNA	complementary DNA
CDRH	Center for Devices and Radiological Health, FDA
CE	competitive exclusion
CFSAN	Center for Food Safety and Applied Nutrition, FDA
CG	cytosine guanine
CHO	Chinese hamster ovary
CIMA	Computational Intelligence, Methods and Applications
CMA	Chemical Manufacturers Association
CNS	central nervous system
CoMFA	comparative molecular field analysis
COMT	catecholamine-o-methyltransferase
Co-PI	Co-Principal Investigator
C_pG	cytosine-phosphate-guanine
CR	caloric restriction
CRADA	Cooperative Research and Development Agreement
CRIMS	chemical reaction interface mass spectrometry
CVM	Center for Veterinary Medicine, FDA
d4T	Stavudine
DAS	diallyl sulfide
ddC	Zalcitabane
DdC	2',3'-dideoxycytidine
ddI	Dideoxyinosine
DD-PCR	differential display PCR
DGRT	Division of Genetic and Reproductive Toxicology
DHHS	Department of Health and Human Services
DHR	Dehydroretronecine
DMA	Diamethylamine
DMBA	Dimethylbenz(a)anthracene
DMN	Dimethylnitrosamine
DNA	Deoxyribonucleic acid
DOD	Department of Defense
DR	dietary restriction
ECD	Electrochemical detection

ECVAM	European Centre for the Validation of Alternative Methods
EDKB	Endocrine Disruptor Knowledge Base
EE2	ethinyl estradiol
EEG	Electroencephalogram
EEMS	European Environmental Mutagen Society
EGFR	epidermal growth factor receptor
EI	electron impact
EKB	Estrogen Knowledge Base
EM	Expectation Maximization
EMS	Environmental Mutagenesis Society
ENAR	Eastern North American Region
ENC	Experimental NMR Conference
ENU	ethyl nitrosourea
EPA	Environmental Protection Agency
ER	estrogen receptor
FAA	Federal Aviation Administration
FDA	Food and Drug Administration
FFA	free fatty acids
FFM	fat free mass
FISH	Florescent NC2 hybridization
FT	Fourier transform
FTMS	Fourier transform mass spectometer
FY	Fiscal year
GAT	Guanine adenine thymidine
GB	Ginkgo biloba
GBS	Guilliane Barr Syndrome
GCRC	General Clinical Research Center
GC-TI	gas chromatography theroionic detector
GD	Gestational day
GGT	guanine guanine thymidine
GLP	Good Laboratory Practice
GS	Goldenseal
GSA	Genotypic selection assay
GSH	Gluthion
GST	Glutathione S-transferase
GSTP1	Glutathione S-transferase P1
GTT	guanine thymidine thymidine
HAAs	Heterocyclic aromatic amines
HaCaT	Keratinocyte cell line
HGSTAl	human glutathione S-transferase Al
hGSTM1	human glutathione S-transferase M1

hGSTT1	human glutathione S-transferase T1
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
HPLC/ECD	high performance liquid chromatography/electrochemical detection
HPLC-ES	high performance liquid chromatography electrospray
HRMS	high resolution mass spectrometry
HS	Histamine
IA/LC/MS	Immuno affinity/liquid chromatography/mass spectrometry
IACUC	Institutional Animal Care and Use Committee
IAG	Interagency agreement
IAOAC	International Association of Official Analytical Chemists
IARC	International Agency for Cancer Research
IC-50	Inhibition curve-50
ICCVAM	Interagency Coordinating Committee for Validation of Alternative Methods
IGF-1	insulin growth factor-1
IgG	immune sera
ILSI	International Life Sciences Institute
ILSI/HESI	International Life Sciences Institute / ILSI Health and Environmental Sciences Institute
IM	Intramuscular
INTOX	Interdisciplinary Toxicology Program
IRB	Institutional Review Board
IV	intravenous
KO	knockout
LC	liquid chromatography
LC/MS	liquid chromatography-mass spectrometry
LC/PDA	liquid chromatography/photo diode array
LC-APCI/MS	liquid chromatography with atmospheric pressure chemical ionization mass spectrometry
LC-ESI/MS	liquid chromatography-electron spray ionization/mass sprectroscopy
LIMS	Laboratory Information Management System
LLNA	Local Lymph Node Assay
LOH	loss of heterozygosity
LPS	Lipopolysaccharide
LRRI	Lovelace Respiratory Research Institute
M&R	metabolic and repair
MAB/MS	metastable atom bombardment/mass spectrometry
MALDI	Matrix-assisted laser desorption/ionization
MALDI FT	Matrix-assisted laser desorption fourier transform
MALDI/MS	Matrix-assisted laser desorption-mass spectrometry

MALDI-ESI-QTof	Matrix-assisted laser desorption ionization electrospray ionization quadrapole time of flight
MALDI-FTMS	Matrix-assisted laser desorption ionization Fourier transform mass spectrometer
MALDI-TOF MS	matrix assisted laser desorption ionization time-of-flight mass spectrometry
MAT	methionine adenosyltransferase
MD	methyl deficiency
MDMA	ecstasy
MeIQx	2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline
MFs	mutant frequencies
MHz	megahertz
mm	millimeters
MMRRC	Mutant Mouse Regional Resource Center
MR	magnetic resonance
MRI	Magnetic Resonance Imaging
mRNA	messenger RNA
MRS	brain magnetic resonance spectra
MS	mass spectrometry
MS	methionine synthase
MSc	Master of Science
MTHFR	methylene-tetrahydrofolate reductase
NA	not applicable
NASA	National Aeronautics and Space Administration
NCCRI	National Cancer Center Research Institute, Japan
NCI	National Cancer Institute
NCI-MMHCC	National Cancer Institute Mouse Models of Human Cancers Consortium
NCTR	National Center for Toxicological Research, FDA
NIA	National Institute on Aging
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NMDA	n-methyl-d-aspartate
NMR	nuclear magnetic resonance
NMU	nitroso methylurea
NOAA	National Oceanic and Atmospheric Administration
N-OH-IQ	n-hydroxy-2-amino-3-methylimidazo[4,5-f]quinoline
NONMEN	nonlinear mixed-effects modelling
NOS	nitric oxide synthase
NRC	Nuclear Regulatory Commission
NTF	neurogrowth/neurotrophic factor

NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
Oltipraz	5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione
ORA	Office of Regulatory Affairs, FDA
ORISE	Oak Ridge Institute for Science and Education
OTA	Ochratoxin A
OTB	operant test battery
OTC	Oxytetracycline
OWH	Office of Women's Health, FDA
PAH	polycyclic aromatic hydrocarbon
PattRec	pattern recognition
PBPK	physiologically-based pharmacokinetic
PC	personal computer
PC-12	cell culture
PCA	Principal Components Analysis
PCB	polychlorinated biphenyl
PCR	Project on Caloric Restriction
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase Chain Reaction Restriction Fragment Length Polymorphism
PD-ANN	parallel distributed artificial neural network
PE	Polyethylene
PFGE	Pulse-field gel electrophoresis
PhIP	2-amino-1-methyl-6-phynelimidazo[4,5-f]pyridine
PhRMA	Pharmaceutical Research and Manufacturers of America
PI	Principal Investigator
ppb	parts per billion
PPI	proton pump inhibitor
PRS	Propyl Sulfonic Acid
PU	Putrescine
PUVA	Psoralen Ultraviolet A
PVC	polyvinyl chloride
Py-MAB-Tof-MS	pyrolysis-metastable atom bombardment-time of flight-mass spectrometry
PyMS	pyrolysis mass spectrometry
QA	Quality Assurance
QSAR	quantitative spectrometric-activity relationship
QSDAR	quantitative spectrometric data-activity relationship
RBA	relative binding affinity
RBC	red blood cells
RFD	rank fish detector
RFLP	Restriction Fragment Length Polymorphism

RNA	ribononucleic acid
Romet-30	sulfadimethoxine-ormetoprim
ROS	reactive oxygen species
RT-PCR	reverse transcriptase – polymerase chain reaction
SA	sulfonamide
SAB	Science Advisory Board
SACATM	Scientific Advisory Committee for the Validation of Alternative Methods
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SAR	Structure Activity Relationship
SAR-SDAR	Structure Activity Relationship-Spectral Data-Activity Relationships
SCR	sample collection report
SD	Sprague Dawley
SDAR	spectral data-activity relationship
SDH	succinate dehydrogenase
SDN	sexually dimorphic hypothalmic nuclei
SFC	supercritical fluid chromatography
SHR	spontaneously hypertensive rat
SNARC	Stuttgart National Aquaculture Research Center, USDA
SNP	single nucleotide polymorphism
SOT	Society of Toxicology
SPE	solid phase extraction
SQ	subcutaneous
SRTR	Scientific Registry of Transplant Recipients
SSCP	single-strand conformation polymorphism
SWEG	Spacecraft Water Exposure Guidelines
TBW	total body water
TCR	transcription coupled repair
TEF	toxic equivalent factors
TER	Transcutaneous Electrical Resistance
TGE	total gestational exposure
TLC	thin layer chromatography
TMA	Trimethylamine
TOBEC	total body electrical conductivity
TOF	time of flight
TSNAs	tobacco specific N-nitrosamines
TSSRC	Toxicology Study Selection and Review Committee
TVB	total volatile bases
UAF	University of Arkansas at Fayetteville
UALR	University of Arkansas at Little Rock
UAMS	University of Arkansas for Medical Sciences

UDP	up-and-down procedure
UK	United Kingdom
Urethane	ethyl carbamate
US or U.S.	United States
USDA	United States Department of Agriculture
UV	Ultraviolet
UVA or UVB	ultraviolet (A or B indicates the region)
VA	John M. McClellan Memorial Veterans Hospital
VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products