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Preface

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

The NCTR conducts FDA mission-critical, peer-reviewed, critical path (translational) research that is targeted to develop a scientifically sound basis for regulatory decisions and reduce risks associated with FDA-regulated products. This research is aimed at evaluating the biological effects of potentially toxic chemicals or microorganisms, defining the complex mechanisms that govern their toxicity, understanding critical biological events in the expression of toxicity, and developing methods to improve assessment of human exposure, susceptibility, and risk. The NCTR's research efforts are primarily directed at supporting the FDA Strategic Goal Framework by implementing the objectives of Strategic Goal 1, that is, to "Increase access to innovative technologies to advance health", and Strategic Goal 2, "Improve product quality and safety through better manufacturing oversight."

Customized bioassessment of chemicals of vital interest to the FDA involves the coordination of expertise in the areas of biochemical and molecular markers of carcinogenicity, quantitative risk assessment, transgenics (mimicking responses in animal models by insertion or ablation of toxicologically relevant genes into a test animal or tissue culture), neurotoxicology, microbiology, chemistry, and genetic or reproductive/developmental toxicology. Using its existing strengths in methods development, statistics, analytical chemistry, and spectroscopy, NCTR is developing and standardizing new technologies, such as genomics, proteomics, metabonomics, and nanotechnology to identify and characterize early biomarkers of toxicity in our traditional toxicological and animal models. In addition, NCTR is using toxicoinformatics (data collection, interpretation, and storage of information about gene and protein expression) to manage and integrate data from these new technologies with traditional toxicological data to provide a basis for better predictive toxicology. Application of these new tools in animal surrogates will provide us with mechanistic biomarkers that will have more relevance for extrapolation of risk to humans; provide a better understanding of the present models used to assess risk in humans; and direct the development of more useful surrogate models that will increase our understanding of toxic responses in humans.

A significant contribution to our research accomplishments is the benefit gained by sharing knowledge through collaborations with scientific staff

in all disciplines in other FDA Centers as well as in other government agencies, academia, and industry. One such example is the use of ArrayTrack™, a software tool developed at the NCTR to store and analyze and interpret DNA microarray data. This tool is being used by the Center for Drug Evaluation and Research (CDER) in assessing pharmacogenomic data voluntarily submitted by the regulated industry. This collaboration is one that identifies the FDA as a catalyst in the development of new standards that will facilitate drug development for the promotion and protection of the public health. ArrayTrack™ is also being considered as a useful regulatory tool for use by other agencies, such as the Environmental Protection Agency. In addition to methods and standards development, the NCTR conducts translational and applied research on compounds nominated by the FDA for evaluation by the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP). All of the studies conducted at the NCTR are intimately associated with Secretary Mike Leavitt's goals of transforming health care, advancing medical research, and securing the homeland. The NCTR views its public health role as a key element in the development and modification of toxicology safety standards through the application of innovative scientific research.

Daniel A. Casciano, Ph.D.
Director, NCTR, 1999–2005

NCTR: A Key Contributor to FDA's Strategic Goals

The FDA is committed to improving public health through increased access to innovative products and technologies. The FDA is also committed to increasing the number of approved safe and effective products—including those for unmet medical and public health needs including emerging infectious diseases, and counterterrorism—by increasing the predictability, efficiency, and effectiveness of product development. These commitments represent two of FDA's strategic goals and lay the foundation for NCTR's research efforts.

The NCTR provides the FDA with the tools and expertise to: 1) develop, validate, and provide guidance for the use of new technologies and integrative approaches for product safety and efficacy; 2) promote the scientific processes moving us toward personalized medicine; 3) provide key research data for high priority safety issues; and 4) provide research for food safety and food defense. NCTR scientists conduct fundamental and innovative laboratory research and the application of the knowledgebase that is generated by these activities serves to solve important issues and advance the regulatory review process. Human subjects and tissues, as well as appropriately selected whole and *in vitro* animal models, are routinely employed.

NCTR is an FDA resource for the conduct of research involving various animal models from rodents to nonhuman primates. NCTR has the facilities, equipment, and expertise to actively support NCTR's vital interdisciplinary work. The NCTR Laboratory Animal Care and Use Program has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International since 1977. This distinction assures the Center's scientists, the FDA, and the American consumer that data generated from animal experiments at NCTR are of the highest quality. NCTR has recently constructed a BSL-3 containment facility in response to the national need for additional capability to conduct food safety and food defense research.

New Technologies and Integrative Approaches for Product Safety and Efficacy

A major focus of NCTR research involves the development of new and innovative technologies and approaches that support the regulatory centers and, in particular, the Critical Path Initiative, which is "FDA's effort to stimulate and facilitate a national effort to modernize the scientific process through which a potential human drug, biological product, or medical device is transformed from a discovery or "proof of concept" into a medical product."¹ This initiative is often called the Critical Path to New Medical Products.

¹ <http://www.fda.gov/oc/initiatives/criticalpath/initiative.html>

NCTR is using new OMICs technologies—those developed via genomics, proteomics, metabolomics, and informatics—in combination with more traditional approaches, to address various public health related research questions. Research is focused on the optimization, integration, and utility of OMICs technologies, with an emphasis on microarray performance and analysis, proteomic tool development and application, and metabolomics. Work in each of these areas has been applied to problems in preclinical development and is being assessed for use in clinical situations.

While current technologies in the fields of carcinogenesis, genetic toxicology, neurotoxicology, hepatotoxicology, microbiology, and reproductive/developmental toxicology generally evaluate single adverse health endpoints, the OMICs technologies, in combination with other functional and structural endpoints, are providing opportunities for simultaneously detecting alterations in multiple health endpoints. NCTR is developing a strategy in which these new approaches to evaluating toxicity will allow for the integration of information across various types of adverse health outcomes. For instance, when these technologies are fully developed, it will be possible to concurrently evaluate chemicals for their ability to cause cancer, to impact the nervous system, to cause birth defects, to cause hepatotoxicity, and to modify immune function.

NCTR scientists play an integral role in developing, standardizing, and integrating microarray and other OMICs data. They have developed ArrayTrack™, an integrated solution for managing, analyzing, and interpreting microarray data, which is currently used as a tool for CDER reviewers. ArrayTrack™ is under continuous development and is currently being enhanced to accept both proteomic and microarray data. Metabolomics group members have been at the forefront in discussions on standards development in this rapidly evolving field. They are working to develop biomarkers for hepatic, renal, and cardiovascular toxicity that might bridge preclinical and clinical tests. The Proteomics group is developing an understanding of its applicability to questions in toxicology, which will lay the basis for future best practices. NCTR scientists were essential partners in the initial Voluntary Genomic Data Submission data analyses. Staff have been involved in other aspects of standardization of microarray technology. Specifically, the MicroArray Quality Control project catapulted NCTR/FDA into front line discussions and contributions toward standardization of microarray use. The existence of an internal expert OMICs panel at NCTR positions the NCTR to play a defining role in the integration of OMICs data into review processes.

Research Leading to Personalized Medicine

Modern evaluation tools will give clinicians the best available information about how to use a product to maximize its benefit and minimize its side effects. Many of these new technologies may help individualize treatment by identifying individuals likely to respond well to specific treatments.

NCTR research on classification algorithms provides the fundamental basis for making personalized medicine a reality by enabling the assignment of therapies to patients to maximize efficacy and minimize toxicity. These algorithms can combine diverse, high-dimensional biomarkers to achieve increased accuracy in predicting which patients will benefit most from medical products and which are most likely to experience toxicities that might outweigh treatment benefits. Research on probabilistic techniques minimizing uncertainty in risk assessment provides risk managers with tools for ensuring adequate public health protection, including protection of sensitive populations. Research on physiologically-based pharmacokinetic/pharmacodynamic models is providing software that enables risk assessors and managers to make scientifically sound decisions based on data from animal studies to characterize the risk to humans of FDA-regulated products. Research on methods and algorithms to analyze and interpret data from genomic experiments can help to establish new high-dimensional genomic biomarkers of risk, disease, and treatment effects.

NCTR researchers envision that applications utilizing gene diversity can be used to define disease susceptibility and predict drug-induced adverse events. They have identified human study populations with specific treatment, toxicity, and outcomes data for breast cancer, colorectal cancer, and multiple myeloma for exploring individualized approaches to cancer therapy. The hypothesis being tested in these human studies is that common genetic polymorphisms of enzymes involved in the metabolism of chemotherapy drugs modify toxicity, cancer survival, and treatment efficacy. The anticipated benefit of this work is the identification of subpopulations that respond favorably to specific drug therapies.

NCTR scientists are determining the impact of selected genetic polymorphisms on colorectal cancer risk, prognosis, and efficacy of treatment, using tumor tissue sections and records of treatment received and outcome from retrospectively identified colorectal cancer cases. Scientists are genotyping DNA isolated from tumor tissue to identify polymorphisms of drug metabolizing enzymes and then evaluating the association between toxicity, cancer survival, and these polymorphisms.

NCTR scientists are developing an *in silico* (modeled on a computer) description of hepatotoxicity. In conjunction with scientists from CDER, they have proposed an innovative clinical trial design of a prospective safety study to develop biomarkers of liver toxicity should they arise in clinical development. It is hypothesized that these biomarkers can then be applied prospectively to predict patients at risk. In addition, scientists are in the process of developing preclinical biomarkers of liver toxicity to determine OMICs biomarker signatures of compounds with a known clinical toxicity profile.

Key Research for High Priority Safety Issues

A significant portion of NCTR research is focused on addressing specific high priority issues. NCTR scientists work collaboratively with individuals in other FDA Centers and in the scientific community to develop research strategies that assist in defining adverse health outcomes and modes-of-action for chemically- or pharmaceutically-induced diseases such as cancer and neurological effects. This research is an integral part of the research supported by the National Institutes of Environmental Health Sciences/National Toxicology Program (NTP)/ NCTR Interagency Agreement (IAG). This IAG provides the mechanism for comprehensive, stakeholder-designed research programs that evaluate FDA-nominated chemicals and pharmaceuticals.

For example, studies on malachite green, an antifungal drug used illegally in fish aquaculture, are being completed. As part of a complete toxicological assessment, the investigators demonstrated that malachite green caused liver tumors in rodents. The Center for Veterinary Medicine used these data in enforcement activities and in establishing residue hazard levels for unapproved animal drugs. A toxicological evaluation of urethane, a natural by-product of fermentation that occurs in the presence of alcohol, was completed. These data were furnished to the Center for Food Safety and Applied Nutrition to develop a risk assessment on urethane and have been used by a recent Food and Agriculture Organization of the United Nations and the World Health Organization Joint Expert Committee on Food Additives review of urethane. Other experiments have focused on α - and β -hydroxy acids, substances added to a large percentage of cosmetic formulations to cause a restructuring of the skin and eliminate fine wrinkles. The results of this study, which have been furnished to CFSAN, indicate that there is no increase in sunlight-induced skin cancer in mice by either α - or β -hydroxy acid. Ongoing assessments include: 1) acrylamide, a known rodent carcinogen and a neurotoxicant that was recently identified in baked and fried starchy foods, notably french fries, potato chips, bread, coffee, and many other consumer food products; 2) *Aloe vera*, a natural product that is incorporated into commercial skin care products and dietary supplements; 3) antiretroviral drugs that are administered to pregnant women and their infants; and 4) permanent make-up and tattoos that are being used by an increasing proportion of the U.S. population.

NCTR scientists have developed noninvasive methods for monitoring biomarkers of adverse drug effects on Central Nervous System (CNS) function, primary events that frequently result in the failure of drugs during clinical trials. The ultimate goal of these efforts has been to develop techniques for the routine monitoring of drug effects on brain function using animal (preclinical) surrogates. Towards this end, the nonhuman primate has been shown to be an excellent predictor of CNS drug effects in humans. The behavioral instrument developed for the

assessment of complex brain function, now known widely as the NCTR Operant Test Battery (OTB), has proven successful not only in the animal laboratory, but also in the clinical setting—primarily in children—where it has been proven to be sensitive not only in differentiating clinical syndromes, such as Attention Deficit Hyperactivity Disorder, but also in detecting the efficacy of drug treatment. In addition, OTB performance correlates significantly with IQ scores in children, further demonstrating relevance. Since this kind of assessment can be performed repeatedly in the same subjects, is noninvasive, is cost-effective and can be performed in laboratory animal surrogates (rodents through nonhuman primates), the utility of this approach becomes more evident daily. Ongoing studies are examining the ability of the OTB to identify specific cognitive profiles in children with anxiety, depression, and childhood abuse. Thus, Critical Path Initiatives concerning biomarkers, assessment of depression, pediatrics, preclinical models, and human surrogates are being directly assessed in these efforts. Currently, our nonhuman primate model is being used to directly assess the consequences of pediatric anesthetic procedures that occur during critical periods of brain development.

NCTR researchers are determining the extent to which the disruption of monoaminergic and glutamatergic neurotransmitter systems, mitochondrial function and alterations in oxidative stress are involved in the progression of Parkinson's and other nervous system diseases. Post-mortem brains of Parkinson's disease subjects and protein extracts from the brains of our Parkinson's disease mouse model will be used to measure post-translational protein modifications which will be verified and quantified via mass spectrometry.

Research for Food Safety and Food Defense

NCTR scientists provide guidance and expert advice to FDA, other national regulatory agencies and the World Health Organization on the potential human health risks associated with the use of antimicrobial agents and competitive exclusion products used in animal husbandry. In addition, investigators developed a risk assessment approach in the safety evaluations of antimicrobial drug residues in food. The Center for Veterinary Medicine and the Food and Agriculture Organization of the United Nations and the World Health Organization Joint Expert Committee on Food Additives use this approach in evaluating data to determine the impact of veterinary drug residues in the food and the human intestinal microflora.

In the areas of counterterrorism and food defense, NCTR scientists plan to collaborate with scientists from other FDA Centers and several federal and state agencies to detect microbial hazards from the intentional contamination of our food supply. Researchers are developing diagnostic microarray gene chips and other rapid molecular techniques for the simultaneous detection of multiple foodborne pathogens and antimicrobial

resistance genes. The food safety research project highlights the impact of preharvest control of foodborne pathogens as the necessary first step in the “farm-to-the-fork” continuum. This research can help poultry producers and regulatory agencies design hazard analysis and critical control point protocols to safeguard fresh poultry against pathogen contamination during processing. NCTR researchers have also developed a proposal to study the metabolism of third-generation cephalosporins by intestinal bacteria. Understanding the interaction between cephalosporins and intestinal microflora will help the FDA evaluate the safety of these antibiotics and further understand the potential for development of antimicrobial resistance.

NCTR researchers, by working in integrated and multidisciplinary teams, will continue to provide the necessary data for science-based decision making. Frequent interactions and collaborations with fellow researchers and regulators in sister centers and the Office of Regulatory Affairs will continue to guide the research agenda. Often the leveraging of resources, both intellectual and capital, is necessary to achieve the desired goal of improving public health. The development of innovative technologies and best practices will continue to allow NCTR scientists to increase the predictability and safety of FDA-regulated product development.

William Slikker, Jr., Ph.D.
Acting Director, NCTR

NCTR: An Internationally Recognized Resource

Established by executive order in 1971, the National Center for Toxicological Research (NCTR) is internationally recognized for research that addresses the mechanisms of toxicity of chemicals and pharmaceutical drugs, defines the risks associated with chemical and microbial food contamination, and identifies biomarkers for terrorism due to biological and/or chemical exposure.

The NCTR Research Divisions are committed to the study of biochemical and molecular markers of cancer, nutritional modulation of risk and toxicity, developmental toxicity, neurotoxicity, quantitative risk assessment, transgenics, applied and environmental microbiology, and solid-state toxicity. Each division works closely with the others in a seamless effort to support the FDA's mission to bring safe and efficacious products to the market rapidly and to reduce the risk of adverse health effects from products on the market.

- Biometry and Risk Assessment (DBRA)
- Biochemical Toxicology (DBT)
- Genetic and Reproductive Toxicology (DGRT)
- Microbiology (DM)
- Pharmacogenomics and Molecular Epidemiology (DPME)
- Neurotoxicology (DNT)
- Systems Toxicology (DST)
- Veterinary Services (DVS)

Science Advisory Board

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.

Chair: Dr. Daniel Acosta, Jr.
Dean, College of Pharmacy
University of Cincinnati
Expertise: Pharmacology and Toxicology

Members: Dr. Nancy Ann Gillett
Sr. Vice-President, Sierra Biomedical
Charles River Laboratories
Expertise: Veterinary Medicine and Pathology

Dr. Alberto Luis Rivera-Rentas
Dean, School of Environmental Affairs

Ana G. Mendez University System
Expertise: Neurobiology and Electrophysiology

Dr. Paul J. Catalano
Associate Professor of Biostatistics
Harvard School of Public Health
Expertise: Biostatistics

Core Capabilities

Internationally Recognized Staff

The NCTR staff includes 110 Ph.D. scientists representing a wide array of scientific expertise. The program is supported by approximately 400 support scientists, on-site contractors, and administrative staff. Many of the senior staff members have more than twenty-five years experience conducting multidisciplinary public health research. Undergraduate and graduate students, post-doctoral fellows, and visiting scientists come to NCTR to learn new technologies and scientific disciplines and to contribute their skills and perspectives to the NCTR research program.

Secure Research Campus

Located adjacent to the Pine Bluff Arsenal in Jefferson, Arkansas, the Jefferson Laboratories of the FDA resides on 500 acres. Jefferson Laboratories of the FDA is home to, and managed by, the staff of the NCTR and also houses the Office of Regulatory Affairs' Arkansas Regional Laboratory (ARL). The facility consists of 30 buildings with approximately one million square feet of floor space and \$20 million in capital equipment.

AAALAC Accredited Animal Research Facility

NCTR is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). NCTR's policies and procedures on animal husbandry, veterinary care, and the physical plant promote animal well-being and enhance the scientific research. NCTR maintains its own breeding colony, has specific pathogen free (SPF) barrier facilities, and a unique dietary preparation facility. There are both general purpose and high containment laboratories.

While the majority of animal research involves rodents, other species (including nonhuman primates) are available or can be obtained and accommodated to meet the goals of specific research projects. The NCTR nonhuman primate research center is a unique resource that is available for collaborative projects.

GLP-Compliant Research

NCTR conducts large scale, Good Laboratory Practice (GLP)-compliant whole animal research studies. The Quality Assurance group provides knowledgeable oversight of experimental studies and assures that the data generated in these studies can be utilized for regulatory purposes. The

chemistry and microbiology analytical staffs have extensive expertise and experience in assuring agent purity, determining accurate dose, and monitoring animal health.

Biological Safety Laboratories (BSL)

In response to the national need for additional capability to conduct high biological hazard research, NCTR has recently completed a state-of-the-art BSL-3 containment facility. The BSL-3 facility has ten individual BSL-3 suites (~120 square feet each) equipped with biosafety cabinets, workbench, and sink. The suites are supported by a shared preparation laboratory of 300 square feet. Currently, in collaboration with the Environmental Protection Agency, NCTR is utilizing this facility to investigate an infectivity model for *cryptosporidium*.

Multidisciplinary Toxicology Research Team

NCTR is a center for toxicology research excellence. Throughout its history NCTR scientists have provided the international health community with research that addresses important public health questions. The NCTR multidisciplinary scientific team conducts research for a number of adverse health outcomes. The capability to conduct GLP and nonGLP studies for cancer, neurological diseases, birth defects, thyroid toxicity, and liver toxicity is widely recognized and utilized. NCTR is noted for its development of new technologies and new assays to evaluate adverse toxicological effects. Methods developed by NCTR scientists are incorporated into regulatory agency policies and guidance documents for industry.

NCTR capabilities include the ability to develop new research standards for and to conduct:

- Mechanistically-based rodent cancer bioassays;
- Multigenerational developmental studies;
- *In vitro* and *in vivo* studies to assess the ability of chemicals to induce mutations;
- Teratology studies;
- Studies to identify genetic variants that make individuals more susceptible to disease or more susceptible to the adverse effects of chemical exposure;
- Neuropathological, neurophysiological, and neurobehavioral assessments;
- Physiological responses; and
- Organ specific toxicities, such as liver and thyroid.

Based upon the specific health issue, NCTR can assemble a team of scientists with the appropriate cross-discipline toxicological expertise to design, conduct, and communicate research for use in making regulatory decisions.

Microbiology and Chemistry Research and Support Team

The NCTR microbiology research team utilizes its extensive scientific expertise and state-of-the-art equipment to address a variety of public health issues and to provide core microbial support to assure the health of the NCTR animal colonies. Research is focused on developing the methods and the capabilities to:

- Detect foodborne pathogens (both naturally occurring and resulting from terrorist acts);
- Evaluate antimicrobial resistance;
- Understand the relationship between gastrointestinal microbiology and host interactions;
- Use microbes for bioremediation;
- Perform microbiological surveillance; and
- Develop microorganisms as models to predict the metabolic pathways by which drugs are metabolized in mammals.

A critical component of the NCTR research capability is the outstanding chemistry expertise. This expertise is applied to both routine chemical analysis for compound composition and stability and to the development of new technologies that can be applied to the detection of pathogen and chemical contaminants, both naturally occurring and applied as a terrorist attack. The chemistry staff, using analytical mass spectrometry, can perform the quality assurance analysis that allows for the conduct of GLP-compliant research. The Counter Bioterrorism Research group has developed and is now applying rapid, reliable, and cost-effective mass spectrometric methods to identify pathogenic agents. These methods use pattern recognition-based biomarker methods to detect pathogens and make it possible to distinguish between real and hoax counterterrorist incidents. In collaboration with the University of Arkansas at Little Rock, the Nanotechnology and Sensory Technology group has developed two nanotechnology-based cancer therapies, several large scale nanoparticle production patents, and a novel nanoparticle-based filter technology to protect the public from chemical and biological contaminants. This group has also developed a sensor technology for food freshness and quality that is now under development for potential commercial application. This sensor technology, which is now being applied to the detection of NO_x (Nitrogen Oxide) and nitroaromatics and is of interest to the Federal Aviation Administration, has great potential and can be developed for the detection of other chemicals.

NTP Center for Phototoxicology

Via an Interagency Agreement with the National Institutes for Environmental Health Sciences (NIEHS), the FDA/NIEHS National Toxicology Program (NTP) Phototoxicology Research and Testing Laboratory was established at NCTR. This facility, one of only two such

facilities in the nation, can be used to expose animals, cell cultures, or chemical mixtures to simulated solar light. The light source can be adjusted to simulate specific exposure scenarios or specific geographic locations. The facility is used to study the potential toxic components in cosmetic ingredients and tattoo pigments.

Risk Assessment and Statistics

NCTR has assembled a team of internationally recognized scientists (statisticians, mathematicians, and risk assessors) who not only utilize existing statistical and risk assessment methodologies but also develop new approaches that can be applied to improve the interpretation and utilization of data for regulatory decision-making.

NCTR scientists develop classification algorithms for biomedical decision making, physiological models of internal dose for improving risk prediction, statistical methods for analyzing high-dimensional OMICs data, and probabilistic approaches for characterizing and managing uncertainty in risk assessment.

Toxicoinformatics and Computational Models

NCTR is internationally recognized for its informatic and computational modeling capabilities. Computational modeling using structural activity relationships (SAR) can be applied to large numbers of chemical classes to predict various toxicity or other biological activity outcomes. A variety of artificial intelligence-based neural net approaches to data analysis are under development. The infrastructure is in place to handle the large quantities of data generated from the new genomic, metabolomic, and proteomic (or OMICs) technologies. NCTR developed ArrayTrack™, a publicly available database of NCTR OMICs data that can not only process but can also integrate OMICs data. It has libraries that can access public toxicology data and tools for analyzing and visualizing the data. One of the visualization tools permits cross-species chromosomal mapping to assist with data interpretation, and the generation of hypotheses concerning the pathways that are perturbed following chemical exposure, and the applicability of rodent model data to humans.

Genomics

NCTR has two Centers of Excellence that address issues related to the genetic makeup of individuals (the genome). The Center for Structural Genomics (CSG) provides the tools for and addresses questions concerning individual genetic differences and the impact that these genetic differences have on the development of disease. The CSG uses molecular techniques to identify single nucleotide polymorphisms (SNPs) in humans. The CSG is developing a genome haplotype map for prostate cancer and esophageal cancer susceptibility. Using this approach, the CSG researchers can provide information that can be used to assess an

individual's risk for developing cancer based on the person's inherited genetic characteristics.

The Center for Functional Genomics (CFG) provides the tools for and conducts research to understand the genetic functional consequences following exposure to chemicals or pharmaceutical drugs. The CFG includes a microarray facility that can print customized arrays for mouse, rat, or human studies. While the majority of the arrays use oligos, the CFG can customize arrays that are optimized to address a wide variety of research questions. The CFG staff of highly experienced individuals interacts effectively with other research scientists to appropriately design, conduct, and interpret the data from microarray experiments.

NCTR scientists are developing microarray technology to identify the presence of microorganisms. This application is not only being developed for use in screening food supplies for naturally occurring pathogenic microorganisms, but also for identifying highly infectious pathogenic microorganisms intentionally released as a part of a terrorist event.

Metabolomics

The NCTR Metabolomic group develops biomarkers of toxicity and disease. The core metabolomic facility is equipped with a Bruker 600 MHz nuclear magnetic resonance (NMR) that has a cryoprobe to analyze endogenous metabolites in biological samples. Capabilities of performing mass spectrometry (MS)-based metabolomic analysis will soon be expanded with the addition of a Waters LTQ Premiere mass spectrometer. The group has initially focused on the development of biomarkers of acute toxicity that can be applied to a variety of research questions. Currently the group is evaluating the effects of renal, liver, and cardiotoxins using serum, urine, and various tissues. This technology is readily applicable to cross-species comparisons and for preclinical and clinical studies. NCTR is using Scaled-to-Maximum, Aligned, and Reduced Trajectories (SMART) analysis to map trajectories of change in physiological space to expand its ability to conduct cross-species analysis.

Proteomics

The Proteomics group includes seven highly experienced staff members dedicated to mass spectrometry-based methodologies for qualitatively and quantitatively analyzing proteins from a variety of biological matrices. With an emphasis on nano LC MS/MS with ion trap mass spectrometers, this group is currently involved in multiple protein profiling projects including rat liver mitochondria, mouse liver, virulence factors in *S. Aureus*, and the characterization of the 19S mouse proteasome. The Proteomics group is developing improved methods for serum biomarker discovery by isolating the low molecular weight peptide components interacting with abundant high molecular weight proteins.

Division of Biochemical Toxicology

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Executive Summary

Introduction

The Division of Biochemical Toxicology (DBT) 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 product centers of the Food and Drug Administration (FDA). This research centers on assessing the toxicities and carcinogenic risk associated with specific chemicals and gene-nutrient interactions and the introduction of new techniques to assess toxicities and carcinogenic risk. The risk assessment research is firmly rooted in mechanistic studies focused on the understanding of toxicological endpoints, an approach that allows greater confidence in the subsequent carcinogenic risk assessments. Research within the Division capitalizes on scientific knowledge in the areas of biochemistry, organic chemistry, analytical chemistry, cellular and molecular biology, immunology, nutritional biochemistry, and pharmacology.

FY 2005 Accomplishments

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

During FY 2005, Division investigators initiated a chronic bioassay on acrylamide, a carcinogen found in certain baked and fried foods, in response to an NTP nomination by the Center for Food Safety and Applied Nutrition (CFSAN). The bioassay includes assessing the carcinogenicity of glycidamide, a genotoxic metabolite of acrylamide. Division investigators also conducted mechanistic studies on acrylamide and glycidamide, including toxicokinetics, DNA and hemoglobin adduct dosimetry, and *in vivo* mutagenesis assays to complement the two-year

bioassay. In addition, PBPK modeling was used to link kinetic and biomarker data from animal studies with the human biomonitoring data that are available to estimate DNA damage and cancer risks in humans consuming acrylamide in the diet. In response to another NTP nomination, Division investigators began a two-year chronic bioassay on *Aloe vera*, a widely used dietary supplement. As part of the assessment of potential toxicities associated with *Aloe vera* ingestion, the expression of cyclooxygenases, alterations in DNA methylation, and the ability of *Aloe vera* polysaccharides to alter the growth of colonic bacteria to ferment nonstarch polysaccharides were examined. Division investigators also completed draft reports on multigeneration and chronic studies on genistein, a component of soy products, including soy-based infant formula and dietary supplements. Investigations were also conducted to elucidate the mechanisms for the protective effects of diets containing soy against renal toxicities elicited by *p*-nonylphenol (NP) and di(2-ethylhexyl)phthalate (DEHP). In addition, pharmacokinetic studies showed that nonnutritive constituents present in soy can modify the Phase II metabolism of isoflavones and alter the estrogenic effects of genistein, the principal isoflavone in soy, on the growth of implanted human mammary tumors in athymic mice.

An area of particular concern to the FDA, in particular CFSAN, is the potential toxicity of cosmetic ingredients due to their interaction with light. To address this concern, the NCTR, in collaboration with the NIEHS/NTP, constructed a phototoxicity facility that is located within the Division. During FY 2005, a final report was completed on the co-carcinogenic effects of simulated solar light and topically-applied α - and β -hydroxy acids, and the MultiGen phases of photocarcinogenesis studies of *Aloe vera* and retinyl palmitate were completed. More recently, experiments were initiated to assess the safety of chemicals found in tattoo inks, including those used in permanent make-up. During FY 2005, a photocarcinogenesis study was conducted with iron oxide, Pigment Orange 13, Pigment Yellow 83, Pigment Orange 36, and Pigment Red 22. As part of this effort, experiments were performed to examine the photochemical properties of specific tattoo dyes and their ability to react with DNA following metabolism. Studies were also initiated to assess the phototoxicity of nanoscale titanium dioxide, a component of certain sunscreens and other cosmetic products.

Antiretroviral drugs are being used to prevent the mother-to-child transmission of human immunodeficiency virus type 1, the virus responsible for acquired immunodeficiency syndrome (AIDS). While effective in preventing viral transmission, the long-term consequences of perinatal exposure to these drugs are presently unknown. During FY 2005, Division investigators continued bioassays to assess the effects of transplacental exposure of the antiretroviral drugs zidovudine and lamivudine in combination with nevirapine and nelfinavir. In addition, range-finding studies were conducted in which the drugs were

administered transplacentally and neonatally. Division investigators have also been measuring other endpoints (DNA incorporation, mutagenicity, and micronuclei induction) to determine the mechanisms for the adverse effects of these drugs.

Tamoxifen is an adjuvant chemotherapeutic agent for the treatment of breast cancer and a chemoprotective agent for breast cancer prevention. Despite being beneficial in regard to breast cancer, tamoxifen is known to increase the risk of endometrial cancer in women. During FY 2005, Division investigators investigated the ability of two tamoxifen analogues, toremifene and GW5638, to form DNA adducts. They also conducted experiments to establish if certain tamoxifen metabolites modify the ability of tamoxifen to form DNA adducts. In further work, possible diet-drug interactions between soy food consumption and tamoxifen efficacy were investigated in an epidemiological study of breast cancer patients.

During FY 2005, Division investigators provided cell biology support for a CFSAN-sponsored project to evaluate the thermal inactivation of ricin, a potent plant toxin, in foods. This project was a component of an Agency-wide directive to advance food defense programs relevant to bioterrorism.

A strong emphasis within the Division has been to determine whether epigenetic changes induced by carcinogens and found in tumors play a causative role in carcinogenesis or are merely a consequence of the transformed state. During FY 2005, Division investigators examined epigenetic changes (e.g., changes in DNA methylation, alterations in histone methylation, expression of histone methyltransferases) in animals administered tamoxifen and the food carcinogen PhIP.

FY 2006 Plans

In FY 2006, Division investigators will complete and defend the final NTP reports on the multigenerational reproductive and carcinogenic effects of genistein. Chronic two-year bioassays will continue on *Aloe vera*, acrylamide, and glycidamide. In addition, a newborn mouse assay will be initiated to compare the carcinogenicity of acrylamide and glycidamide. Chronic studies will begin to determine the effects of transplacental and neonatal exposure to zidovudine and lamivudine in combination with nevirapine and nelfinavir.

Investigators associated with the NCTR Center for Phototoxicology will continue to study the interaction of light with tattoo pigments. Specifically, photocarcinogenesis studies will continue on various tattoo inks using full-spectrum simulated solar light. Investigations will be initiated to determine if tattoo inks can elicit an immune response, either directly or through metabolism or photoactivation. Experiments will continue to investigate potential dermal penetration and toxic properties of nanoscale materials. Studies will also continue on the characterization of

transgenic mouse models for photocarcinogenesis, with an emphasis on the induction of cutaneous and ocular melanoma.

In collaboration with investigators at the Environmental Protection Agency, Division staff will develop HPLC methods coupled with tandem mass spectrometry for the determination of unstable DNA adducts from the ubiquitous carcinogen benzo[*a*]pyrene. These methods will then be used to assess the levels of stable and unstable DNA adducts in mice administered benzo[*a*]pyrene. As part of this effort, the levels of oxidative DNA damage will be determined. HPLC/tandem mass spectrometry methods will also be developed to assess DNA adducts arising from pyrrolizidine alkaloids that are found in herbal plants and dietary supplements.

Investigations will continue to investigate the pharmacokinetics and potential toxicities following intravenous administration of DEHP, a plasticizer found in certain medical products, to neonatal nonhuman primates. These experiments will indicate if this plasticizer poses an undue risk to infants. Pharmacokinetic models will also continue to be developed for acrylamide and glycidamide, with emphasis on rodent-to-human extrapolations. In addition, the short-term effects of acrylamide exposure on hormone levels in rats will be correlated with histopathological changes, genome expression changes in the brain and thyroid, and metabonomic markers in serum and urine. In additional studies, the pharmacokinetics of methylphenidate (Ritalin) will be determined in monkeys and mice.

Experiments will also continue to study the relationships between genetic and epigenetic changes in carcinogenesis. These studies will include analyzing the gene-expression profile, the extent of DNA damage and repair, and the expression and methylation status of imprinted genes in liver tissue of tamoxifen-treated rats.

Investigations of the prominent soy isoflavone metabolite, equol, will be continued through studies of pharmacokinetics and pharmacodynamics of implanted mammary tumor growth in mice. Bioavailability studies of soy isoflavones will be conducted using soy protein isolates, a major commodity used by the U.S. food industry.

Contribution to FDA's Strategic Goals

The majority of the Division's research is focused upon Patient and Consumer Protection. For example, during FY 2005, Division investigators completed studies on malachite green, an antifungal drug used illegally in fish aquaculture. As part of a complete toxicological assessment, the investigators demonstrated that malachite green caused liver tumors in rodents. The Center for Veterinary Medicine used these data in enforcement activities and in establishing residue hazard levels for unapproved animal drugs. The information was also used by the United

Kingdom and Asian governments in establishing aquaculture residue hazard standards. Division investigators also completed the toxicological evaluation of urethane, a natural by-product of fermentation, in the presence of alcohol. These data were published by NTP as a technical report, were furnished to CFSAN to develop a risk assessment on urethane, and have been used by a recent Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) review of urethane. Other experiments focused on α - and β -hydroxy acids, substances added to a large percentage of cosmetic formulations as keratolytic agents to cause a restructuring of the skin and eliminate fine wrinkles. The results of the study, which have been furnished to CFSAN, indicate no increase in sunlight-induced skin cancer in mice by either α - or β -hydroxy acids. Ongoing assessments include: 1) acrylamide, a known rodent carcinogen and a neurotoxicant that was recently identified in baked and fried starchy foods, notably french fries, potato chips, bread, coffee, and many other consumer food products; 2) *Aloe vera* a natural product that is incorporated into commercial skin care products and dietary supplements; 3) antiretroviral drugs that are administered to pregnant women and the babies; and 4) permanent make-up and tattoos that are being used by an increasing proportion of the U.S. population.

Ongoing Research Projects

Beland, Frederick A.	<p>Perinatal Carcinogenicity of Drug Combinations Used to Prevent Mother-to-Child Transmission of HIV (E0214111)</p> <p>Objective(s): To determine the carcinogenicity, genotoxicity, and metabolism of antiretroviral drug combinations administered to mice transplacentally, perinatally, or neonatally.</p>
Beland, Frederick A.	<p>Genotoxicity and Carcinogenicity of Acrylamide and its Metabolite, Glycidamide, in Rodents Range-Finding/Subchronic/Two-Year Chronic Carcinogenicity Studies (E0215001)</p> <p>Objective(s): To compare the carcinogenicity of acrylamide and its metabolite glycidamide in B₆C₃F₁ mice and F344 rats treated chronically for two years.</p>
Beland, Frederick A.	<p>DNA Adducts of Tamoxifen (E0701101)</p> <p>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. 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.</p>
Beland, Frederick A.	<p>Detection of DNA Adducts in Mice Treated with Benzo[a]pyrene at Low Exposure Levels (E0723701)</p> <p>Objective(s): Define dose-response curves for benzo[a]pyrene DNA adducts in the A/J mouse lung utilizing the application of HPLC-ES-MS/MS methodologies developed at NCTR.</p>
Boudreau, Mary D.	<p>Effects of <i>Aloe Vera</i> Components on Cell Proliferation and DNA Adduct Formation in SKH-1 Mice Following Simulated Solar Light Exposure (E0214001)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determine the dose-response and acute kinetics of topical exposure to <i>Aloe vera</i> 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 <i>Aloe vera</i> 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 <i>Aloe vera</i> 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 <i>Aloe vera</i> plant components following simulated solar light tumor initiation; and 5) Determine the influence of <i>Aloe vera</i> components on simulated solar light-induced tumor formations in mice.

Boudreau, Mary D.

Bioassays in the F344 Rat and the B₆C₃F₁ Mouse Administered *Aloe Vera* Plant Constituents in the Drinking Water (E0214201)

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

Chou, Ming W.

A Study of Genotoxic Mechanisms of Carcinogenic Pyrrolizidine Alkaloids and Pyrrolizidine Alkaloid *N*-Oxides (E0710401)

Objective(s):

- 1) Characterize the structures of the eight DHP-derived DNA adducts;
- 2) Study metabolism of retronecine-based pyrrolizidine alkaloids, heliotridine-based pyrrolizidine alkaloids, otonecine-based pyrrolizidine alkaloids, and pyrrolizidine alkaloid *N*-oxides by liver microsomes of F344 rats, B₆C₃F₁ mice, and humans of both sexes, and compare metabolism profiles;
- 3) Study the DNA adduct formation *in vitro* (from liver microsomal metabolism of the pyrrolizidine alkaloids described above in the presence of calf thymus DNA) and *in vivo* and determine whether or not the same set of DHP-derived DNA adducts is formed;
- 4) Determine whether or not the levels of DHP-derived DNA adducts from different types of necine-based pyrrolizidine alkaloids formed in target tissues (liver) are significantly higher than those in nontarget tissues;
- 5) Determine whether or not pyrrolizidine alkaloid *N*-oxides can be metabolized by rat and mouse liver microsomes to the parent pyrrolizidine alkaloids and whether or not DHP-derived DNA adducts are formed in significant amounts both *in vivo* and *in vitro*;
- 6) Determine whether or not some dietary supplements sold in the United States contain genotoxic pyrrolizidine alkaloids;
- 7) Determine the effect of liver carboxyesterases on DHP-derived DNA adduct formation from rat and human liver microsomal metabolism in the presence of calf thymus DNA;
- 8) Determine the effect of liver carboxyesterase inhibitors on DHP-derived DNA adduct formation from rat and human liver microsomal metabolism in the presence of calf thymus DNA; and
- 9) Determine the effect of Chinese herbs, such as liquorice, and their active components, such as glycyrrhizin and glycyrrhetic acid, on inhibition of DHP-derived DNA adduct formation *in vivo* and *in vitro*.

Delclos, K. Barry

Genistein: Evaluation of Reproductive Effects over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages (E0213201)

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; and
- 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).

Delclos, K. Barry

p-Nonylphenol: Evaluation of Reproductive Effects over Multiple Generations (E0213501)

Objective(s):

- 1) Determine the effects of *p*-nonylphenol, an intermediate in the production of surfactants and other industrial products, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations;
- 2) Determine if subtle effects observed in the dose range-finding study are magnified through multiple generations; and
- 3) Evaluate the reversibility of any observed effects.

Delclos, K. Barry

Ethinyl Estradiol: Evaluation of Reproductive Effects over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages (E0213801)

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, and
- 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.

Delclos, K. Barry

Effects of Endocrine Active Agents on Bone (E0710601)

Objective(s): To determine if the administration of the endocrine active agents genistein and ethinyl estradiol will alter bone growth and remodeling and if the direction and extent of the effect depends on the window of exposure to the compounds.

Delclos, K. Barry

Dietary Modulation of the Renal Toxicity of *p*-Nonylphenol and Di(2-ethylhexyl)phthalate (DEHP) (E0714201)

- Objective(s):**
- 1) To demonstrate that the cystic kidney disease previously shown to be induced by *p*-nonylphenol in developing NCTR CD rats fed a soy-free diet is decreased in incidence and/or severity in rats fed soy-containing diets;
 - 2) To evaluate the renal toxicity of dietary DEHP in developing rats maintained on a soy-free diet;
 - 3) To evaluate potential early markers of renal cystogenesis in *p*-nonylphenol- and DEHP-treated rats and their modulation by soy-containing diets;
 - 4) To evaluate the roles of modulation of antioxidant defenses and cyclooxygenase activities in the protective effect of soy against *p*-nonylphenol; and, if demonstrated, DEHP-induced renal toxicity; and
 - 5) As secondary objectives in the above studies, the effect of diet on hepatic, testicular, and lung toxicity of DEHP will be assessed.

Doerge, Daniel R.

Genotoxicity, Mutagenicity and Exposure Biomarkers of Acrylamide and Its Metabolite, Glycidamide, in Rodents (E0214601)

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

Doerge, Daniel R.

Neuroendocrine Mechanisms For Acrylamide Carcinogenicity (E0214631)

- Objective(s):**
- 1) Dopamine and its metabolic turnover products will be measured in the hypothalamus and pituitary to determine the effects of acrylamide on neurotransmitter release. In addition, circulating levels of pituitary-regulated hormones, the thyroid hormones, and sex steroid hormones (estradiol, progesterone, testosterone) will be determined.
 - 2) Acrylamide-induced changes in expression of hormone-related genes will be determined, including those important for brain dopaminergic responses and the thyroid.

	<ol style="list-style-type: none"> 3) Acrylamide-induced changes in normal metabolites present in urine, serum, and brain will be investigated to identify a accessible biomarkers of effect for possible extrapolation to human biomonitoring studies. 4) Dose-dependent changes in testicular function (sperm motility and morphology) caused by acrylamide will be investigated.
Doerge, Daniel R.	<p>Development of Methods for Analysis and Confirmation of β-Agonists (E0694501)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Develop determinative and confirmatory procedures using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCI/MS) for multiresidue screening β-agonists in livestock tissues; 2) Develop synthetic procedures to produce authentic β-agonist standards for use in regulatory screening. These methods will provide the flexibility to adapt to the potential for “designer drug” modifications by clandestine laboratories; and 3) Explore the use of packed column supercritical fluid chromatography coupled to APCI/MS as a more efficient technique for chromatographic separation in the screening of large numbers of β-agonists in livestock tissues.
Doerge, Daniel R.	<p>Effect of Soy-Containing Diets on Ammonium Perchlorate-Induced Thyroid Toxicity in Sprague-Dawley Rats (E0716301)</p> <p>Objective(s): Determine the effect of dietary soy and genistein, the principal soy isoflavone, on the dose-response characteristics for perchlorate-induced thyroid toxicity in male Sprague-Dawley rats.</p>
Doerge, Daniel R.	<p>Phytoestrogens and Aging: Dose, Timing, and Tissue (E0721001)</p> <p>Objective(s): Evaluate the potential benefits or detrimental effects of dietary phytoestrogens on breast cancer progression, adipose tissue, and the brain, using well-established laboratory animal models.</p>
Doerge, Daniel R.	<p>Human Studies of Isoflavone Safety and Efficacy (S00607)</p> <p>Objective(s): Bioanalytical analysis of soy isoflavones (and metabolites) in support of clinical trials at the University of Miami and Wayne State University.</p>
Fu, Peter P.	<p>Effect of Topically Applied Skin Creams Containing Retinyl Palmitate on the Photocarcinogenicity of Simulated Solar Light in SKH-1 Mice (E0214301)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) To determine whether or not the application of creams containing retinyl palmitate to the skin of SKH-1 hairless mice alters the tumor incidence induced by simulated solar light or fluorescence lamp generated UV light; and 2) To determine the mechanisms in the alteration of tumor incidence in treated mice.

Fu, Peter P.

The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells (E0687901)

- 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 B₁;
 - 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; and
 - 4) Transgenic human lymphoblastoid cell lines expressing appropriate CYP isozymes will also be employed to study the mutations and DNA binding of the subject drugs.

Howard, Paul C.

Methodology for Safety Testing of Pigments used for Tattooing, Including Permanent Make-up (E0710501)

- Objective(s):**
- 1) Determine the chemicals in tattoo pigments and their metabolism *in vitro*;
 - 2) Develop methodology for tattooing SKH-1 hairless mice in a quantitative and reproducible manner;
 - 3) Determine the extent of inflammation induced by the implanted pigment, and determine the time of recovery following tattooing;
 - 4) Determine the acute toxicity of several tattoo inks and permanent make-up inks in SKH-1 hairless mice in the presence and absence of simulated solar light; and
 - 5) Determine if tattoo pigments are photocarcinogenic in the SKH-1 hairless mouse using simulated solar light.

Howard, Paul C.

Historical Database of Skin Tumor Formation in SKH-1 Mice (S00213)

- Objective(s):**
- 1) Transfer photocarcinogenicity data from 12 studies at Argus Research Laboratories to NCTR and enter into NCTR MultiGen database;
 - 2) Develop statistical methods for analysis of tumor incidence in these photocarcinogenesis studies;
 - 3) Dhere with Argus Research Laboratories methods that are developed for data analysis. NCTR and Argus Research Laboratories will also share information on the generation of simulated solar light.

Pogribna, Marta V.

Folic Acid Metabolism in Children with Down Syndrome (E0708501)

- Objective(s):** To determine whether supplementation with the nutrients folic acid and betaine will increase plasma levels of methionine, S-adenosylmethionine, and S-adenosylhomocysteine, which have shown to be low in children with Down Syndrome.

Pogribny, Igor P.

Global and Locus-specific DNA Hypomethylation: A Common Mechanism Involved in Genotoxic and Nongenotoxic Rat Hepatocarcinogenesis
(E0718101)

- Objective(s):**
- 1) Determine if the temporal alterations in genomic methylation profile in preneoplastic liver tissue observed in the folate/methyl deficient model of rat endogenous hepatocarcinogenesis also occur in other carcinogenesis model;
 - 2) Identify genes that are consistently up-regulated or down-regulated in target tissue during the promotion stage of carcinogenesis; and
 - 3) Evaluate whether or not the global and locus-specific DNA hypomethylation, along with aberrant expression of related genes and changes in chromatin conformation, is specific only to target tissues and may be used for early detection of chemicals with carcinogenic potential.

Tolleson, William H.

Photoinduction of Cutaneous Malignant Melanoma in TP-ras/ink4A (+/-) Transgenic Mice (E0708901)

- 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; and
 - 4) Determine whether UVA+UVB exposure at an early age creates a greater risk for developing cutaneous melanoma in TP-ras (+)ink4a(+/-) mice compared with chronic UVA+UVB exposure of older animals.

Tolleson, William H.

Thermal Inactivation of Ricin Cytotoxic Activity in Infant Formula Samples
(P00653)

- Objective(s):** Detect and quantify residual cytotoxic activity present in thermally-treated ricin-contaminated infant formula samples. This project will:
- 1) Determine temperature/time requirements for thermal inactivation of ricin in infant formula;
 - 2) Provide adaptable methods for food safety surveillance with particular regard to bioterrorism agents; and
 - 3) Validate the reliability of *in vitro* techniques to substitute for animal experiments.

Research Projects Completed in FY 2005

Delclos, K. Barry

The Effects of Dietary Genistein on the Growth of Chemically Induced Mammary Tumors in Ovariectomized and Intact Rats (E0702701)

Results:

The initial purpose of this experiment was to evaluate the ability of the soy isoflavone genistein to affect mammary tumor growth at various times after carcinogen exposure. Although a standard tumor induction protocol with the carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) was used, there was a high mortality rate in the rats shortly after carcinogen treatment that was attributed to adrenal toxicity. We hypothesized that the soy-free diet used in our studies exacerbated the well-known adrenal toxicity of DMBA. In subsequent studies we demonstrated that the soy-free diet enhanced the metabolism of DMBA to toxic metabolites by adrenal microsomes and modulated apoptosis and necrosis in the adrenal following DMBA treatment. The result emphasizes the critical nature of the diet used in toxicity studies.

Delclos, K. Barry

Effects of Endocrine Active Agents on Bone (E0710601)

Results:

The purpose of this experiment was to determine if the administration of the endocrine active agents genistein and ethinyl estradiol would alter bone growth and remodeling and if the direction and extent of the effect depended on the window of exposure to the compounds. Tissues evaluated in the study were from two-year chronic feed studies of genistein and ethinyl estradiol that included three exposure windows: continuous exposure from conception through two years; exposure from conception through 20 weeks followed by control feed to two years; and exposure from conception through weaning followed by control feed to two years. The effects observed were minimal, but continuous exposure to the high doses of the compounds (500 ppm in the feed for genistein, 50 ppb for ethinyl estradiol) resulted in decreased bone size, which could reduce the force required to cause breaks.

Howard, Paul C.

Effect of Topically Applied Skin Creams Containing Glycolic and Salicylic Acid on the Photocarcinogenicity of Simulated Solar Light in SKH-1 Mice (E0213701)

Results:

Studies were conducted to test the impact of topical application of creams containing glycolic or salicylic acid (α - and β -hydroxy acid, respectively) on the carcinogenicity of simulated solar light. SKH-1 hairless mice were topically treated at 8 weeks of age with no cream, control cream, creams containing 4% or 10% glycolic acid, or 2% or 4% salicylic acid, and no solar light or two levels of simulated solar light. Mice were treated 5 days per week for 40 weeks. Test metrics were time-to-first-skin tumor, tumor multiplicity, and tumor diagnosis. Glycolic acid did not affect the formation of tumors by sunlight in the mice. Salicylic acid in some cases was protective against formation of simulated solar light induced skin tumors.

Thermal Inactivation of Ricin Cytotoxic Activity in Infant Formula Samples
(P00653)

Results:

Methodology was developed to quantify residual cytotoxic activity remaining in thermally treated ricin-contaminated infant formula samples. Enhanced thermal stability was observed for ricin dissolved in the infant formula sample matrices tested compared to ricin dissolved in phosphate-buffered saline.

FY 2005 Publications

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- Ang, C.Y. and Jhoo, J., Challenges in assessing bioactive botanical ingredients in functional beverages: an update of recent development, *ACS Book entitled "Challenges in Chemistry and Biology of Herbs"*, in press. (E0716101)
- Beland, F.A., Churchwell, M.I., Von Tungeln, L.S., Chen, S., Fu, P.P., Culp, S.J., Schoket, B., Gyorffy, E., Minárovits, J., Poirier, M.C., Bowman, E.D., Weston, A., and Doerge, D.R., 2005. High performance liquid chromatography electrospray ionization tandem mass spectrometry for the detection and quantitation of benzo[a]pyrene-DNA adducts, *Chemical Research in Toxicology*, 18:1306-1315. (S00198)
- Boudreau, M.D. and Beland, F.A., An evaluation of the biological and toxicological properties of *Aloe barbadensis* (Miller), *Aloe Vera: evaluation of the toxicological potential from topical and oral administration*, *Journal of Environmental Science and Health-Part C*, in press. (E0214201)
- Brezna, B., Kweon, O., Stingley, R.L., Freeman, J.P., Khan, A.A., Polek, B., Jones, R.C., and Cerniglia, C.E., 2005. Molecular characterization of cytochrome P450 genes in the polycyclic aromatic hydrocarbon degrading *Mycobacterium vanbaalenii* PYR-1, *Applied Microbiology and Biotechnology*, Nov. 30, 1-11. (E0711801)
- Chen, T., Mittelstaedt, R.A., Beland, F.A., Heflich, R.H., Moore, M.M., and Parsons, B.L., 2005. 4-Aminobiphenyl induces liver DNA adducts in both neonatal and adult mice but induces liver mutations only in neonatal mice, *International Journal of Cancer*, 117:182-187. (E0709001)
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Division of Biometry and Risk Assessment

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Executive Summary

Introduction

The Division of Biometry and Risk Assessment (DBRA) conducts research to develop new and improved methods for assessing human health risks associated with exposure to chemicals and biological organisms. The Division currently is comprised of four mathematical statisticians, two research biologists, two postdoctoral fellows, and one program support specialist. Division scientists conduct both individual research within the division and collaborative research with scientists from other NCTR Divisions, other FDA Centers, other government agencies, and academic institutions.

The main functions of the Division of Biometry and Risk Assessment include:

- Developing statistical methods for identifying health hazards, including inferring the association of effects with the expression of specific genes;
- Developing biometrical methods for setting safe exposure levels of toxic substances and for modeling and managing the underlying uncertainties;
- Developing mathematical models and computer systems for analyzing the pharmacokinetic and pharmacodynamic components of toxic mechanisms;
- Developing classification algorithms for biomedical decision making, including identifying food hazards and assigning patients to drug therapies;
- Conducting animal studies to evaluate health risks associated with microbiological organisms, including risks to sensitive subpopulations;
- Providing analytical expertise to NCTR scientists on the design, conduct, and analysis of research studies to evaluate the toxicity of regulated products;
- Assisting other FDA Centers in conducting risk assessments for the regulation of specific products; and
- Participating in interagency risk assessment activities, and in activities of scientific organizations, to promote the best practice of risk assessment.

FY 2005 Accomplishments

Division Initiated Research

During FY 2005, Division scientists engaged in research addressing a variety of problems in biometry and risk assessment relevant to science-based regulation.

Research projects included:

- Developing statistical classification algorithms for assigning subjects to defined categories based on high-dimensional predictor sets, including identifying carcinogenic chemicals based on structure-activity relationships and assigning patients to treatment therapies based on genomic profiles;
- Investigating the effect of an imbalance of positive and negative samples in a training/learning set on the classification of unknown samples, and developing remedial methods to balance sensitivity and specificity;
- Developing probabilistic constructs for correctly propagating uncertainty in the pharmacokinetic/pharmacodynamic-modeling component and the interspecies/intraspecies-extrapolation component of quantitative risk assessment;
- Developing Windows-based software for simultaneously implementing and linking as many as four physiologically based pharmacokinetic (PBPK) models, each of which incorporates postnatal growth and includes linkage for simulation of pharmacodynamic (PD) effects;
- Conducting animal experiments to acquire data for developing a sensitive-subpopulation model for the transmission kinetics of infection by *Cryptosporidium parvum*, a key microbiological contaminant of drinking water that also contaminates food products through secondary transmission; and
- Developing statistical procedures for assessing model uncertainty when fitting dose-response models for benchmark-dose estimation, and model-averaging techniques for setting safe exposure levels.

Collaborations with other NCTR Divisions

During FY 2005, scientists in the Division engaged in collaborative research with scientists from other NCTR Divisions to address a number of regulatory issues. Research projects included:

- Effect of *p53* Genotype on Gene Expression Profiles in Mice Exposed to the Model Mutagen, ENU—with the Division of Genetic and Reproductive Toxicology.
- Evaluation of the Genetic Toxicity and Behavioral Effects of Chronic Methylphenidate Exposure in Juvenile Male Rhesus Monkeys (*Macaca mulatta*)—with the Division of Genetic and Reproductive Toxicology.

- Development of a PBPK/PD model of acrylamide and glycidamide in rats and mice by various routes of exposure—with the Division of Biochemical Toxicology.
- Model independent pharmacokinetic analysis of 13-cis-retinoic acid or all-trans-retinoic acid exposure to male and female rats—with the Division of Neurotoxicology.
- Determining the neurotoxic profile-specific changes in cortical gene expression resulting from amphetamine exposure—with the Division of Neurotoxicology.
- Development of a MitoChip, a glass-based oligonucleotide microarray containing mitochondrial and nuclear genes associated with mitochondrial function—with the Division of Systems Toxicology.
- Measurement of cancer-associated gene mutation in colon tumor and non-tumor tissue—with the Division of Genetic and Reproductive Toxicology.

FY 2006 Plans

Division Initiated Research

During FY 2006, Division scientists will conduct research on new methods of data mining and classification, improved methods for propagating uncertainty in risk assessment, user-friendly software for complex PBPK/PD analyses, improved methods for hazard identification, and new methods for the statistical analysis and interpretation of data from genomic experiments.

Specific planned activities include:

- Developing novel statistical classification algorithms, with special emphasis on making personalized medicine a reality by providing practical tools for assigning patients to treatment therapies based on high-dimensional genomic and other biomarkers;
- Developing a probabilistic hierarchy for propagating uncertainty when moving from dose-response assessment to risk characterization in quantitative risk assessment of regulated substances;
- Conducting beta testing of Windows-based software for simultaneously simulating four physiologically based pharmacokinetic (PBPK) models that incorporate postnatal growth and linkage to pharmacodynamic (PD) effects, designing and coding the Windows® interface for a prenatal PBPK model, and making software available to scientific community;
- Developing improved survival-adjusted Poly-k and Peto-type tests for tumorigenicity, and estimation of the lag time between tumor onset and tumor death by attribution of tumor lethality when cause of death is not assigned;

- Evaluating the statistical significance of treatments on a group of correlated genes by implementing computer algorithms to compute adjusted p-values that eliminate or mitigate the deleterious effect of correlations for the large number of subsets defined through a gene ontology; and
- Developing statistical models and computational methods to construct genetic regulatory networks, by identifying genes and subsets of genes that are most influential in the classification of samples based on genomic profiles.

Collaborations with other NCTR Divisions

During FY 2006, Division scientists will continue to devote significant effort to collaboration with scientists from other NCTR Divisions.

Contribution to FDA's Strategic Goals

The Division's research contributes in several ways toward the FDA's goal to increase access to innovative technologies to advance public health. Research on classification algorithms can help to make personalized medicine a reality by enabling the assignment of therapies to patients to maximize efficacy and minimize toxicity. These algorithms can combine diverse, high-dimensional biomarkers to achieve high accuracy in predicting which patients will benefit most from medical products and which are likely to respond badly or to experience toxicities greater than benefits. Research on probabilistic techniques for propagating uncertainty in risk assessment provides risk managers with tools for ensuring adequate public health protection, including protection of sensitive subpopulations. Research on PBPK/PD models provides software to enable risk assessors/managers to make scientifically sound characterizations of risk to humans from FDA-regulated products based on data from animal studies. Research on methods and algorithms to analyze and interpret data from genomic experiments can help to establish new high-dimensional genomic biomarkers of risk, disease, and treatment effects.

Ongoing Research Projects

Chen, James J.	<p>Network Algorithms to Analyze Gene Expression Data (E0715901)</p> <p>Objective(s): Develop statistical models and computational methods for class prediction, class discovery, and genetic regulatory networks.</p>
Kodell, Ralph L.	<p>Dose-Response Modeling for Microbial Risk Assessment (E0704501)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) To evaluate existing dose-response models for microbial risk assessment; 2) To develop improved models for estimating probabilities of infection and disease; and 3) To develop methods for incorporating model uncertainty into microbial risk assessment.
Kodell, Ralph L.	<p>Modification and Application of Quantitative Risk Assessment Techniques to FDA-regulated Products (S00174)</p> <p>Objective(s): In response to requests from scientists and regulators at CDRH, CDER, CBER, CFSAN, and CVM, using available toxicological data, conduct cancer and noncancer risk assessments of FDA-regulated products to assist in establishing “safe” conditions of exposure to toxic substances.</p>
Moon, Hojin	<p>Development of Improved Survival-adjusted Tests for Animal Carcinogenicity/Tumorigenicity Data (E0717101)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Develop new statistical methods for investigating the carcinogenic potential of drugs and other chemical substances; and 2) Develop a statistical testing methodology for a dose-related trend in tumor incidence rates of an occult tumor.
Moon, Hojin	<p>Estimation of Lag Time Between Onset of and Death from an Occult Tumor via Attribution of Tumor Lethality (E0717201)</p> <p>Objective(s): Develop new statistical methods for estimating the elapsed time between onset of, and death from, an occult tumor when cause of death (COD) for each animal or context of observation for each tumor is not available.</p>
Moon, Hojin	<p>Optimal Tree-Based Ensemble Methods for Class Prediction (E0722101)</p> <p>Objective(s): The first goal of this study is to produce an ensemble of decision trees, each constructed from a different set of predictors from a random partition of the predictor space, by statistically pruning to optimal size using cross-validation. The second goal is to use Monte Carlo simulation techniques to compare the performance of the proposed ensemble classifier to the performance of other popular classifiers. A primary area of application is the classification of subjects into risk categories in class prediction problems occurring with genomics and proteomics data.</p>

Turturro, Angelo

Development of a Model for the Transmission Kinetics of Infection by *Cryptosporidium parvum* with Acquisition of Data on Key Parameters
(E0708201)

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

Young, John F.

Computational Predictive System for Rodent Organ-Specific Carcinogenicity (E0708301)

- Objective(s):** Using modern SAR technology and statistical approaches, develop an expert system to predict rodent carcinogenicity.

Young, John F.

Bio-Preg to Windows® Upgrade (E0713001)

- Objective(s):** Design and code a Windows® interface to NCTR's existing PBPK software program called Bio-Preg.

Research Projects Completed in FY 2005

Experimental Design and Analysis of GeneArray Expression Data (E0711201)

Results:

Four studies were conducted under this project:

- 1) A study on various microarray normalization methods was conducted. The method with a global *lowess* fit for intensity adjustment combined with subset median for location adjustment, if necessary, appears to perform well. In addition, a generalized additive model for normalization of nuisance effects, such as, dye, day, or array effects, was developed. This model provides a formal statistical model for the *lowess* method; it incorporates the best aspects of the *lowess* fit and the ANOVA model method. This model can be applied to one channel or two channel data from experiments with multiple treatments or multiple nuisance factors.
- 2) A study on the Type I error and power of the one- and two-sample t-test and permutation test for microarray data analysis was conducted. For data from the two-color dye-swap experiment, the one-sample test performs better than the two-sample test since expression measurements from the same spot are correlated. For data from independent samples, such as one-channel array or two-channel array using a reference design, the two-sample tests are more powerful. When the number of arrays is sufficient, the permutation test performs better than the corresponding t-test since the distribution of the normalized intensities often does not follow a normal. In addition, a general procedure for estimating the number of arrays needed was proposed. The sample sizes needed for a two-sample z-test were computed for independent and equally correlated models. The procedure is useful for planning microarray experiments.
- 3) A study on the analysis variance component approach to investigate animal-to-animal, between-array, within-array, and day-to-day (or week-to-week) variations was conducted. The largest variation observed is the week-to-week effect and the animal-to-animal variation. A method to establish optimal numbers of animals, arrays per animal, and section per array for experimental planning was described.
- 4) A study on methods to determine a cut-off for selection of altered genes was conducted. A statistical model for the number of false rejections of truly altered genes for the false discovery rate (FDR) estimation was developed. This model provided a theoretical framework for different FDR error measures and a connection between classical and Bayesian approaches.

A final report was submitted and approved on September 14, 2005.

Kodell, Ralph L.

Statistical Analysis of Tumor Multiplicity Data (E0706101)

Results:

A statistical test for photocarcinogenicity experiments with multiple induction times was developed to isolate differences in the distribution of the *number* of induced tumors and the distribution of their *times* to observation. This “frequency-latency” procedure was shown to perform well in terms of controlling Type I error and achieving power comparable to common tests that can test for overall differences but can’t separate frequency effects from latency effects. The test may be used to infer whether cosmetics and other skin treatments, when interacting with sunlight, cause an increase/decrease in the number of induced skin tumors and/or cause an increase/decrease in the time to observation of such tumors. A final report was submitted December 20, 2004, and was accepted January 5, 2005.

Kodell, Ralph L.

Interagency Agreement on Developing and Evaluating Risk Assessment Models for Key Waterborne and Foodborne Pathogens and Chemicals
(P00422)

Results:

Two experiments were conducted under this umbrella project. In the latter experiment, methods were developed to implement EPA’s Relative Potency Factor approach to risk/safety assessment for mixtures of chemicals. Strategies were provided to EPA for grouping chemicals into similar classes and for combining the classes. This experiment was completed prior to FY 2005. In the former experiment, just completed, animal studies were conducted to acquire data to flesh out a model for the transmission kinetics of infection by *Cryptosporidium parvum*. The data were used to show that secondary transmission among subpopulations of different sensitivities was a key factor in accurately predicting cases of cryptosporidiosis in an actual human outbreak. A final technical report on this experiment has just been submitted to EPA. This umbrella project has been closed.

FY 2005 Publications

- Akerman, G.S., Rosenzweig, B., Domon, O.E., Tsai, C., Bishop, M.E., McGarrity, L.J., MacGregor, J.T., Sistare, F., Chen, J.J., and Morris, S.M., 2005. Alterations in gene expression profiles and the DNA damage response in ionizing radiation-exposed TK6 cells, *Environmental and Molecular Mutagenesis*, 45:188-205. (E0712901)
- Bowyer, J.F., Delongchamp, R.R., and Jakab, R.L., 2004. Glutamate N-methyl-D-aspartate and dopamine receptors have contrasting effect on the limbic versus the somatosensory cortex with respect to amphetamine-induced neurodegeneration, *Brain Research*, 1030:234-246. (E0707301)
- Chen, D., Chen, J.J., and Soong, S., 2005. Probe rank approaches for gene selection in oligonucleotide arrays with a small number of replicates, *Bioinformatics*, 21:2861-2866. (E0715901)
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Division of Genetic and Reproductive Toxicology

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Executive Summary

Introduction

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

As experts in the field of genetic toxicology, scientists in DGRT are actively involved in national and international efforts to harmonize the conduct of genetic toxicology tests and to improve their interpretation and use for regulatory decision making. DGRT scientists frequently provide expert advice to the FDA Centers, and other government organizations, and various other public and private organizations. They are active participants in the FDA Genetic Toxicology Network and the CDER Genetic Toxicology Network.

There are four basic focus areas in the Division research program. Genetic toxicology research addresses the development of methods to assess the potential for chemicals to negatively impact human genetic material or the function of the genetic material.

Reproductive/developmental toxicology focuses on methods to understand normal human development and how chemicals might alter normal development. In addition to these disciplinary research areas, the Division conducts research to understand the impact of diet (including dietary supplements) and to incorporate the new OMICs technologies for the development of biomarkers and for hazard assessment. Dietary research primarily focuses on understanding the physiological and genetic consequences of dietary modulation and the potential hazards of dietary supplements. The OMICs research is rapidly increasing in Division emphasis and is coupled with the more traditional approaches to improve

the ability of FDA to incorporate these new and powerful technologies into regulatory decision-making.

DGRT activities provide both direct support to the generation of new approaches that are used by the other FDA Centers and, in particular, provide research and expertise that are directly related to the FDA's Critical Path Initiative.

FY 2005 Accomplishments

In 2005, DGRT scientists actively participated in providing genetic toxicology advice to CDER, CVM, CFSAN, CBER, and CDRH. These consultations included both general advice concerning the conduct and interpretation of data from specific assays and the evaluation of data from submissions to FDA. DGRT scientists participated in the International Workshop for Genotoxicity Tests and are authors on manuscripts providing harmonized guidance for the conduct of specific assays and on the appropriate follow-up strategies for chemicals (primarily pharmaceuticals) that are found to be positive in genetic toxicology tests conducted as part of the drug safety evaluation. They also participated in an international meeting in Germany evaluating the existing information for the *in vivo* transgenic mutation assays and their suitability for the development of an OECD guideline specifically for transgenic mutation assays.

Specific research areas are highlighted as follows:

- 1) DGRT scientists have developed a series of allele-specific competitive blocker-polymerase chain reaction (ACB-PCR) n (ACB-PCR) genotypic selection methods that can directly measure specific mutations in genes involved in tumor induction (oncogenes and tumor suppressor genes). These assays were used to measure mutations in tumors resulting from solar light exposure and in mice treated with 4-aminobiphenyl. ACB-PCRn (ACB-PCR) can detect mutations when they occur in as few as 1 cell in 100,000 cells. These studies have yielded significant new information. For instance, all the skin tumors resulting from solar exposure had relatively high frequencies of *p53* mutations, indicating that, while this mutation was not the initiating event for the tumor, it was important for tumor formation. This research was conducted in collaboration with the NCTR Center for Phototoxicity. Two companion studies evaluating the presence on *p53* mutations in colon cancer from both mice and humans are well underway. Collaboration was initiated involving researchers in Biochemical Toxicology (NCTR) and investigators in the Carcinogenesis Division, National Health and Environmental Effects Research Laboratory, Environmental Protection Agency (EPA). This study will address the shape of the dose-response curve for low-dose exposure to carcinogens. To date, NCTR research using this new

ACB-PCRn (ACB-PCR) technology indicates that this approach provides the opportunity to detect the rare mutations involved in the etiology of cancer prior to the development of the actual visible tumor. Thus, this appears to be a promising new biomarker that may ultimately lead to the replacement of the traditional 2-year cancer bioassay. Such an approach would hasten the development, safety assessment, and approval of new drugs.

- 2) Studies were completed indicating that the neonatal mouse is more sensitive than the adult mouse to the induction of mutation following exposure to the human carcinogen, 4-aminobiphenyl. This supports the hypothesis that young humans are more susceptible than adult humans to developing drug- or other chemically-induced cancer.
- 3) DGRT scientists published manuscripts providing guidance and review articles, including: 1) the mutagenicity of bromate and implications for cancer risk assessment, 2) an assessment of progress using transgenic rodent mutation models, 3) the use of genetic toxicology information for mode of action analysis in risk assessment 4) internationally harmonized guidance for the interpretation of data from the mouse lymphoma mutation assay, and 5) a standard reference multicolor spectral karyotype for the mouse lymphoma cell line.
- 4) A study was completed and published that provided a methodology for using the *in vitro* mouse lymphoma mutation assay to assess photomutagenicity, and which showed that retinyl palmitate can be activated by ultraviolet (UV) light to a mutagenic form.
- 5) In collaboration with scientists in the Biometry and Risk Assessment Division, a manuscript was published describing an experimental design and an approach for the analysis of gene array expression data.
- 6) In collaboration with scientists in the Division of Biochemical Toxicology, a study was completed and published describing C-glycoside flavonoids as potential mutagenic compounds in the dietary supplement Kava.
- 7) Division scientists completed and published results from studies evaluating the genotoxicity of several additional chemicals (zidovudine, didanosine, malachite green, leucomalachite green, azathioprine, comfrey, and riddelliine) of regulatory interest to the FDA. Many of these compounds were nominated by the FDA for evaluation, and the research on them was supported through an IAG with the NTP/NIEHS. Studies evaluating the *in vivo* mutagenicity of acrylamide, which has recently been detected in baked goods, also are ongoing, and results on the *in vivo* genotoxicity of acrylamide, and its major metabolite glycidamide, were recently published.

FY 2006 Plans

- 1) In collaboration with scientists in the Division of Biometry and Risk Assessment, a new approach for using the quantitative analysis of *in vivo* mutation data to inform the mode of action assessment for carcinogens was developed in 2005. This approach will be evaluated for its utility in a cooperative research agreement (CRADA) with Toxicology Excellence for Risk Assessment (TERA) and an informal collaboration with Environ, International.
- 2) DGRT scientists will continue with the development of a new two-transgene *in vivo* assay using a fluorescent green protein, as a reporter of mutation, and another gene that controls its production as the mutational target sequence. When a chemical causes a mutation in the controlling gene, the cell produces a fluorescent protein that becomes visible under UV light and can be quantified using flow cytometric instrumentation.
- 3) DGRT scientists will continue studies applying the new genotypic selection technology measuring specific rare mutations in cancer-causing genes to studies involving colon cancer in humans, a skin cancer model in mice, and a colon cancer model in rats. The rat studies will involve examining mutations in cells with specific morphologies related to cancer induction; the cells will be isolated using laser capture microdissection. The collaboration with EPA to apply this technology to understanding the shape of the dose-response curve at low carcinogen dose will continue. New emphasis will be placed on developing this technology so that it might be used as a biomarker to identify potential carcinogens, thus providing an alternative to the 2-year cancer bioassay.
- 4) DGRT scientists will investigate the possibility of using the new genotypic selection technology to determine the number of specific tumor mutations that are still present following the treatment of tumors with cancer chemotherapeutics. Because this technology can detect these cancer biomarkers when they are present at a low frequency, it should be possible to use this approach to evaluate the efficacy of cancer treatment. Ultimately, it should be possible to tailor this type of approach to an individual patient. The technology could readily determine whether a particular treatment is effective for that particular patient, thus providing a personalized medicine approach to evaluating the efficacy of cancer therapy.
- 5) Another new approach for analyzing *in vivo* mutations will be developed. This assay uses fluorescent probes to detect mutation in the endogenous, X-linked *PIG-A* gene. In theory, detection of mutations in this gene does not require cell culture (as do many other *in vivo* mutation detection methods) and lends itself to both *in situ* and high through-put analyses in humans and animal models. These properties make *PIG-A* an attractive reporter gene for *in vivo* mutation studies. Experiments are being conducted to

determine the level of sensitivity of the analysis using cultures of cells that are wild-type and mutant for *PIG-A*.

- 6) In collaboration with Division of Biochemical Toxicology scientists, the AIDS drug project will be continued. The drug treatments in these studies will model the use of these agents to prevent the transmission of the HIV virus from infected pregnant women to their children. Human clinical data suggest that a major target for the toxicity of AIDS therapeutic agents is the mitochondria, and studies will be conducted to evaluate the long-term effects of perinatal treatments to mice on mitochondrial DNA copy number and mutation. In an additional study, an NTP-sponsored experiment will evaluate the ability of *p53* haplodeficient (*p53*^{+/-}) mice to detect the tumorigenicity of AIDS drugs in a relatively short-term bioassay.
- 7) DGRT scientists are collaborating with Division of Neurotoxicology scientists to assess the potential for methylphenidate (a drug commonly used to control attention deficient disorder in children) to induce mutagenic damage. This study is being conducted as a part of an interagency agreement with the National Institute for Child Health and Development.

In addition, work will continue on several ongoing projects, including:

- 1) An Office of Women's Health project that is investigating whether genistein can decrease the induction of carcinogen-caused mutations;
- 2) A collaborative project with the University of Arkansas for Medical Sciences investigating the influence of biotin on the developing embryo;
- 3) The further development and characterization of the Φ X174 transgenic mouse, particularly as it applies to detecting mutation in mouse skin following exposure to solar light;
- 4) NTP projects investigating the genetic toxicity of AIDS therapeutic drugs and the developmental toxicity of bitter orange;
- 5) Evaluation of flow cytometry for the high through-put analysis of micronucleus frequency in mice, rats, monkeys, dogs, and humans; and
- 6) A project to investigate whether the developing embryo and/or the neonate is particularly sensitive to the induction of mutation following exposure

Contribution to FDA's Strategic Goals

DGRT provides expert advice and innovative research to the other FDA Centers, thus contributing to FDA's mission of advancing public health. Several of the DGRT research projects involve the development of new and innovative technologies and approaches that support the regulatory centers and, in particular, the FDA's Critical Path Initiative.

Genetic toxicology is concerned with the ability of chemicals to alter genetic material. The FDA requires that petitioners provide data evaluating the potential genetic toxicity of their products as a part of the product approval process. Because genetic damage is believed to be

important in tumor development, this information is used as a part of the evaluation of suspected carcinogens. Regulatory decisions are based not only on the identification of potentially genotoxic substances, but also on an understanding of their mode of action. Research within the Division centers on the development and validation of new methods to assess genetic risk. Bacterial and tissue culture approaches are commonly used to detect potential genotoxicity and to generate hypotheses concerning the basic mechanisms of genotoxicity. While the Division utilizes *in vitro* approaches, it specializes in the development and validation of *in vivo* mammalian systems and the incorporation of these methods into risk assessment strategies. An increased understanding of mutational mechanisms, combined with test systems with an increased ability to detect genetic damage, will provide the FDA with better information for decision making. As new assays are validated, Division scientists will continue to work with international scientists to assure the harmonization of protocols and the development of guidelines to assess genetic hazards.

Reproductive/developmental toxicology is important to the Agency because one of the difficult challenges facing the FDA is the identification and regulation of chemicals, food additives, and biological therapies that may produce birth defects. Such defects affect 7% of humans 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 vitamin folic acid plays 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 interaction between diet and toxicants may be important in producing certain birth defects.

Genomic technologies are beginning to provide new tools for making better public health decisions. International research efforts are providing the scientific and medical community with an increased understanding of the genetic material and how it functions in both humans and rodents. Utilizing this information, new molecular technologies are being rapidly developed and can be used to evaluate structural and functional changes to the genetic material of both rodents and humans. The Division is using new technologies in combination with more traditional approaches to address various research questions. While current technologies in the field of genetic and reproductive/developmental toxicology generally evaluate single endpoints, these new genomic technologies are providing the opportunity to detect alterations in a number of different endpoints. In the future, these new approaches to evaluating toxicity will allow for the integration of information across the various types of adverse health outcomes. For instance, when these technologies are fully developed, it will be possible to concurrently evaluate chemicals for their ability to cause cancer, to impact the nervous system, to cause birth defects, and to modify the immune function.

Ongoing Research Projects

Aidoo, Anane

The Development of a Genotypic Selection Assay and Analysis of the Age-Specific Patterns of Mutant Accumulation (E0706301)

- Objective(s):**
- 1) To develop a genotypic selection assay (GSA) allowing a direct measurement of mutant frequencies and molecular analysis of mutation in any nonpolymorphic endogenous sequence and in any tissue;
 - 2) To determine the spontaneous mutant frequencies (MFs) and age-associated accumulation rates (ARs) in highly (Exon 3) and poorly (Exon 4) mutable regions of *hprt* coding sequence in the *hprt* lymphocyte mutation assay; and
 - 3) To compare the *in vivo* persistence of elevated MFs in *hprt* exons 3 and 4 induced after exposure to ENU.

Aidoo, Anane

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)

- Objective(s):** Determine whether daidzein and genistein or estradiol supplementation, singly or in combination, to ovariectomized rats would alter mammary tissues:
- 1) DNA adducts produced by DMBA;
 - 2) The frequency and types of mutations produced by DMBA; and
 - 3) Tumor formation by DMBA and types of *p53* and *H-ras* mutations in tumors.

Aidoo, Anane

An Efficient Regulatory Method for Evaluating Chromosomal Damage: Analysis of Micronucleus in Different Rat Strains by Flow Cytometry (E0714001)

- Objective(s):** Provide the information necessary to establish a new standard for pre-market evaluation of genotoxic potential by:
- 1) Establishing the validity of the flow cytometric scoring of micronuclei in Sprague-Dawley and Fischer 344 rats;
 - 2) Determining the kinetics of the appearance and elimination of micronucleated cells in both strains; and
 - 3) Determining whether the frequency of micronuclei in the young circulating reticulocytes accurately reflects the frequency in the primary bone marrow cell population from which they are derived.

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)

- Objective(s):**
- 1) Determine the mutant frequencies in the *cII* gene of lambda/*lacI* transgenic mice treated with ethylnitrosourea, a direct-acting carcinogen, and the modifying role of age, sex and target organ;
 - 2) To compare the mutant frequencies in the *cII* gene of livers from the transgenic mice exposed as neonates and adults to different doses of aflatoxin B1, a human hepatocarcinogen that requires a metabolic activation;
 - 3) Determine the effect of exposure of neonatal and adult Big Blue[®] mice to 17 b-estradiol, a human hormone carcinogen, on subsequent spontaneous and carcinogen-induced mutations in the *cII* gene of the target organs; and
 - 4) Determine the types of *cII* mutations in the mutants from Objectives 1, 2, and 3.

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

Chen, Tao

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

Dobrovolsky, Vasily N.

Transgenic Mouse Model for Detecting *In Vivo* Mutation Using a Green Fluorescent Protein Reporter (E0713801)

- Objective(s):**
- 1) Produce two lines of transgenic mice expressing the tetracycline-repressor protein;
 - 2) Investigate the efficiency of *in vivo* repression of green

	<p>fluorescent protein (GFP) in various tissues of different lines of the double-transgenic mice; and</p> <p>3) Determine the frequency of spontaneous and y-ray-induced TetR mutation in lymphocytes of double-transgenic mice using flow cytometry.</p>
Duffy, Peter H.	<p>Effect of Diet and Different Levels of Caloric Restriction (CR) on Physiological, Metabolic, Biochemical, Immunological, Molecular, and Body Composition Variables in Rats (E0692401)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 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) Determine the relationship between body fat (BF), fat free mass (FFM), total body water (TBW), and total body electrical conductivity (TOBEC) as a function of strain, age, mass, and nutritional status in rats; 3) Validate and automate the use of a new noninvasive electromagnetic scanning device to measure BF, FFM, and TBW and compare the results to a conventional chemical fat extraction technique; 4) Determine if CR alters the relative quantity and disposition of various types of lipids such as cholesterol, phospholipids, free fatty acid, etc. in various tissues, as well as in urine, feces, and blood serum; 5) Develop control data related to CR that can be used by CFSAN to evaluate the toxicity and efficacy of low calorie foods, food additives, and food substitutes; 6) Determine temporal and environmental factors that modulate the effects of CR; 7) Develop experimental methods for utilizing a low level of CR to increase the survival rate and to decrease variability in the chronic bioassay; to provide the concomitant control data for comparison; and 8) Develop control data for a reference purified diet that has been formulated to conform to long-term nutrient requirements of rodent animal models typically utilized in toxicology and nutrition studies.
Hansen, Deborah K.	<p>Developmental Toxicity of Bitter Orange in Rats (E0214701)</p> <p>Objective(s): To determine potential developmental toxicity of synthetic synephrine and citrus aurantium extract in rats.</p>
Hansen, Deborah K.	<p>Physiological Effects of Bitter Orange in Rats (E0214901)</p> <p>Objective(s): To determine potential physiological effects of synthetic synephrine as well as an extract from the botanical citrus aurantium alone and in combination with caffeine in rats.</p>
Hansen, Deborah K.	<p>Mechanism(s) of Folate-Responsive Dysgenesis (E0707401)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 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-hr windows on each day of gestation from GD 5 to GD 15, and to determine if the loss of these cohorts of cells during these windows of antifolate exposure gives rise to recognizable neural tube defects and neurocristopathies in the fetus at term;

- 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 overexpression from GD 8 to GD 16 during maternal folate deficiency has an adverse impact on cytotrophoblastic proliferation leading to small placentas and global growth retardation of fetuses; and
- 4) To demonstrate that neural tube closure and neural crest cell function in the whole mouse embryo 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.

Hansen, Deborah K.

Examination of Embryonic Gene Expression during Neural Tube Closure
(E0710901)

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

Hansen, Deborah K.

Mechanism of Biotin Deficiency-induced Malformations (E0713301)

- Objective(s):**
- 1) Determine if palatal tissue from biotin-deficient embryos is able to fuse *in vitro* in either biotin-sufficient or -deficient medium;
 - 2) Determine if arachidonic acid increases palatal fusion and improved limb development and increases the length of the long bones *in vitro* from biotin deficient mouse embryos;

	<ol style="list-style-type: none"> 3) Determine if prostaglandin E2 increases palatal fusion and improved limb development and increases the length of the long bones <i>in vitro</i> from biotin deficient mouse embryos; 4) Determine if malonyl CoA increases palatal fusion and improves limb development and increases the length of the long bones <i>in vitro</i> from biotin deficient mouse embryos; 5) Determine fetal arachidonic acid content and synthesis <i>in vivo</i>; and 6) Determine if arachidonic acid is able to prevent biotin deficiency-induced orofacial clefting and limb hypoplasia <i>in vivo</i>.
Hass, Bruce S.	<p>Identification of Target Sites for UVB Irradiation in Gene A of ΦX174 contained as a Transgene in Mouse Embryonic Cell PX-2 (E0710101)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determine the dose-survival response of PX02 cells to UVB/UVA light in order to determine UV doses that optimize mutation induction and cell survival; 2) Determine the induced mutant frequency in gene A of ΦX174 by a forward mutation assay using cultures of PX2 exposed to UVB; and 3) Sequence the UVB/UVA-induced mutants from treated and untreated cultures to identify specific target sequences.
Hass, Bruce S.	<p>UV-Induced Mutations in Mouse Epidermis using Gene A of ΦX174: Proof of Principle (E0718701)</p> <p>Objective(s): Establish that a UVB-induced dose-response in mutant frequency of mouse epidermis can be detected by the forward assay for ΦX174 analyzed by single bursts.</p>
Heflich, Robert H.	<p>Effect of Azathioprine on Somatic Cell and Germline <i>Hprt</i> Mutant Frequencies in the Mouse (E0709901)</p> <p>Objective(s): Test the hypothesis that <i>in vivo</i> selection by azathioprine affects both somatic cell and germline <i>Hprt</i> mutant frequencies using the mouse.</p>
Mckinzie, Page B.	<p>ACB-PCR n (ACB-PCR) Measurement of Azoxymethane-induced Rat K-ras codon 12 GGT-->GAT and GTT-->GTT Mutations in Colonic Aberrant Crypt Foci Isolated using Laser Capture Microdissection (E0714901)</p> <p>Objective(s): Use newly established PCR-based methods to quantify the rat K-ras codon 12 GGT ' GAT and GGT ' GTT mutant fractions in rat colonic mucosa, aberrant crypt foci, and tumors at specified times after colon tumor initiation by azoxymethane treatment. Use this data in conjunction with K-ras mutant fraction data generated from studies of human colon to determine how rodent data can be extrapolated to human disease.</p>
Moore, Martha M.	<p>Genetic Toxicology Evaluations in Support of FDA Centers for Evaluating Substances for their Genotoxic Potential (S00677)</p> <p>Objective(s): To provide direct research to FDA Centers.</p>

Morris, Suzanne M.	<p>Effect of <i>p53</i> Genotype on Gene Expression Profiles in Mice Exposed to the Model Mutagen, N-ethyl-N-nitrosourea (ENU) (E0712901)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) To determine the effect of mutation in the <i>p53</i> tumor suppressor gene on gene expression profiles in young and aged mice; and 2) Determine the effect of mutation in <i>p53</i> tumor suppressor gene on gene expression profiles in young and aged mice exposed to the model mutagen, N-ethyl-N-nitrosourea.
Morris, Suzanne M.	<p>Phosphatidylinositol Glycan–Complementation Group A (<i>PIG-A</i>) Mutagenesis: Development of Methods for the Identification and Molecular Characterization of Mutations in the <i>PIG-A</i> Gene in Human Lymphoblastoid Cells and C57Bl/6 Mice (E0720901)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) To develop flow cytometric methods for the detection of cells with mutations in the <i>PIG-A</i> gene using wild-type and mutant human lymphoblastoid cells, TK6 and WTK1, as a model; and 2) To develop flow cytometric methods for the detection of hematopoietic cells with mutations in the <i>PIG-A</i> gene in C57Bl/6 mice.
Morris, Suzanne M.	<p>Evaluation of the Genetic Toxicity and Behavioral Effects of Chronic Methylphenidate Exposure in Juvenile Male Rhesus Monkeys (<i>Macaca mulatta</i>) (E0723401)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) To determine the baseline frequency of measures of genetic damage in a population of juvenile rhesus monkeys; 2) Determine the frequency of these measures of genetic damage in a population of juvenile rhesus monkeys at defined intervals during a chronic exposure to methylphenidate; 3) Determine if chronic exposure to methylphenidate results in measurable effects on the behavior of juvenile rhesus monkeys utilizing the NCTR Operant Test Battery; and 4) Determine the plasma concentration of methylphenidate and its major metabolite, ritalinic acid, during the chronic exposure of juvenile rhesus monkeys to the drug.
Morris, Suzanne M.	<p>Evaluation of the Genotoxicity and Pharmacokinetics of Methylphenidate in Male Big Blue[®] Mice (E0723501)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) To determine the metabolites of methylphenidate at early times after exposure in B₆C₃F₁ mice to compare the major metabolites in the human, monkey and the mouse; 2) Determine the plasma levels of methylphenidate and its major metabolites in the B₆C₃F₁ mouse after 28 days of exposure; 3) Determine the effect of exposure to methylphenidate on body and organ weights of the B₆C₃F₁ mouse after 28 days of exposure; 4) Determine if long-term exposure to methylphenidate results in a dose-responsive increase in the liver <i>c11</i> gene mutant frequency of Big Blue[®] mouse; and 5) Determine the pharmacokinetics of methylphenidate and its major metabolite, ritalinic acid, in B₆C₃F₁ mice.

Morris, Suzanne M.	<p>A Feasibility Study for the Analysis of Cytogenetic Damage by Chromosome Painting in Lymphocytes of the Rhesus Monkey (<i>Macacca mulatta</i>) (P00672)</p> <p>Objective(s): Assess the feasibility of performing chromosome painting analyses on the lymphocytes of rhesus monkeys.</p>
Parsons, Barbara	<p>Analysis of <i>p53</i> Codon 270 CGT to TGT Mutation in Simulated Solar Light-induced Skin Tumors and Exposed Mouse Skin (E0715201)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Develop the ACB-PCR detection of mouse <i>p53</i> codon 270 CGT->TGT mutation; 2) Measure the frequency of detection and levels of this mutation in mouse skin tumors; 3) Measure the frequency of this mutation in skin tissue from tumor-bearing animals; and 4) Measure the frequency of this mutation in skin exposed to decreasing levels of SSL.
Parsons, Barbara L.	<p>Measurement of Cancer-Associated Gene Mutation in Colon Tumor and nontumor Tissue (E0716001)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determine <i>k-ras</i> codon 12 GGT to GAT and GGT to GTT mutant frequencies in colonic ACF, adenomas, and carcinomas; first by DNA sequencing and, if mutation is not detected, then by ACP-PCR; 2) Determine <i>K-ras</i> codon 12 GGT to GAT and GGT to GTT mutant frequencies in tumor margin samples and tumor-distant, normal-appearing colonic epithelium from colon cancer patients; and 3) Determine <i>K-ras</i> codon 12 GGT to GAT and GGT to GTT mutant frequencies in autopsy samples of colonic epithelium from colon-disease-free individuals.
Valentine, Carrie R.	<p>Evaluation of the Potential of the Gene A Forward Mutational Assay of PhiX174 for Improving Sensitivity of Transgenic Mutation Assays (E0711501)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determine the appropriate experimental conditions to identify single bursts of mutations fixed <i>in vivo</i>; 2) Develop a microplate scoring method that will identify <i>in vivo</i> bursts within numerous aliquots; 3) Determine the spontaneous mutant frequency and ENU-induced mutant frequency by single burst analysis for mouse splenic lymphocytes; and 4) Continue development of a frameshift assay for ΦX174 in gene <i>J</i> by our collaborator Dr. Bentley Fane.
Valentine, Carrie R.	<p>Creation of a Web-based Database for Mutations Associated with Exon-skipping (E0720101)</p> <p>Objective(s): Create and update a public web site database with reported exonic mutations associated with exon loss. The database will be posted on a public web site that is searchable by gene characteristics and will be monitored for use.</p>

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Division of Microbiology

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Executive Summary

Introduction

The Division of Microbiology (DM) at the National Center for Toxicological Research (NCTR) serves a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of the Food and Drug Administration's (FDA's) responsibility in toxicology. The Division of Microbiology also responds to microbial surveillance and diagnostic needs for research projects within the NCTR and FDA. The Division of Microbiology has a multidisciplinary staff including 13 research scientists and 16 research support staff, postdoctoral fellows, undergraduate/graduate students, visiting scientists and program support specialists. In the Microbiology Division, we have the staff, the know-how, and the facilities to help address the scientific challenges encountered by the FDA and other government organizations. Some examples of the research projects within the Division and collaborative research with scientists from other NCTR Divisions, FDA Centers and academic institutions are described below. Projects are 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) Antimicrobial resistance; 3) Gastrointestinal microbiology and host interactions; 4) Environmental biotechnology; and 5) Microbiological surveillance and diagnostic support of research.

FY 2005 Accomplishments

Foodborne Pathogens, Food Safety, and Methods Development

In recent years, there has been increasing concern by the public, industry, and regulatory agencies concerning the safety and quality of food. Division of Microbiology scientists have collaborated with the Center for Veterinary Medicine (CVM), Center for Food Safety and Applied Nutrition (CFSAN), and Office of Regulatory Affairs (ORA) on a variety of projects to identify and characterize foodborne hazards more rapidly and accurately. We are committed in developing an integrated approach to food safety and biosecurity consistent with the FDA mission.

Molecular typing is a valuable epidemiological tool in delineating possible movement and transmission pathways of *Salmonella* from poultry farms (breeder flocks and hatcheries to processing plants) and in determining possible links with clinical and outbreak strains. We investigated the

efficacy of the multilocus sequence typing (MLST) method in discriminating serovars, which could not be differentiated by the pulsed-field gel electrophoresis (PFGE) method. Five housekeeping and virulence genes were used to discriminate allelic clones of *Salmonella* serovars.

With the University of Arkansas in Fayetteville, we have initiated a study to develop high-resolution molecular typing methods based on the restriction site polymorphism flanking ribosomal RNA (*rrn*) operon to discriminate *Salmonella* genotypes. The premise for the study is that there are seven copies of the *rrn* operon in *Salmonella*, and multiple copies of these highly homologous sequences frequently serve as targets for chromosomal rearrangement, giving rise to polymorphism surrounding the operon. We have already performed the fingerprint analysis on Heidelberg strains using the statistical analyses of the DNA fingerprints obtained by Bionumerics software. Once the data are collected, we will compare the fingerprint analyses between the PFGE pattern and the pattern generated by restriction site polymorphism flanking of the *rrn* operon in *Salmonella* strains. This study can then be used as an alternative typing protocol for generating a DNA fingerprint database to archive *Salmonella* genotypes from sampled sources.

A summer intern found that *Salmonella* isolates recovered from a recent multistate tomato outbreak and from egg houses were susceptible to all standard National Antimicrobial Resistance Monitoring System (NARMS) antimicrobials tested, suggesting that the sources from which these pathogens were recovered may not have been exposed to antimicrobials. The student also found that *Salmonella* serovars possessed a wide range of virulence genes, rendering these strains highly pathogenic with the potential to cause infections in humans.

In collaboration with the Division of Systems Toxicology, we have investigated the rapid phenotypic characterization of *Salmonella enterica* and *Vibrio* strains by pyrolysis metastable atom bombardment mass spectrometry with multivariate statistical and artificial neural network pattern recognition.

We are characterizing 220 *Salmonella* and 120 *Vibrio* spp. isolated from seafood samples by molecular techniques, such as restriction fragment length polymorphism (RFLP), PFGE, ribotyping, ERIC-PCR, and RAPD methods. After characterization, a rapid microarray (gene chip) method will be developed to detect these pathogens in ocean-derived products. We choose to focus on *Salmonella* and *Vibrio* in this study due to our past research success with these organisms and their significance in foodborne illness. The results of this study will be used as a template for development of a diagnostic gene chip capable of simultaneous detection of multiple foodborne pathogens.

Antimicrobial Resistance

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

Antibiotics are extensively used for treatment and prevention of infectious diseases in humans and pets, as well as in food-producing livestock, poultry, and fish. *Escherichia coli* is a commensal bacterium of the gut microflora of the chicken. Some serotypes are pathogenic if they are inhaled into the respiratory tract and are probably the most frequent and economically significant cause of bacterial diseases, particularly colisepticemia, in broiler chickens. Fluoroquinolones are broad-spectrum antimicrobial agents effective in the treatment of a wide range of infections. Two fluoroquinolones, sarafloxacin and enrofloxacin, were approved by FDA in 1995 and 1996 for veterinary use to control morbidity and mortality associated with *E. coli*-related colibacillosis infections. The prevalence of fluoroquinolone-resistant *E. coli* in poultry ecosystems must be characterized because drug-resistant *E. coli* may play a role in the transfer of resistance to its clinical counterparts and may play a role in complicating the clinical treatment of *E. coli* infections in humans. We isolated and characterized nineteen fluoroquinolone-resistant *E. coli* strains from poultry litter. Sixteen of the nineteen strains were serotyped to groups 6, 8, 53, 56, 153, and 174. Three strains were not serotyped to any known group. All isolates were resistant to multiple antibiotics. Most strains were resistant to gentamicin, kanamycin, chloramphenicol, and streptomycin. Ribotyping of the multidrug-resistant isolates with the restriction enzyme *PvuII* showed five different ribogroups, suggesting independent development of resistance instead of clonal spread. Quinolone resistance was associated with mutations in the quinolone resistance-determining region (QRDR) of the *gyrA* gene in all cases. Fluoroquinolone resistance was present among different serotypes and ribotypes, and drug resistance profiles suggest that the incidence of resistance does not indicate a clonal population in avian *E. coli*.

Investigation of the drug-resistance profiles of *Salmonella typhimurium* DT104 from human and animal sources indicated that most strains of this serotype are resistant to multiple antibiotics, such as ampicillin, chloramphenicol, florfenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT-type). A multiplex PCR method was developed to identify these genes from clinical, food, and environmental samples. Currently, this method is used by ORA field labs to detect *S. typhimurium* DT104 in food samples.

130 *Salmonella* spp. isolated from seafood samples were analyzed for twenty antibiotics and found to be resistant to several antibiotics including fluoroquinolones and sulfonamides. Serotype analysis of *Salmonella* spp. grouped them into sixteen serogroups. PFGE analysis with *Xba*I digested serogroup B and C strains, showing genetic diversity among these strains. We have also found several plasmids in antibiotic-resistant *Salmonella* strains. Further characterization of these seafood *Salmonella* isolates for integrons, invasive genes, and virulence plasmids are in progress.

We developed a Rep-PCR method to differentiate vancomycin resistant (VRE) isolates at the genus and species levels. The method was able to determine the differences at the genus and species levels but, unlike the PFGE method, it was unable to differentiate the strains at the intra-species level. However, considering the ease of use, cost, and time, the Rep-PCR method appears to be effective for quick screening of VREs.

We have shown that a strain of *Enterococcus faecalis* had different MIC values on different media when tested by the E-test and broth dilution assays. The E-test showed vancomycin MICs in the range of 1-1.8 µg/ml, while the broth dilution assays indicated MICs in the range of 256-512 µg/ml. Reasons for the differences in MIC values in different media are not well understood. Recently, we isolated three variants from the zone of clearance. When retested, these variants, unlike the parental strain, had higher MIC values (> 256-512 µg/ml) by both the E-test and the broth-dilution assays. Mutations in the regulatory elements (*vanRS*) and the resistance determinant region (*vanHAX*) can cause hetero-resistance to vancomycin.

The *van* operon of *E. faecalis* (8.7-kb) was also cloned, and *Eco*R1 RFLP analysis of the cloned operon indicated differences between the wild type and variant 2. When the clones were grown on kanamycin and kanamycin-vancomycin plates, further differences were seen in the RFLP profiles. To date, there has only been circumstantial evidence about the involvement of vancomycin in causing hetero-resistance and RFLP variations, but our results clearly indicate a direct involvement of vancomycin in causing point mutations in the *vanR*, *vans*, and *vanH* genes. All the mutations are novel. The results suggest that the clinical use of vancomycin could have serious consequences for patients undergoing vancomycin therapy. The use of vancomycin may generate hyper-resistant VRE strains that are difficult to manage. The results also shed light on how clonally-related VRE strains originate with differences in resistance, PFGE, and RFLP profiles.

The Center for Functional Genomics and the Division of Microbiology have a collaborative research project for microarray detection of multiple antibiotic resistance markers. The microarray slides are printed and used in our research for the detection of resistance markers in bacteria of different ecological backgrounds. Extra probes were designed for

different genes that had indicated nonspecific hybridization in earlier microarray experiments. These were printed and tested for their specificity. PCR primers that were used for the amplification of various antibiotic resistance markers were also printed on the slides to determine their hybridization characteristics in microarray experiments.

We have continued working on molecular epidemiology and characterization of multiple antibiotic-resistant *Salmonella* strains from turkey production facilities. *Salmonella* serovars were isolated from turkeys and different environmental sources and genetically fingerprinted in an attempt to delineate the bacterial transmission pathways. *Salmonella* serovars appeared to originate from the birds and cross-contaminate other birds, drinkers, and litter samples. In addition to conventional serovars of *Salmonella*, unknown strains could also contaminate turkey production facilities. The prevalence of *Salmonella* serovars in this study appeared to be flock specific and source specific, and depended on the stages of the production cycle.

In collaboration with CFSAN, we are developing and validating a *Salmonella* biochip using the microarray technology. Development of a biochip for rapid and accurate identification of virulence and antimicrobial resistance genes in *Salmonella* and other infectious pathogens using microarray technology will meet the future challenges of counterterrorism. A *S. typhimurium* biochip has been developed that identifies 79 virulence genes using whole genome labeling and chemical modification of guanine with a fluorescent tag. A microbiologist in the Division has been trained in designing oligonucleotide probes used in the gene expression profile, DNA chip fabrication, DNA labeling using fluorescent tags, chip hybridization, scanning, and data interpretation. In this study, we will be using the technology to validate the chip's ability to detect virulence genes on emerging *Salmonella* serovars isolated from FDA field laboratories and from our culture collection. The effort will be useful in transferring microarray technology to the FDA field laboratories and law enforcement mobile labs.

Tetracycline, a broad-spectrum antibiotic, is widely prescribed for the treatment of numerous infections in humans. Analogs or derivatives of the drug have also been approved by CVM for use in aquaculture. Extensive use of the drug in aquaculture has resulted in the prevalence of tetracycline-resistant bacteria, which may complicate the antibiotic treatment of clinical infections. Thus, the spread and transfer of antibiotic-resistant genes from animals to humans and vice versa is a major public health problem. The FDA and Centers for Disease Control and Prevention (CDC) want to minimize the prevalence of drug-resistant bacteria and protect the efficacy of antibiotics. In addition, contaminated seafood is one of the causes of diarrheal infections, especially in immunocompromised individuals. The FDA wants to protect the safety and hygiene of the nation's food supply and requires data on the

prevalence of drug-resistant pathogenic bacteria, resistance profiles of these bacteria, types of gene transfer, and mechanisms of resistance to antibiotics.

We isolated 81 tetracycline-resistant *Aeromonas* spp. strains from farm-raised catfish. Morphological and biochemical characteristics indicated that 23 of the 81 aeromonads were *A. hydrophila*, seven isolates were *A. trota*, six isolates were *A. caviae*, 42 isolates were *A. veronii* and three isolates were *A. jandaei*. However, the RFLP patterns of the PCR amplified rRNA genes from all 81 tetracycline-resistant aeromonads from catfish were identical to the RFLP banding patterns of *A. veronii*, indicating that all 81 isolates were strains of *A. veronii*. All isolates were resistant to tetracycline, ampicillin, and penicillin and most were also resistant to bacitracin. A multiplex PCR was designed to screen and amplify tetracycline-resistance determinants (*tetA-E*) from the genomic DNA of all 81 isolates. The assay amplified a 221-bp *tetA*, a 391-bp *tetB*, an 897-bp *tetC*, and an 844-bp *tetD*. The protocol detected a 744-bp *tetE* from the cell extracts of 90.0% of the aeromonads. Plasmids were isolated from 51 of the 81 isolates. The aeromonads were further characterized by PFGE after their genomes were digested with the *SpeI* restriction enzyme. Sixteen of the 81 aeromonads were untypable by the PFGE method. Based on the *SpeI*-PFGE profiles and dendrogram analysis of 65 isolates with the Bionumeric software, 15 distinct macrorestriction patterns (mrps) were detected. Our results indicate the need for use of 16S rRNA-based methods in the identification of *Aeromonas* spp. and the prevalence of catfish as a reservoir.

We have isolated 37 *Citrobacter* spp. from catfish. Morphological and biochemical characterization indicated that 26 isolates were *C. freundii*, six were *C. braakii*, and five were *C. amalonaticus*. All isolates were resistant to multiple antibiotics. Most isolates contain plasmids. Currently, we are investigating the transferability of tetracycline-resistant determinants to *E. coli* and *Aeromonas* by conjugation.

We are collaborating with the Arkansas Regional Laboratory to isolate tetracycline-resistant bacteria from imported fish samples. Antibiotic-resistant microflora from fish farms in foreign countries may be exported to the U.S. We are determining if imported fish and their microflora contain drug-resistant pathogenic bacteria.

Fluoroquinolones are commonly prescribed for a variety of bacterial infections. Such treatment practices may be increasing the prevalence of fluoroquinolone-resistant strains, especially of anaerobic bacteria from the gastrointestinal tract, which are not normally susceptible to this group of compounds. These bacteria are likely to cause subsequent clinical infections in colonized individuals.

The efficacy of fluoroquinolones against anaerobic bacteria depends on the molecular structure. By the generation of mutants resistant to various concentrations of fluoroquinolones of different structures, we have investigated the role that specific fluoroquinolone modification plays in the induction of resistant mutants in anaerobic bacteria. We have used fluoroquinolones of similar structure with molecular variations to induce mutations in *Clostridium perfringens*, which is a pathogen implicated in food poisoning and fatal human and poultry infections. We have found effects specifically related to the type of structural alteration and the concentration of fluoroquinolone. These have resulted in alteration in two of the main fluoroquinolone targets, gyrase and topoisomerase. The major changes have occurred in the specific portions of the enzyme molecules, which bind to fluoroquinolones. Addition of a methoxy group has been proven to be important in the induction of mutation. We have shown that various strains of these bacteria differ in resisting fluoroquinolones and generating mutants in response to these compounds. *C. perfringens* strains have produced much more readily the types of mutations that are rare in facultative and aerobic bacteria. Even a single mutation generated in response to low concentrations of older fluoroquinolones protects *C. perfringens* bacteria against large concentrations of more effective newer fluoroquinolones. This work has been in collaboration with U.S.D.A.

In addition to the mutations in targets, we have studied other factors involved in fluoroquinolone resistance in anaerobic bacteria. Multidrug resistance (MDR) efflux pumps are tools that bacteria use to cope with intracellular toxic molecules. The expression of these protein pumps is up-regulated during exposure to the drugs. These proteins pump out structurally diverse compounds, including fluoroquinolones, thereby preventing drug accumulation in the cells and access to the intracellular targets. By screening colonic microflora for fluoroquinolone-resistant strains of anaerobic bacteria, we have identified a *Clostridium* mutant that can grow with large concentrations of these compounds. The efflux of drugs in this strain and its parent strain has been determined by measuring fluoroquinolone sensitivity, drug accumulation, and the effects of different concentrations of fluoroquinolones on the kinetics of growth in the presence and absence of known inhibitors of MDR efflux pumps.

Gastrointestinal Microbiology and Host Interactions

The human gastrointestinal tract is populated with a complex and diverse population of anaerobic bacteria. These bacteria play an important role in human health, acting as a barrier to colonization of the intestinal tract by pathogenic bacteria, as well as contributing to the digestion of dietary components and metabolism of drugs, xenobiotics, and nutrients.

We obtained a collection of oligonucleotide probes that target bacterial genera rather than individual species of the predominant microflora in the human gastrointestinal tract. These probes were affixed to a membrane support and hybridized to PCR-amplified DNA samples extracted from

pure cultures and mixed cultures of fecal bacteria. The probes designed to identify *Bifidobacterium* and *Bacteroides* were sensitive and specific, while the probes for *Lactobacillus*, *Enterococcus*, *Clostridium*, and several other groups gave variable results.

The art of tattooing has a long history. The pigments used in tattoos and topically applied colorants are subject to FDA regulation. The metabolism of the tattoo pigments and topically applied colorants by the skin and intestinal microflora and the potential toxicity of the reaction products to the body are currently being investigated. This investigation provides valuable information on the toxicity of tattoo pigments and topically applied colorants that are metabolized by bacteria and their enzymes. We have characterized a tetrameric NADPH-dependent azoreductase from a skin bacterium, *Staphylococcus aureus*. The properties of the azoreductase indicate that it is FMN-dependent and has a broad spectrum of substrate specificity capable of degrading a wide variety of azo dyes. We have demonstrated that the anaerobic skin bacterium *Propionibacterium acnes* is able to convert tattoo pigment PY 74 to an aromatic amine, which potentially is carcinogenic. To understand the mechanisms of dye degradation, it is essential to know the structures of the azoreductases. In cooperation with the University of Georgia, the azoreductase from *Enterococcus faecalis* was successfully crystallized in the presence of FMN and the crystal was diffracted at 2.0 Å. Its structure was resolved using an expressed selenomethionine-substituted *E. faecalis* azoreductase protein.

We are conducting research on biodegradation of potentially carcinogenic and mutagenic Sudan dyes found as contaminants in chili powder and other food products by intestinal microflora. We have demonstrated that some of these Sudan dyes can be metabolized by intestinal microflora to produce genotoxic compounds. We are also developing a method using HPLC and LC/MS to detect five Sudan dyes in contaminated food products.

Herbivorous animals depend for their survival on a symbiotic association with microorganisms in their alimentary tract. The anaerobic fungi represent an important part of the gut microflora. They produce a wide range of enzymes capable of hydrolyzing many compounds. In cooperation with the U.S.D.A., we are studying important hydrolytic enzymes from an anaerobic fungus, *Orpinomyces* sp. We also developed a method for large-scale isolation of extremely AT-rich genomic DNA and analysis of genes encoding several important hydrolytic enzymes from this fungus.

Environmental Biotechnology

A major focus in the environmental biotechnology area in the Division has been the investigation of the biodegradability of a wide range of pollutants

with special emphasis on polycyclic aromatic hydrocarbons and antimicrobial agents.

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of organic compounds whose environmental fate is of concern because some PAHs have mutagenic, ecotoxic, and carcinogenic potential. Studies have been conducted to elucidate the biodegradative pathways of benzo[*a*]pyrene, benz[*a*]anthracene, and 7, 12-dimethylbenz[*a*]anthracene and the enzymes involved in PAH metabolism. *Mycobacterium vanbaalenii* is capable of degrading a number of PAHs to ring cleavage metabolites via multiple pathways. Proteomic and genomic techniques have been developed to identify proteins and genes involved in the bacterial metabolism of PAHs. Molecular cloning and functional characterization of dioxygenases, dehydrogenases, cytochromes P450, and other putative enzymes involved in the degradation of benz[*a*]anthracene, fluoranthene, pyrene, and phenanthrene have been determined. This research increases our understanding of the environmental fate of PAHs for developing practical bioremediation strategies in the future.

The environmental fates of veterinary drugs and factors that influence the biodegradation of antibiotics used in farm animals and aquaculture have been investigated. Flumequine is an antibacterial fluoroquinolone drug that is used in aquaculture and veterinary medicine in Europe but has been reported to cause liver tumors in mice. We have been investigating the ability of the fungi *Wolfiporia cocos* and *Cunninghamella elegans* to transform flumequine. We separated the metabolites by high-performance liquid chromatography and identified them by ultraviolet spectroscopy, mass spectrometry, and nuclear magnetic resonance spectroscopy. The products included two isomers of 7-hydroxyflumequine, which is known to be a mammalian metabolite with greatly reduced antibacterial activity, and 7-oxoflumequine, which has not been found previously.

N-Phenylpiperazine is a model heterocyclic compound that is structurally related to some fluoroquinolone drugs and pollutants produced by the pharmaceutical and dye industries. We extracted cultures of eight different strains of *Mycobacterium* that had been dosed with *N*-phenylpiperazine; high-performance liquid chromatography showed that all of the strains produced the same two metabolites. Mass spectrometry and nuclear magnetic resonance spectroscopy identified them as *N*-acetyl-*N'*-phenylpiperazine and *N*-(2-anilinoethyl)acetamide. The mycobacteria may play a role in the metabolism of piperazinyll drugs and the detoxification of industrial effluents containing piperazine.

The potential for environmental bacteria to biotransform fluoroquinolone antibacterial agents was investigated. We grew 18 *Mycobacterium* cultures with norfloxacin, extracted them with ethyl acetate, and analyzed them by high-performance liquid chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy. Eight strains produced *N*-

acetylnorfloxacin, and two of them also produced *N*-nitrosonorfloxacin. *N*-Acetylated fluoroquinolones are known to have reduced antibacterial activity. The antibacterial activity of *N*-nitrosonorfloxacin was tested against Gram-negative and Gram-positive bacteria and found to be much less than that of norfloxacin; however, as a nitrosamine, it is potentially carcinogenic to mammals.

Microbiological Surveillance and Diagnostic Support of Research

The primary mission of the Surveillance and Diagnostic Program continues to be the assurance that NCTR research data is not compromised by the use of infected or unhealthy experimental animals. To that end, during FY 2005, the Surveillance/Diagnostic Program prevented pathogen introduction by quarantined animals by 1) detecting pathogenic endoparasites and pathogenic bacteria in Rhesus monkeys received from a commercial vendor and preventing these animals from being introduced into the NCTR primate colony until they had been treated, 2) detecting bacterial pathogens in the cage water from commercially purchased mice, 3) monitoring the health, environment and food of the animals in the established NCTR breeder colonies, and 4) working closely with Veterinary Services to monitor the success of a bacterial pathogen eradication program in the NCTR breeder mouse population.

We also provided microbial cultures to researchers in the United States and foreign countries as well as to researchers at NCTR. We provided technical support and assistance to NCTR researchers by 1) supplying and maintaining microbial cultures used for research, 2) supplying culture media and reagents, and 3) providing culture identification and antibiotic sensitivity testing.

FY 2006 Plans

Foodborne Pathogens, Food Safety, and Methods Development

We will characterize *Salmonella* and *Vibrio* spp. isolated from seafood samples by pulsed-field gel electrophoresis (PFGE), multiplex PCR ribotyping, ERIC-PCR, multilocus sequencing, and RAPD methods. Multidrug-resistant bacteria, including sulfonamide and ciprofloxacin-resistant *Salmonella* spp., will be characterized for integrons. The amplified integrons will be sequenced to determine any unique characteristics.

After characterization of *Salmonella* spp. and *Vibrio parahaemolyticus*, a rapid microarray method will be developed to detect these pathogens in seafood. *Salmonella* and *Vibrio* are important pathogens that can cause illness in humans after consumption of contaminated seafood. The results of this study will be used for development of a diagnostic gene chip capable of simultaneous detection of multiple foodborne pathogens.

We will do sequence typing on the remainder of *Salmonella* serovars and analyze the fingerprint patterns using the Bionumerics software. In addition, we will validate the efficacy of a *Salmonella* biochip on selected *Salmonella* serovars.

As part of a study with the Marshfield Clinic Research Foundation, Marshfield, Wis., North Dakota State University, and the ORA's Arkansas Regional Laboratory, we will continue to examine the epidemiology and molecular genetics of *Salmonella* Heidelberg, an emerging pathogen, in populations in the U.S.

Antimicrobial Resistance

We plan to improve some of the details in the microarray screening method for antibiotic resistance markers. As a result of initial screening, a number of resistance markers hybridized nonspecifically with other markers. The probes for these markers have been redesigned along with the probes for fourth-generation cephalosporins and beta-lactamases. Once the microarray slides are printed, these will be tested to detect antibiotic resistance markers on foodborne pathogens and human clinical isolates.

The Van operons from 17 human VRE isolates were amplified and tested for differences by a PCR-RFLP method. These isolates indicated a difference in the operons. The van operon from these isolates will be cloned and sequenced to determine differences at the sequence level.

Aeromonas spp. isolates obtained from the CVM will be analyzed by PFGE, PCR, and hybridization.

We plan to study host-pathogen interactions, especially the expression of host and pathogen genes, by microarray analysis. Human intestinal epithelial cells and foodborne pathogens will be used as model systems.

We will conduct conjugation experiments to determine the rate of *tet* gene transferability from *tet*-resistant *Aeromonas* spp. to *tet*-sensitive *E. coli*. Similar studies will be done with *tet*-resistant *Citrobacter* isolates. In addition, we will characterize *tet*-resistant *Citrobacter*, *E. coli* and *Pseudomonas* isolates from aquaculture samples.

We will continue evaluation of the effect of fluoroquinolones on resistance development in bacteria from the human intestinal tract. Analysis of the fluoroquinolone-resistance mechanisms in anaerobic bacteria from the human intestinal tract and other sources will also be determined.

Our future endeavors include the development of proteomic approaches to identify *Staphylococcus aureus* extracellular proteins responsible for staphylococcal pneumonia and ways to improve protein coverage. One approach is to improve our quantitative analysis of proteins by utilizing

$^{12}\text{C6}$ - and $^{13}\text{C6}$ -arginine incorporation. In addition, we will begin to examine those extracellular proteins found to be regulated by *sar* and *agr* genes and determine their roles in disease. Future studies will involve examining those proteins found to play a role in disease for their antigenic properties and determining whether or not these proteins would be suitable candidates for passive immunotherapy development.

We will also continue to collaborate with St. Jude Children's Research Hospital on the threat of pandemic influenza. Over the past seven years, human infections, as a result of exposure to avian influenza viruses, have raised serious concerns of an approaching pandemic. While influenza and pneumonia represent a leading cause of death worldwide, rarely does a primary viral infection result in death. Much more common is the exacerbation of a preexisting condition or the predisposition to a secondary bacterial infection. In many cases, the bacterial agent encountered is *S. aureus*. Currently our collaboration with St. Jude involves the use of mouse and guinea pig models of pneumonia to understand the interactions between influenza and *S. aureus* and how these two infectious agents result in elevated mortality rates.

Gastrointestinal Microbiology and Host Interactions

We will examine the competition of endogenous steroid compounds with typical exogenous drug substrates for multidrug efflux pump function. In addition, we will determine whether steroid compounds can influence efflux pump expression. We have already obtained commercial *E. coli* whole-genome microarrays and plan to profile the bacterial transcriptomic response to mammalian hormones through collaboration with the NCTR's Center for Functional Genomics. We also plan to profile the proteomic response with membrane and cytosolic extracts through collaboration with the Division of Systems Toxicology's Proteomics Facility. This may yield new information regarding the human-microbe interaction. Drug resistance in *Lactobacillus* serves as an important indicator of GI and vaginal tract health. *Lactobacilli* are heavily used by consumers intentionally as probiotic supplements or unintentionally in microbially fortified foods. Lately, we have turned increasingly to *E. coli* because of its ease of manipulation as a commensal microbe. Less complicated studies where minimal molecular manipulation is required, such as assessing growth fitness or identifying metabolites of endogenous steroid molecules (hormones and bile acids), will be conducted in *Lactobacillus*, as will studies assessing resistance to microbicides and spermicides in conjunction with vaginal health. *Lactobacillus* microarrays (NimbleGen Systems, Inc.) will be used to address questions similar to those we have addressed recently in *E. coli*. Thus, we will have a perspective on this issue from both a commensal Gram-positive aerotolerant anaerobe and a Gram-negative facultative anaerobe.

We will analyze degradation products of tattoo dyes and Sudan dyes by skin and intestinal microorganisms, using specific enzymatic treatments,

HPLC, and LC-MS/GC-MS methods. Since the three-dimensional structure of the azoreductase from *Enterococcus faecalis* has been solved, site-directed mutagenesis will be employed to study the enzyme activity center and FMN binding motif. We will continue to use DNA probes and antibodies from *E. faecalis* and *S. aureus* azoreductases for screening similar genes in skin and intestinal microflora to determine the distribution of the azoreductase genes among predominant bacteria and the enzyme expression levels in these microorganisms. We will continue to study structure and function of the azoreductases from *S. aureus* and environmental microorganisms.

We are collaborating with the University of Arkansas in Fayetteville on the effects of *Echinacea* on the human intestinal microflora. Fecal samples obtained from test subjects whose diet had been supplemented with *Echinacea* were plated on selective and nonselective media to detect population changes in major aerobic and anaerobic bacterial species. Statistical analysis suggests significant changes in several bacterial groups.

We plan to develop and validate real-time PCR assays to monitor changes in the populations of different bacteria in fecal cultures.

We will continue gene cloning and PCR analyses of antimicrobial drug-resistance genes in *Lactococcus lactis*.

We propose to acquire a few breeding pairs of germfree mice from the Medical University of South Carolina. After sufficient animals are raised, experiments described in current protocols can be completed.

We plan to develop an *in vitro* project to study chemokine expression activation in intestinal epithelial cell lines after contact with intestinal bacteria and the enteric opportunistic pathogen *Candida albicans*.

Environmental Biotechnology

For proteomic approaches to elucidate PAH biodegradative pathways, we will identify proteins that show different expressions, when induced by PAHs, and conduct comparative studies on functions and expressional regulation of enzymes and genes involved in the initial steps of PAH degradation.

We will identify microorganisms that either completely degrade fluoroquinolones or modify the fluoroquinolone molecule so as to reduce its toxicity to bacteria.

Microbiological Surveillance and Diagnostic Support of Research

The goal for FY 2006 is the development and implementation of new techniques for the detection and identification of pathogenic microorganisms.

CRADA

We have spent substantial time this year to develop a CRADA with Pfizer Animal Health to study the metabolism of third-generation cephalosporins by normal intestinal bacteria. Through meetings and conversations with Pfizer, and the efforts of a team of microbiologists in the Division of Microbiology, we have written a research plan and full CRADA document that have recently been approved by the FDA Commissioner.

Preliminary evidence from Pfizer suggests that the drug ceftiofur is very rapidly degraded in the bovine gut. Understanding the interaction between extended spectrum cephalosporins, such as ceftiofur, and the intestinal microflora will help the FDA to evaluate the safety of these antibiotics for different applications and better understand the potential for the development of clinical resistance.

Contribution to FDA's Strategic Goals

The Division of Microbiology uses an interdisciplinary approach to be responsive to FDA regulatory needs. The Microbiology Division staff has a number of projects in conjunction with other FDA Centers to provide critical research to address FDA's strategic goals.

Foodborne Pathogens, Food Safety, and Methods Development

Salmonella and *Vibrio* cause thousands of infections and illnesses every year. In the past two years, there were more than 3000 confirmed *Salmonella* infections in the United States. A total of 220 *Salmonella* spp. contaminated food products were identified by the PRL-SW FDA laboratory, and 71% of these were associated with contaminated seafood. In 1999, there were 218 confirmed illnesses resulting from *Vibrio* and 93% of the patients had consumed seafood. In an effort to promote FDA's mission by better ensuring that the nation's food supply is safe and sanitary, we will address microbial pathogen contamination in seafood and the development of improved molecular diagnostic methods for detecting their presence. The long-term goal is to develop a diagnostic gene chip capable of simultaneous detection of hundreds to thousands of foodborne pathogens that will replace all current diagnostic methods for pathogen detection in food products.

The Center for Veterinary Medicine (CVM) reviews and approves the use of antibiotics in veterinary practice. Recently, multidrug-resistant *S. typhimurium* DT104 strains have been isolated at higher frequency in food-producing animals, poultry, and humans. Since this is a safety issue, as defined by the Federal Food, Drug and Cosmetic (FFD&C) Act, the FDA needs adequate information on the prevalence of drug-resistant bacteria and the factors that contribute to their spread. Most of the *S. typhimurium* DT104 strains are characterized by chromosomal resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT-resistant). Recent reports published by the National

Salmonella Antimicrobial Resistance Monitoring System (CDC) suggested that almost 28% of the *S. typhimurium* isolated had the R type ACSSuT as compared to 7% in 1990. Several strains isolated from animal farms, poultry, and human isolates were studied for these multiple antibiotic genes. We found that all of the DT104 isolates contain at least two integrons, one containing the aminoglycoside resistance gene cassette *ant (3'')-la*, which encodes resistance to streptomycin, and one containing a beta lactamase resistance gene cassette, *pse-I*, which encodes resistance to ampicillin. A gene encoding sulfonamide resistance (*sul-I*) was found in the 3' conserved sequences of both integrons. We also found that all definitive DT104 strains were resistant to florfenicol. Florfenicol is a fluorinated analog of chloramphenicol approved by FDA in 1996 for the treatment of bovine respiratory pathogens. The florfenicol resistance gene encodes resistance to both chloramphenicol and florfenicol. Current diagnostic methods used for the identification and characterization of ACFSSuT-type DT104 strains require several days. Since DT104 ACFSSuT-type strains are becoming widespread globally, development of a rapid and sensitive method for the identification of DT104 strains was important. Based on our genetic studies on these isolates, we designed a multiplex PCR method to simultaneously amplify four genes (florfenicol, virulence plasmid, invasion gene, and integron). Twenty-two ACFSSuT-resistant DT104 isolates in our collection gave 100% positive reaction to this PCR assay. The invasion gene operon *invA* is essential for full virulence of *Salmonella*, and our results indicated that all multidrug-resistant *Salmonella* strains were positive for this gene. A plasmid *spvC* that is present in *Salmonella* spp. interacts with the host immune system and is responsible for an increased growth rate in host cells. Our PCR results indicated that the *spvC* gene was present in 97% of *S. typhimurium* strains, and all multidrug-resistant ACFSSuT-type strains were positive for the *spvC* plasmid. This method can be used for rapid and specific identification of ACFSSuT-type DT104 strains from food, clinical, and environmental samples. This method is now successfully used in the New York ORA laboratory.

The food safety research project highlights the impact of preharvest control of foodborne pathogens as the necessary first step in the “farm-to-the-fork” continuum. This research can help poultry producers and regulatory agencies design hazard analysis and critical control points (HACCP) protocols to safeguard fresh poultry against pathogen contamination during processing. Monitoring genotypes and virulence gene determinants of bacterial pathogens generated from this research will yield important information to the FDA and U.S.D.A. to assess the distribution of genetic variability and pathogenicity among these pathogens in sampled sources. The bacterial fingerprinting studies will ultimately supplement on-going surveillance programs such as PulseNet and FoodNet in implementing newer food safety regulations and consumer education efforts in protecting the public health in the U.S. The baseline studies generated by this project can be used by the FDA in monitoring the

prevalence of drug-resistant pathogens and developing public policies for the use of antimicrobials in food producing animals in the U.S. The drug-resistance surveillance and monitoring programs will play a key role in the development of national and perhaps international regulatory policies for the containment of antimicrobial-resistant bacterial pathogens from animals and ecological niches. The data from humans, livestock, and FDA-regulated food animals could be compared and analyzed for patterns of novel emerging phenotypes. Rapid and cost-effective detection of biological agents using PCR, microarray, and MS technologies will be useful to the first responders of the Departments of Health and Human Services and Homeland Security and the Federal Bureau of Investigation (FBI) in limiting the potential of an outbreak (accidental or bioterrorism), thereby saving lives and dramatically reducing the medical costs involved in treating illnesses.

Antimicrobial Resistance

In July 1999, an interagency task force on antimicrobial resistance co-chaired by the Centers for Disease Control and Prevention (CDC), the FDA, and the National Institutes of Health (NIH) formulated a document titled ***A Public Health Action Plan to Combat Antimicrobial Resistance***. The document identified 84 different action items covering surveillance, prevention and control, research and product development, and assigned various federal agencies as coordinators. Of the 84 action items identified, 13 were categorized as top priority. The research being conducted in one project falls within two of the 13 top action items categorized as research: 1) Provide the research community with genomics and other powerful technologies to identify targets in critical areas for the development of new rapid diagnostic methodologies, novel therapeutics, and interventions to prevent the emergence and spread of resistant pathogens (Action Item #70); and 2) encourage applied and clinical research in support of the development and appropriate use of vaccines in human and veterinary medicine in partnership with academia and the private sector (Action Item #77).

In keeping with the Task Force action plan, which coincides with FDA's mission, we have established collaborations with the Proteomic Center at NCTR to develop a proteomic approach to generate a protein profile for the Gram-positive bacterium, *Staphylococcus aureus*. This bacterium remains an important pathogen, responsible for numerous disease syndromes in humans and animals worldwide.

The incidence of infection and colonization with vancomycin-resistant enterococci (VRE) in U.S. hospitals has increased in the last five years, and 12% of all nosocomial infections are caused by *Enterococcus* spp. that are highly resistant to vancomycin and readily acquire resistance to other antimicrobials. In addition, the transfer of vancomycin resistance genes from VRE to other Gram-positive microorganisms, especially *Staphylococcus aureus*, by plasmid or transposon-mediated mechanisms

both *in vitro* and *in vivo*, is a serious public health concern. Therefore, it is important to study the mechanism of vancomycin resistance in these organisms and develop methods to detect the presence of multiple antibiotic resistance markers. This will also help in assessing the risk factors associated with the spread of drug-resistant organisms.

The use of microarray technology in monitoring the drug resistance and potential resistance transfer markers will help FDA track the emerging pattern of antibiotic resistance in foodborne bacteria and assist in making sound regulatory decisions about the agricultural use of certain antibiotics. From the public health point of view, the monitoring of bacterial isolates from food and human clinical sources would help in designing strategies to combat the problem of emerging antimicrobial resistance and have a positive impact on animal and human health. The development of such a chip and its use would be of general interest to the scientists working in antibiotic resistance and of particular interest to the scientists in the regulatory areas at CVM. The results of the study will help the FDA in achieving its strategic goals by safeguarding the food supply from the contaminating multidrug-resistant bacteria and regulating the misuse of antibiotics in farm animals and humans.

Conducting scientific research on antimicrobial agents provides FDA with fundamental information needed for making regulatory decisions on these compounds. Resistance to antimicrobial drugs among anaerobes is increasing, especially for commonly prescribed drugs. Newer fluoroquinolones have activities against a broad range of aerobic and anaerobic bacteria. These drugs are useful for treatment of mixed infections, such as serious intra-abdominal infections, in which both anaerobes and aerobes are involved. However, strains resistant to newer fluoroquinolones have been also found and newer, more effective, drugs are being developed and will need to be regulated by FDA.

Understanding the mechanisms of drug resistance is critical, not only for the development of effective new drugs, but also to provide a tool to find out if the new drugs overcome these mechanisms. Intrinsically fluoroquinolone-resistant bacteria, such as those found in our investigations, are good candidates for assaying the potency and effectiveness of new fluoroquinolone drugs that are developed. In addition, inhibition of efflux pumps as a novel approach to overcome drug resistance is of interest to pharmaceutical researchers. Bacteria, such as *Clostridium*, that use a multidrug efflux pump are useful tools for the development of these pump inhibitors and regulation of their use.

Gastrointestinal Microbiology and Host Interactions

Assessing the safety of drugs and other exogenous compounds involves understanding their effects on the GI tract microbiota, manifested in FDA guidance #52. This research will determine whether steroid compounds can influence the resistance phenotype of commensal bacteria in the GI tract or transient pathogens. This class of molecules, when used in drug

therapy, is not typically antibacterial, thereby diminishing any selective pressure to induce resistance; yet, the newly discovered connection in our lab between intrinsic bacterial resistance mechanisms and steroids (bile acids and hormones) suggests otherwise. Because multidrug efflux pump resistance mechanisms are so powerful, effectors that can influence their expression and/or function can also alter the resistance to a plethora of clinically significant drugs. Since steroids are used extensively in human drug therapy, it is necessary to address their threshold levels as outlined in FDA guidance #52.

The intestinal microflora play an important role in human health, acting as a barrier to infection as well as contributing to the digestion of dietary components and metabolism of drugs. We are developing the methods to predict and monitor changes in this complex bacterial population. These approaches will allow the FDA to gain a clearer understanding of how drug residues or xenobiotic substances affect the intestinal microflora and how changes in this population may affect human health.

The *in vitro* competitive exclusion (CE) assay provides a technique for evaluating mixtures of defined bacteria for use as competitive exclusion products before the use of *in vivo* testing in poultry. This assay addresses the FDA strategic goal of “improving product quality and safety through better manufacturing oversight”. The information gained on the use of 16S rRNA sequence identification of bacteria also aids in this goal by showing that the technology improves the characterization of CE products, which is necessary for their regulatory approval.

The cell culture-based assay provides an additional method to assess the concentrations of antimicrobial drugs present as food residues that can perturb the protective functions of the intestinal bacterial population against foodborne pathogen colonization. This project addresses the FDA strategic goal of “increasing access to innovative technologies to advance health”.

Another project is giving us important knowledge of the biological effects of probiotic products on the immune system of the consumer and on the microbial ecology of the consumer’s intestinal bacterial populations. The FDA strategic goal of “improving product quality and safety through better manufacturing oversight” may be addressed by this project if the legal impediment to regulation of “food additives” changes. Probiotic products are not currently regulated as drugs, even though their use implies claims of health benefits.

We are also investigating the capacity for antimicrobial drug-resistant bacteria isolated from a CE product to transfer resistance genes among themselves and to other bacteria likely to be present in the intestinal bacterial populations of poultry consumers. The project addresses the

FDA strategic goal of “improving product quality and safety through better manufacturing oversight”.

The pigments used in tattoos and topically applied colorants are subject to FDA regulation; however, at this time the FDA has not exercised regulatory authority concerning the chemicals used in tattoo inks. The data generated on the metabolism of azo colorants by the skin and intestinal microflora and the potential toxicity of the reaction products to the human body will provide valuable information on the fate of the dyes, which, with other related studies in the FDA, will initially develop sound scientific knowledge for determining the safety and toxicity of the tattoos and topically applied colorants.

Food safety represents one of the main issues of the FDA. Sudan azo dyes are classified as category 3 carcinogens that may not be used as color additives in food products. A sensitive method to simultaneously detect Sudan dyes in contaminated food products could be used by FDA laboratories to prevent the importing of contaminated products. Research on biodegradation of the possible human carcinogenic and mutagenic Sudan dyes found in the contaminated food products by intestinal microflora could provide useful information regarding the potential toxicity of these dyes as well.

The microbiological safety of food is a high priority for FDA. Novel rapid and sensitive molecular approaches, including microarray and PCR methods, will be developed for the detection of intestinal bacteria. These methods could be applied for monitoring foodborne pathogens in food products.

Environmental Biotechnology

PAHs are one of the largest classes of chemicals present in the environment and have been considered a health risk for humans for a long time. Exposure to PAHs occurs by food intake, cigarette smoke, smoke from the burning of fossil fuels, and occupational exposure. The carcinogenic properties of PAHs have been extensively studied and it is now widely known that PAHs are metabolized to intermediates that may react with DNA and other macromolecules. This process is accomplished not only by mammalian but also by fungal and bacterial enzymes. For example, the PAH benzo[*a*]pyrene (BaP) is bioactivated by enzymes, such as cytochrome P450 to BaP diol epoxide, which binds to DNA and induces GC→TA transversions. PAHs in cosmetics have been one of the concerns of FDA and are regulated as a potential carcinogen. In our Division, metabolism of several PAHs has been investigated by using fungi and bacteria to understand the mechanisms of PAH biotransformation. Studies also include identification of PAH intermediates, elucidation of PAH metabolic pathways, and cloning/expression of genes and enzymes involved in PAH degradation. Investigation of microbial biotransformation reveals the pathways by

which these compounds are transformed to harmful metabolites. It increases our understanding of conversion of these compounds by parallel pathways accomplished by mammalian enzymes, which impact human health.

Research on the biotransformation of fluoroquinolones suggests that fungi that grow on poultry litter may degrade residues of antimicrobial drugs, reducing the pressure for the selection of drug-resistant strains of bacteria.

Ongoing Research Projects

Cerniglia, Carl E.

Proteomic Approaches to Elucidate Biodegradative Pathways (E0711801)

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

Chen, Huizhong

Novel Molecular Approaches for the Detection and Analysis of the Predominant Bacterial Species in the Human Gastrointestinal Tract (E0711901)

- Objective(s):**
- 1) Develop a rapid method for quantification of intestinal bacteria;
 - 2) Conduct qualitative analysis of the communities for several major genera and discover the species which are noncultivated;
 - 3) Isolate and identify the bacterial species from probiotics used for human or animal health; and
 - 4) Develop a microarray method for the detection of intestinal bacteria.

Chen, Huizhong

Genomic Approaches to Determine the Role of Skin Microflora in the Metabolism of Tattoo Dyes (E0717901)

- Objective(s):** The research will be focused on metabolic capacity and enzyme expression in human skin microflora. The objectives of the project are:
- 1) Biodegradation and bioconversion of pigments used for tattooing and permanent make-up pigments;
 - 2) Determine the effects of the skin microflora on tattoo and topically applied dyes that reside in the dermis;
 - 3) Isolate, clone, and over-express genes encoding for azoreductases and nitroreductases, which are able to decolorize the pigments;
 - 4) Determine physicochemical properties of the purified native enzymes from the bacteria and/or the expressed recombinant enzymes cloned in *E. coli*; and
 - 5) Elucidate the role of the microflora with potential genotoxic effects of tattoo and permanent make-up pigments.

Elkins, Christopher

Assessment of Membrane-Associated Antibiotic Resistance Mechanisms in Lactobacilli (E0718001)

- Objective(s):**
- 1) Evaluation of intrinsic drug resistance of *Lactobacillus* isolates from various sources;
 - 2) Determine *Lactobacillus* resistance to microbicides and spermicides in relation to vaginal health;
 - 3) Profile transcriptomic and proteomic responses of *Lactobacillus* and *E. coli* in the presence of various steroids (bile acids and hormones), microbicides, and spermicides;
 - 4) Examine whether endogenous gastrointestinal tract or

	<p>synthetic steroid drug compounds can induce efflux pump expression in either bacterial background; and</p> <p>5) Analyze drug competition for efflux pump function in the presence of endogenous or synthetic steroid compounds.</p>
Erickson, Bruce D.	<p>Determining the Effect of Low Levels of Antibiotic Residues on the Human Intestinal Microflora using an <i>in vitro</i> Continuous Culture System (E0709201)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Develop and refine methods for monitoring changes in the complex mixture of bacterial species that comprise the intestinal population; and 2) Determine the concentration of selected antimicrobial compounds that produce no adverse effect on the human intestinal microflora using several <i>in vitro</i> culture approaches including batch, fed-batch and continuous flow culture systems to simulate the human intestinal environment.
Erickson, Bruce D.	<p>Evaluation of the Mechanisms of Inactivation and Degradation of Third Generation Cephalosporins by the Bovine Intestinal Microflora (E0709201)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Evaluate the ability of the bovine intestinal microflora to inactivate ceftiofur using pure culture isolates and mixed fecal cultures; 2) Identify primary metabolites of ceftiofur degradation; 3) Isolate ceftiofur-resistant bacteria and determine the primary mechanisms of drug inactivation; 4) Investigate the metabolic potential of anaerobic fungi isolated from bovine fecal samples to degrade ceftiofur; and 5) Compare the metabolism of ceftiofur with the human third generation cephalosporin, ceftriaxone.
Hart, Mark E.	<p>Development of Proteomic Approaches to Identify <i>Staphylococcus aureus</i> Extracellular Proteins Responsible for Staphylococcal Pneumonia (E0717501)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Develop a proteomic approach of identifying proteins by first fractionating proteins in spent media using isoelectric focusing followed by nonporous, reverse phase HPLC. Proteins isolated in this manner will be submitted to protease degradation, and peptide profiles will be generated using LC/MS/MS. Peptide profiles will be submitted to various protein databases to identify the proteins; and 2) Generate a proteomic profile for <i>S. aureus</i> RN6390 and its <i>agr</i> and <i>sar</i> isogenic mutants. These profiles will be compared to identify differences between strains and thus, preliminarily identify potential proteins responsible for the lethality observed in the mouse model of pneumonia.
Khan, Ashraf A.	<p>Molecular Characterization of <i>Salmonella</i> spp. and <i>Vibrio</i> spp. Isolated from Seafood and Development of Microarray Detection Method (E0720801)</p> <p>Objective(s): Characterize representative isolates of <i>Salmonella</i> and <i>Vibrio</i> spp. by molecular techniques, such as pulsed-field gel electrophoresis (PFGE), multilocus sequencing, ERIC, and REP-PCR methods. The results of this study will be used as a template of development</p>

	of a diagnostic gene chip capable of simultaneous detection of multiple foodborne pathogens.
Khan, Saeed A.	<p>Development of a Microarray Chip for the Detection of Multiple Antibiotic Resistance Markers (E0715101)</p> <p>Objective(s): Develop a microarray-based method for the detection of 150 genes associated with 22 antibiotics; some of which are used to promote growth in poultry and animal farming while others are used to treat infections in both humans and animals. The data generated by the use of the chip in monitoring and tracking the spread of resistance markers may be helpful for the FDA in making regulatory decisions that require banning and/or approving the use of certain antibiotics in poultry and farm animals.</p>
Nawaz, Mohamed S.	<p>The Fate and Degradation of Antimicrobials, Oxytetracycline (OTC), and Sulfadimethoxine-Ormetoprim (Romet-30) from Aquaculture Environmental Samples (E0707501)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) To determine the biodegradation rates and metabolic fate of antimicrobials, oxytetracycline (OTC) and sulfadimethoxine-Ormetoprim (Romet-30) (SDO), used in fish farming systems; and 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 regulate resistance to the drugs.
Nayak, Rajesh R.	<p>Molecular Epidemiology and Characterization of Multiple Antibiotic-Resistant <i>Salmonella</i> Isolated from Turkey Production Environment (E0717301)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) To determine the preharvest sources and/or vectors of horizontal transmission of <i>Salmonella</i> in turkey flocks; 2) To evaluate the intrinsic resistances of <i>Salmonella</i> isolates to multiple antibiotics; 3) To assess the genetic diversity and epidemiological profiles of <i>Salmonella</i> strains isolated in a turkey production environment; and 4) To develop DNA-based and microarray assays to detect genes in <i>Salmonella</i> isolates that are involved in antibiotic resistance and pathogenicity.
Paine, Donald D.	<p>Microbiological Diagnostic Methods: Development, Testing, and Evaluation (E0026200)</p> <p>Objective(s): To improve diagnostic and epidemiological capabilities in bacteriology, parasitology, mycology, virology, and serology as applicable to NCTR programs and projects.</p>
Paine, Donald D.	<p>Special Epidemiology Investigations of Potential Microbiological Contamination Problems (S00185)</p> <p>Objective(s): To investigate potential microbiological contamination problems. To report nonroutine sample time, which is not recorded on the Sample Collection Report.</p>

Rafii, Fatemeh	<p>Elucidation of the Mechanism of Resistance Development in Anaerobic Bacteria from the Human Intestinal Tract (E0709301)</p> <p>Objective(s): The evaluation of the effects of fluoroquinolones on the resistance development in bacteria from the human intestinal tract and analysis of the fluoroquinolone-resistance mechanisms in anaerobic bacteria from the human intestinal tract.</p>
Sutherland, John B.	<p>Microbial Degradation of Fluoroquinolone Antimicrobial Agents (E0722701)</p> <p>Objective(s): Identify microorganisms that either completely degrade fluoroquinolones or modify the fluoroquinolone molecule so as to reduce its toxicity to bacteria.</p>
Wagner, Robert D.	<p>Measurement of Antimicrobial Drug Concentrations that Inhibit Colonization Resistance (E0708601)</p> <p>Objective(s): An enterocyte culture model of colonization resistance by enteric microbial flora against <i>Salmonella</i> sp. colonization/invasion will be adapted to measure concentrations of antimicrobial drugs as food residues that would inhibit the barrier effect of the consumer's intestinal flora.</p>
Wagner, Robert D.	<p>Probiotic Effects on Host Defense Against Enteric Pathogens (E0709701)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Establish a model intestinal bacterial population in mice that consists of human intestine-derived bacteria; 2) Observe the fate of members of the model bacterial population when probiotic bacteria are fed to the mice; 3) Observe the fate of the probiotic bacteria fed to the human flora-associated mice; 4) Observe the effects of the human-derived bacteria on the host protective systems of immunodeficient and immunocompetent mice; 5) Observe effects of adding probiotic bacteria to the mice on immunodeficient and immunocompetent host protective systems; and 6) Observe the roles of normal intestinal bacteria and probiotic bacteria to modulate host protective systems of immunodeficient and immunocompetent mice from <i>Salmonella enterica</i> and <i>Campylobacter jejuni</i>.
Wagner, Robert D.	<p>Characterization of Antimicrobial Drug Resistance Genes from <i>Lactococcus lactis</i> P1-79 (E0716201)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determine whether the antimicrobial resistance genes are encoded on the bacterial chromosome or on episomes; 2) Screen for the presence of common resistance genes; 3) Clone the resistance genes in <i>E. coli</i> and evaluate their DNA sequence; and 4) Evaluate the potential for <i>L. lactis</i> P1-79 to transfer antimicrobial resistance genes to <i>Enterococcus faecium</i> or <i>Staphylococcus aureus</i>.

Research Projects Completed in FY 2005

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

Results:

Antibiotics are extensively used for treatment and prevention of infectious diseases in humans and pets, as well as in food-producing livestock, poultry, and fish. *Escherichia coli* is a commensal bacterium of the gut microflora of the chicken. Some serotypes are pathogenic if they are inhaled into the respiratory tract and are probably the most frequent and economically significant cause of bacterial diseases, particularly colisepticemia, in broiler chickens. Fluoroquinolones are broad-spectrum antimicrobial agents effective in the treatment of a wide range of infections. Enrofloxacin, danofloxacin, and sarafloxacin are synthetic drugs belonging to the fluoroquinolone class of compounds. Two fluoroquinolones, sarafloxacin and enrofloxacin, were approved by FDA in 1995 and 1996 in the United States for veterinary use to control morbidity and mortality associated with *E. coli*-related colibacillosis infections. The prevalence of fluoroquinolone-resistant *E. coli* in poultry ecosystems must be characterized because drug-resistant *E. coli* may play a role in the transfer of resistance to its clinical counterparts and may play a role in complicating the clinical treatment of *E. coli* infections in humans. In this study, we isolated and characterized high-level fluoroquinolone-resistant *E. coli* from broiler chicken litter. Nineteen fluoroquinolone-resistant *Escherichia coli* strains were isolated from poultry litter. Sixteen of the nineteen strains were serotyped to groups 6, 8, 53, 56, 153, and 174. Three strains were not serotyped to any known group. All isolates were resistant to multiple antibiotics. Most strains were resistant to gentamicin, kanamycin, chloramphenicol, and streptomycin. Ribotyping of the multidrug-resistant isolates with the restriction enzyme *PvuII* showed five different ribogroups, suggesting independent development of resistance instead of clonal spread. Quinolone resistance was associated with mutations of the quinolone resistance-determining region (QRDR) of the *gyrA* gene in all cases. Fluoroquinolone resistance was present among different serotypes and ribotypes, and drug resistance profiles suggest that the incidence of resistance does not indicate a clonal population in avian *E. coli*.

Investigation of the drug resistance profiles of *Salmonella typhimurium* DT104 from human and animal sources indicated that most strains of this serotype are resistant to multiple antibiotics, such as ampicillin, chloramphenicol, florfenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT-type). A rapid molecular method multiplex PCR was developed to identify these genes from clinical, food, and environmental samples. Currently, this method is used by field labs (ORA) to detect *S. typhimurium* DT104 in food samples.

Importance of Human Intestinal Microflora in Conversion of Phytoestrogens to Estrogenic Compounds (E0700701)**Results:**

Phytoestrogen supplements have become popular as alternatives for hormone replacement therapy based on their potential as prevention of hormone dependent diseases. Isoflavonoids found in legumes, such as soybeans, are converted by intestinal bacteria to metabolites with increased or decreased estrogenic activity. Microbial biotransformation plays a central role in regulating the biological activity of isoflavonoid phytoestrogens. They can convert them to potent estrogens or break them to nonestrogenic metabolites. Microbial activities are also involved in prolonging enterohepatic circulation of isoflavonoids by deconjugation of the liver isoflavonoid metabolites. These activities result in delay in excretion, consequently prolonging the period of exposure of target tissues, such as reproductive organs. Detection of the specific bacteria from the human intestinal tract that are involved in the metabolism of phytoestrogens has been the subject of this study. During this investigation, using an *in vitro* system, the importance of bacterial transformation of phytoestrogens has been shown. The bacteria that produce primary and secondary metabolites from isoflavonoids, which render them less or more estrogenic than their parent compounds, have been detected. Specific bacteria involved in biotransformation of three natural isoflavonoids, biochanin A, formononetin and glycitein, to their primary more estrogenic metabolites (genistein, daidzein, and 6,7,4'-trihydroxyisoflavone) by demethylation, which also enhances their absorption, have been found. Screening methods have been devised to detect those species that metabolize two other naturally occurring isoflavonoids, genistin and daidzin. Using these methods, bacteria that convert daidzin and genistin to their more estrogenic deconjugated products, daidzein and genistein, have been detected. The bacteria that produce the secondary metabolites dihydrodaidzein and dihydrogenistein from natural isoflavonoids have been identified. Both of these compounds are intermediates in the hypothetical pathways for complete isoflavonoid metabolism. One of the reasons for the lack of beneficial effect of phytoestrogens has been their conversion by bacteria to nonestrogenic metabolites. Bacteria have been isolated that cleave the C-ring of the isoflavonoid daidzein to produce the nonestrogenic *O*-demethylangolensin. The study established the pathway for this process by using the predicted intermediate, dihydrodaidzein, and showing its conversion to a ring-cleavage product by the same bacterium. The importance of identifying specific bacteria involved in isoflavonoid metabolism is apparent, since their prevalence dictates the effectiveness of phytoestrogen treatment for prevention of hormone dependent diseases.

We need to conduct scientific research for FDA to obtain fundamental information for making regulatory decisions on compounds with beneficial health claims for the general public. FDA has been petitioned and has granted the petition for a claim that the use of soy protein is safe, however, it still does not have a ruling on isoflavonoids for consumers. In addition to advancing the study of phytoestrogen metabolism, the data obtained provide background information that FDA can use when evaluating data on the beneficial or detrimental effects of phytoestrogens for regulatory purposes.

Sutherland, John B.

Biotransformation of Fluoroquinolones by Fungi (E0705201)

Results:

Because fluoroquinolone antimicrobial agents may be released into the environment, the potential for environmental fungi to biotransform these drugs was investigated. Four different environmental fungi were shown to transform fluoroquinolone and cinnolone antimicrobial agents. The metabolites were identified by high-performance liquid chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy.

Umbelopsis ramanniana (*Mucor ramannianus*) transformed ciprofloxacin to *N*-acetylciprofloxacin; enrofloxacin to enrofloxacin *N*-oxide, *N*-acetylciprofloxacin, and desethylene-enrofloxacin; and sarafloxacin to *N*-acetylsarafloxacin and desethylene-*N*-acetylsarafloxacin. *Pestalotiopsis guepini* transformed ciprofloxacin to *N*-acetylciprofloxacin, desethylene-*N*-acetylciprofloxacin, *N*-formylciprofloxacin, and 7-amino-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid; and norfloxacin to *N*-acetylnorfloxacin, desethylene-*N*-acetylnorfloxacin, *N*-formylnorfloxacin, and 7-amino-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. In poultry-litter materials composed of rice hulls, *P. guepini* produced the same four metabolites from norfloxacin. *Trichoderma viride* formed conjugates that were identified as 4-hydroxy-3-oxo-4-vinylcyclopent-1-enyl ciprofloxacin and 4-hydroxy-3-oxo-4-vinylcyclopent-1-enyl norfloxacin. *Beauveria bassiana* transformed cinoxacin to 1-ethyl-1,4-dihydro-3-(hydroxymethyl) [1,3] dioxolo [4,5-*g*] cinnolin-4-one and 1-ethyl-1,4-dihydro-6,7-dihydroxy-3-(hydroxymethyl) cinnolin-4-one. The results indicate that the metabolism of fluoroquinolones by fungi may reduce the antibacterial properties of these drugs, decreasing the selection pressure for bacterial resistance caused when fluoroquinolones reach the environment.

Wagner, Robert D.

***In vitro* Model and Molecular Analysis of Competitive Exclusion Products** (E0704901)

Results:

The *in vitro* model showed that mixtures of bacteria could be tested for efficacy as competitive exclusion (CE) products before expensive *in vivo* testing is performed, facilitating the development of defined CE products. Identification of the typically anaerobic bacteria in a CE product with 16S rRNA sequence analysis was more accurate than conventional technology, facilitating the ability of producers to comply with the requirement to define their CE products. Isolation of antimicrobial drug-resistant bacteria from CE products indicated that they need to be characterized for resistance mechanisms that are transferable to bacteria in the intestines of the poultry consumer.

This study addresses the FDA strategic goal of “improving product quality and safety through better manufacturing oversight”.

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Division of Neurotoxicology

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Executive Summary

Introduction

Brain and other nervous system-related disorders account for more hospitalizations than any other major disease group in the U.S.A. Approximately 25 percent of all Americans will suffer a brain-related disorder during their lifetime with the estimated annual cost to the national economy for treatment, rehabilitation, and related consequences being in excess of \$400 billion. Currently, however, recent advances in biomedical research have provided our scientists with a variety of new tools with which to better study and understand the etiology of brain-related disorders and to further reduce the risks associated with neurotoxic events.

It has long been known or suspected that the causes of brain-related disorders include exposures to chemicals such as therapeutic drugs, food additives, foods, cosmetic ingredients, pesticides, and naturally occurring substances. This was acknowledged several years ago in a report compiled by the Congressional Office of Technology Assessment entitled “Neurotoxicity: Identifying and Controlling Poisons of the Nervous System.” The number of potential neurotoxicants that require FDA regulation is estimated to be in the thousands; thus, identifying methods and approaches for assessing neurotoxicity is critical for the development of guidelines applicable for the assessment of neurotoxic risk. It is clear that chemicals from the categories listed above are vital to the national economy and our quality of life; therefore, the challenge is to determine at what dose and under what conditions these compounds can be used while minimizing the likelihood that they will cause nervous system-related toxicity.

The overall goals of the Division of Neurotoxicology (DNT) are to develop and validate quantitative biomarkers and identify biological pathways associated with the expression of neurotoxicity. An increased understanding of the processes associated with neurotoxic outcomes will provide opportunity for improved assessment of risk and identification of potential therapeutic approaches. The strategy employed for achieving these goals has been to employ multidisciplinary approaches that capitalize upon the expertise of Division personnel, which include: neurochemistry, molecular neurobiology, neuropathology, neurophysiology, and behavior. Some of the more unique features of our research capabilities are the ability to: determine chemical concentrations and cellular level interactions in target tissue; determine changes in gene and protein expression associated with chemical exposures; employ multiple species, including nonhuman

primates, rodents and, in some cases humans, in the risk assessment process to reduce the uncertainty associated with extrapolating findings across species.

FY 2005 Accomplishments

Research protocols were implemented to provide data important for the regulatory needs of the Agency with respect to the ubiquitous food contaminant, acrylamide, the pediatric anesthetics, including ketamine, and the acne medication, retinoic acid. In addition, we continued to address several main areas of fundamental research designed to examine the involvement of: monoamine neurotransmitter systems as targets for neurotoxicity; mitochondrial dysfunction and oxidative stress as mechanisms of neurotoxicity; and the NMDA receptor complex as a mediator of adult and developmental neurotoxicity.

Preliminary data from our rodent lifetime neurotoxicology studies on acrylamide suggest that few behavioral effects can be detected out to six months of age. In pre-weaning rats, subtle effects were noted at the highest doses tested (5 and 10 mg/kg/day) on certain developmental tests. Importantly, a significant dose-related decrease in brain weight was noted after three and six months of treatment (in some cases in the absence of concomitant changes in body weight) and a significant delay in vaginal opening was noted in females, suggestive of a delay in puberty (5 mg/kg).

In collaborative studies with colleagues at CDER, ketamine-induced neural cell death in the developing rat cell culture model was shown to be largely apoptotic in nature, and a manuscript on these finding was published. These studies are providing confirmation that the *in vitro* rat model should prove useful for future screening studies. Based on synaptogenesis, time of exposure becomes important when comparing rodent, nonhuman primate, and human neural cell toxicity outcome. Preliminary *in vivo* nonhuman primate studies demonstrate that measurement of apoptosis is feasible, and that cell death is likely both apoptotic and necrotic in nature. Current studies are examining development-based exposure times and exposure durations to determine safe anesthetic exposure scenarios. A regulatory briefing has been conducted to determine data needs and possible labeling changes based on this new information.

The first definitive studies using the fluorescent anterograde tracer Fluoro-Ruby and an improved version of the neurodegenerative stain Fluoro-Jade (C-version) were recently completed and show that dopaminergic axons and terminals can be destroyed—not just damaged—by amphetamine. These new stains will be used in the future to determine whether insults from doses of fenfluramine that are toxic to serotonergic axons and terminals also destroy the cell bodies themselves. The technique of pre-labeling axons and terminals with Fluoro-Ruby prior to insult and then subsequent staining with Fluoro-Jade C to identify frank degeneration should be applicable to all types of neurotoxicants that damage axons and terminals.

It was demonstrated that even in the absence of hyperthermia, seizures, or stroke, amphetamine can induce neurodegeneration, but this effect is restricted to discrete areas of the cortex and involves parvalbumin- and GABA-containing inhibitory neurons, not excitatory pyramidal neurons as might be expected. Under such conditions, the peri- and post-neurodegenerative genomic response to amphetamine has been described in several regions of the rat brain. Genes with increased expression (confirmed using RT-PCR) included neuropeptide Y precursor protein in the parietal cortex and insulin-like growth factor binding protein 1 in the amygdaloid cortex. Genes with decreased expression included nerve growth factor-inducible protein IA and IB and activity-regulated cytoskeletal protein expression in the parietal cortex. The observation that NMDA antagonists protect the somatosensory cortex from neurodegeneration but not the limbic cortex provides further evidence that the mechanisms involved in neurodegeneration—and the physiological responses to it—are different in these two brain areas. Using a “threshold” neurotoxic 9-day amphetamine exposure regimen, the most sensitive indices of insult again appear to be the loss of gene responsiveness or upregulation for several genes related to synaptic development and plasticity. In the parietal cortex, for example, the expression of neuropeptide Y was increased when neurodegeneration was observed. Such effects in the neocortex appear to be very sensitive indicators of the onset of neurotoxic events. The collection of individual cell types or groups using laser capture microdissection (LCM) may be needed to observe larger fold-changes in gene expression induced by the selective effects of the indirect-acting monoaminergic agents.

In support of all of our areas of research, genomic, proteomic, and bioinformatics approaches were developed or enhanced to allow for the identification of gene and protein expression profiles associated with neurotoxic events. Two manuscripts each were published in the areas of monoamine neurotransmitter system neurotoxicity and mitochondrial dysfunction and oxidative stress as mechanisms of neurotoxicity. One publication was the first that used real-time PCR to demonstrate that MPTP selectively alters gene expression in mice. This study provides information that may be beneficial to future clinical studies of Parkinson’s disease. Another publication reported on the use of newly developed bioinformatics tools to examine mechanisms of toxicity in PC12 cells treated with the neurotoxicant MPP⁺. Improved proteomics techniques resulted in a publication, which describes an improved labeling method using phosphoprotein isotope-coded solid-phase tags (PhIST). This improved labeling method for neuronal cell applications makes it amenable to the study of protein phosphorylation in an *in vitro* model of Parkinson’s disease. Importantly, the Division played a critical role in the FDA MicroArray Quality Control (MAQC) project, which aims to establish QC metrics and thresholds for objectively assessing the performance achievable by different microarray platforms and evaluating the merits and limitations of various data analysis methods. The

microarray datasets generated from the MAQC project will be used for assessing the precision and cross-platform/laboratory comparability of microarrays. The QRT-PCR datasets will enable evaluation of the nature and magnitude of systematic biases that may exist between microarrays and QRT-PCR datasets. The findings from the MAQC study will be made readily accessible to the microarray community and allow individual laboratories to more easily identify and correct procedural problems. Division participation in the MAQC project allowed our laboratories to implement “cutting-edge” genomic technology, which was then applied to existing projects. One area that has particularly benefited from these improved genomic methods involves the NMDA receptor complex as a mediator of adult and developmental neurotoxicity. During the past year, genomics-based approaches have been utilized to investigate the cellular changes in rats that underlie the behavioral toxicity associated with developmental exposure to NMDA receptor antagonists, sodium channel blockers, and compounds active at both sites. Prolonged exposure of juvenile rats and monkeys to these agents has been shown to cause severe and long-lasting changes in critical aspects of brain function, including learning and simple discriminations. The application of genomic analyses to these issues may provide invaluable information concerning the fundamental biology underlying these aspects of brain function.

RT-PCR was used to evaluate changes in gene expression in the hippocampus and striatum of rats after exposure to toxic regimens of either 3-nitropropionic acid (3-NPA) or methamphetamine. This work represents a continuation of studies on the effects of an enhancer of mitochondrial metabolism (L-carnitine) on drug-induced mitochondrial dysfunction. It appears that carnitine exerts an inhibitory effect on D₁ dopamine receptor formation, which may be relevant to its ability to protect against both 3-NPA and methamphetamine-induced neurotoxicity.

An important long-term study was completed, which described markedly different responses in the rat model to chronic exposure to the sodium channel blocker and NMDA receptor antagonist, remacemide, from those seen in earlier monkey studies. In monkeys, chronic exposure to remacemide was shown to have profound adverse effects on the ability of subjects to learn to perform several complex brain function tasks, whereas in rats remacemide had virtually no effects. These findings could have far reaching implications should it be confirmed that the rodent model is fundamentally different from the primate model.

Studies on the neurochemical and behavioral alterations associated with Accutane[®] (13-cis-retinoic acid) treatment were completed. These studies, specifically requested by CDER, used validated tests to monitor depression-related behaviors in the typical laboratory rat strain, Sprague-Dawley. There was no indication that Accutane[®] treatment enhanced depression-type behaviors.

Studies on the assessment of human brain function using the NCTR Operant Test Battery, continued, primarily in children. Data collected from children with major depression suggest that this disorder is associated with significant decreases in short-term memory.

Collaborations with other Divisions

During FY 2005, scientists in the Division collaborated on several studies with colleagues from other NCTR Divisions. These included:

- ACB-PCR n (ACB-PCR) Measurement of Rat *K-ras* Codon 12 GGT—GAT and GGT—GTT Mutations in Normal Rat Colon and Aberrant Crypt Foci of Carcinogen Treated Rates;
- Measurement of Cancer-Associated Gene Mutation in Colon Tumor and nontumor Tissue; and
- The MAQC project: MicroArray Quality Control.

FY 2006 Plans

Work for the coming year will continue to focus on Agency regulatory needs with respect to acrylamide and the pediatric anesthetics, as well as our three areas of fundamental research. Further utilization of genomic (and other OMICs) techniques should allow for the identification of specific genes and pathways involved in the expression of neurotoxicity. Data analyses and manuscripts from the MAQC study should contribute an invaluable scientific resource to the microarray community and provide a foundation for developing a roadmap for the future use of microarrays. Continued collection of brain function data from humans using the NCTR Operant Test Battery will further our knowledge base concerning interspecies comparisons and extrapolations.

A life-time (two year) acrylamide exposure study in rodents will begin during which a host of functional (behavioral), morphological, and other measurements will be obtained. Multiple simple and complex behavioral assessments will occur throughout the study. At sacrifice (after one and two years), a variety of histological and neurochemical assessments will be carried out on nervous tissue. The data obtained will be critical for agency regulatory decisions and colleagues at CFSAN maintain active interactions with Division staff over this issue.

The NMDA receptor complex, as a mediator of developmental neurotoxicity, is the focus of an ongoing protocol developed in collaboration with CDER. This protocol will provide the opportunity to further explore the nature of the ketamine-induced neuronal cell death observed in the developing nonhuman primate, an animal model more closely related to the developing human. Control and ketamine-treated animals will be assessed using histochemical, functional, genomic, and proteomic approaches whenever possible.

The NMDA-mediated excitotoxic response in the adult rat will be used to isolate and characterize the “neurodegeneration protein” expressed by FJ-positive neurons following neurotoxic insult. The role of apoptosis versus necrosis as a pathway of neuronal death will be more clearly defined with the use of the cytotoxic marker, FJ, and proteomic approaches.

Attempts are underway in the rat to identify changes in gene expression that correlate with the behavioral changes associated with long-term exposure to ketamine, remacemide, and other related compounds. In addition, gene changes associated with acute exposure to ketamine during the peak of the brain growth spurt are also being examined. The results from these studies will not only provide fundamental insight into normal developmental processes, but should also provide important data that can inform regulatory decisions for this and related compounds.

Specific studies will focus on gene expression both *in vivo* (in animal models) and *in vitro* (cell cultures) that may be affected by treatment with compounds of interest. These will include: the anesthetic ketamine and related NMDA receptor antagonists; remacemide; MPTP and MPP⁺; methamphetamine; and 3-NPA. Where appropriate, these studies may include examining gene expression in specific cell types using laser capture microdissection (LCM) technology.

An ongoing protocol will allow for continued research in the areas of monoamine neurotransmitter systems and oxidative stress. The extent to which the disruption of monoaminergic systems and alterations in oxidative stress are involved in the progression of Parkinson’s disease will be addressed. Proteomic analyses will be conducted on samples of both mouse and human tissue to develop profiles of the various proteins that are affected by neurotoxic insults producing Parkinsonism or Parkinson-like symptoms. Postmortem brains of Parkinson’s disease subjects, and protein extracts from the substantia nigra and the striatum isolated from methamphetamine- and MPTP-treated mice, will be used to measure post-translational protein modifications using a phosphoprotein isotope-coded solid-phase tag (PhIST) technique. Quantification and identification can then be accomplished by matrix-assisted laser desorption/ionization (MALDI)-mass spectrometry. In addition, protein/DNA arrays will be used to examine specific transcription factors involved in methamphetamine- and MPTP-induced neurodegeneration. Recent data from our laboratory utilizing PC12 cell cultures indicate that changes in dopamine content correlate with selective alterations in specific transcription factors that regulate monoaminergic systems. Nigrostriatal regions from MPTP-treated mice are currently being evaluated to determine if these changes in transcription factor expression are present and if they correlate with alterations in the dopaminergic system.

In continued fundamental research into the consequences of mitochondrial dysfunction and oxidative stress, investigations will focus on

posttranscriptional and translational regulation occurring during early responses to metabolic stress induced by 3-NPA and methamphetamine. cDNA arrays, RT-PCR, and metabolomic profiles obtained using NMR technology will be used in attempts to identify biologically significant changes in gene expression that accompany mitochondrial dysfunction. Such analyses will place emphasis on the importance of apoptotic and inflammatory responses to these processes.

Protocols are in place to begin assessing the brain function of children with anxiety disorder using the NCTR Operant Test Battery (OTB). In addition, further collaborations are being developed to begin OTB assessment of the consequences of infant opiate treatment for pain on subsequent brain function later in life.

Contribution to FDA's Strategic Goals

Over the last decade, increasing expertise, technologically advanced equipment, and improved facilities have been interwoven to further the goals of neurotoxicology research at NCTR. Substantial effort is being brought to bear to specifically address issues of regulatory concern around the food contaminant, acrylamide, and several anesthetic agents, particularly those used in the pediatric setting. In addition, efforts in support of fundamental research continue in several areas critical for our understanding of neurotoxic responses. The development and continued pursuit of these fundamental research areas is based on the prevailing scientific knowledge concerning mechanisms of neurotoxicity and the importance of each area to public health and regulatory concerns. They include mechanistically based approaches for defining and understanding the potential of a broad range of drugs and other chemicals to produce neurotoxic effects during all stages of development and senescence.

Division staff will continue to 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 gene expression, proteomic, and other tools will be further developed and incorporated into traditional toxicology protocols. Training will continue for existing staff so that new technologies can be incorporated into our research efforts.

An interdisciplinary approach, the use of multiple established animal models and innovative biomarkers that can in some cases also be used in humans subjects, coupled with an in-depth working knowledge of, and experience with, mechanistically based fundamental research areas will enable the Division of Neurotoxicology to be responsive to FDA regulatory needs in a timely fashion. Several ongoing or planned studies, many in conjunction with other FDA Centers, exemplify the application of the Division's approach to providing critical research information applicable to FDA's regulatory concerns.

Ongoing Research Projects

Ali, Syed F.

Evaluation of Novel Genetic Changes and Post-Translational Modification in the Protein Products of Specific Genes in Parkinson's Disease and in Substituted Amphetamine Neurotoxicity using Quantitative Proteome Analysis in Mice Models and Human Subjects (E0712101)

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

Binienda, Zbigniew K.

The Role of Mitochondrial Energy Disruption in the Mechanism of Neurotoxicity: Neurophysiological, Neurochemical, and cDNA Microarray Approaches (E0711001)

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

Bowyer, John F.

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

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

Ferguson, Sherry A.

Assessment of Depression Risk Associated with Accutane® (13-cis-retinoic acid or isotretinoin) and all-trans-retinoic Acid Treatment: Measurement of Behavioral and Neurochemical Alterations in Adult Sprague-Dawley and Flinders Sensitive and Insensitive Line Rats (E0714501)

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

	8) Quantitate the neurochemical alterations induced by 13-cis-retinoic acid and/or all-trans-retinoic acid treatment.
Patterson, Tucker A.	<p>Neurotoxicological and Behavioral Assessment of the Human Immunodeficiency Virus (HIV) Suppressors 2',3'-dideoxycytidine (ddC) and Thalidomide in Rhesus Monkeys (E0250201)</p> <p>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.</p>
Patterson, Tucker A.	<p>Analyses of the Rat Hippocampus via DNA Microarrays and a Novel Antibody Array, coupled with Laser Capture Microdissection (LCM)–Evaluation of the Effect of Aging on Gene and Protein Expression Associated with Learning (E0713901)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Measure gene and protein expression in regions of the hippocampus to determine regional distribution; 2) Determine the effect of aging on regional distribution of hippocampal proteins in three strains of rats; 3) Determine if aging, behavioral performance, and alterations in gene and protein expression in the hippocampus are related; and 4) Correlate the differences in gene and protein expression with behavioral performance of young adult and aged rats in a learning task previously shown to be sensitive to changes in protein expression.
Paule, Merle G.	<p>Developmental Neurotoxicity Assessment of Acrylamide in Rats: Range-Finding Studies (E0214801)</p> <p>Objective(s): To determine acrylamide doses to be used in subsequent long-term developmental neurotoxicity studies by identifying those that will not result in overt toxicity as determined by alterations in body weight gain and a variety of physiological, developmental, and behavioral parameters of either pups or dams.</p>
Paule, Merle G.	<p>Developmental Neurotoxicity Assessment of Acrylamide in Rats: Long-Term Studies (E0215101)</p> <p>Objective(s): To determine the consequences of long-term exposure to acrylamide on a variety of developmental milestones and measures of nervous system integrity throughout life.</p>
Paule, Merle G.	<p>Effects of Prenatal Cocaine on Behavioral Plasticity (E0663307)</p> <p>Objective(s): Determine whether chronic exposure to cocaine <i>In utero</i> 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.</p>

Paule, Merle G.	<p>Effects of Chronic Methylphenidate (Ritalin) Administration on ‘Cognitive’ Functions in the Rhesus Monkey (E0683700)</p> <p>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.</p>
Paule, Merle G.	<p>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)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 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; and 5) To determine if the acute behavioral effects of the exogenous compounds of interest differ with regard to gender in the rhesus monkey.
Paule, Merle G.	<p>Complex Brain Function in Children as Measured by Performance in the NCTR Operant Test Battery (E0703301)</p> <p>Objective(s): A battery of automated tests (games) will be given to measure aspects of learning, short-term-memory and attention, motivation, time perception, and color and position discrimination.</p>
Paule, Merle G.	<p>Pharmacological Countermeasures for Space Motion Sickness (E0712401)</p> <p>Objective(s): Establish effectiveness and quantify side effects for potential drug countermeasures for Space Motion Sickness (SMS).</p>
Paule, Merle G.	<p>Automated Cognitive Assessment of Persons with Alzheimer’s Disease (AD) (E0715301)</p> <p>Objective(s): Investigate whether performance on a variety of behavioral tests that measure timing ability, memory, and learning is different between persons with mild to moderate AD and persons who have no diagnosis of AD. This research will also determine which of these tasks is most sensitive to disease severity.</p>

Paule, Merle G.

Evaluation of Changes in Gene Expression in the Brain Associated with Normal Development and the Behavioral Toxicity Caused by Developmental Exposure to the N-methyl-D-aspartate (NMDA) Receptor Antagonists, Sodium Channel Blockers, and Combinations (E0716501)

Objective(s):

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

Paule, Merle G.

Complex Brain Function Study in Children With and Without Major Depression (E0717701)

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

Paule, Merle G.

Cognitive Assessments of Several Psychotropic Compounds using the NCTR Operant Test Battery (OTB) (E0721101)

Objective(s):

- 1) Determine the acute dose-effect relationships of several psychotropic drugs on a battery of operant behavioral tasks in rhesus monkeys;
- 2) Characterize the relative sensitivities of the various behavioral end-points in NCTR’s Operant Test Battery (OTB) to these agents; and
- 3) Compare the behavioral profiles of these agents to those of a variety of reference compounds with well-characterized mechanisms of action.

Paule, Merle G.

Effects of Anxiety of Complex Brain Function in Children (E0721701)

Objective(s): Determine if children with high levels of anxiety perform differently than children without anxiety on tests of motivation, simple visual discriminations, timing ability, memory, and learning.

Paule, Merle G.	<p>Novel Studies on Sites of Action and Mechanisms in Chronic Balance Dysfunction (E0722301)</p> <p>Objective(s): Develop and implement a comprehensive assessment of all levels of the neuraxis in an effort to determine CNS deficits due to balance disorder and vertigo and develop and assess strategies to restore those deficits.</p>
Paule, Merle G.	<p>NCTR Representation on the UAMS Institutional Review Board (S00640)</p> <p>Objective(s): To provide an administrative tracking number for hours served by NCTR's representatives on the University of Arkansas for Medical Sciences Institutional Review Board (IRB).</p>
Schmued, Laurence C.	<p>Proteomics of Toxicant Induced Neuronal Degeneration (E0711101)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 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 chemical identity of the fluorescent component in Fluoro-Jade B responsible for the high affinity labeling of degenerating neurons; and 4) To resolve the metabolic pathway by which the "degeneration protein" is generated.
Slikker, William	<p>Preliminary Studies for the Effects of Chronic Dexfenfluramine Administration in the Rhesus Monkey (E0702601)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determine if the rhesus monkey demonstrates cardiac valve changes due to chronically administered dexfenfluramine; and 2) Determine if the rhesus monkey demonstrates neurobiological changes due to chronically administered dexfenfluramine.
Slikker, William	<p>Assessment of Ketamine in the Developing Nonhuman Primate (E0718901)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determine, using neurohistochemical approaches, if, and at what developmental stages, ketamine exposure increases neuronal apoptosis/proliferation; 2) Determine, using neurohistochemical approaches, the dose-response for ketamine to produce apoptosis at the most sensitive developmental stage; 3) Determine the reversibility or permanence of the response using behavioral, imaging, and neurohistochemical approaches; and 4) Determine, at the most sensitive stage and dose, genomic and proteomic responses to ketamine treatment.
Wang, Cheng	<p>NMDA Antagonist/GABA Agonist-induced Cell Death in the Developing Rat Brain (E0215501)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Screen and evaluate pediatric anesthetic agents; 2) Determine if a one-time bolus dose or prolonged exposure of the developing rat to NMDA antagonist, GABA agonist alone,

or their combinations will induce long-term behavioral deficits, as well as long-lasting pathological changes;

- 3) Determine the dose, temporal and pathophysiological relationships between MDA antagonist/GABA agonist-induced neurotoxicity and long-term behavioral changes;
- 4) Determine the neurotransmitter receptor mechanisms involved in the neuron degeneration and behavioral deficits caused by these agents, particularly the role of altered NMDA receptor function;
- 5) Determine by *in situ* hybridization and immunoblot the relative densities of NMD receptor NR1, NR2A, and NR2B subunits following anesthetic drug administration; and
- 6) Identify mechanisms that could link altered NMDA receptor function, elevation of superoxide free radicals to anesthetic drug-induced apoptosis, inhibitors will be added at various times to determine the contribution and temporal distribution of several elements of the proposed pathway leading to cell death.

Xu, Zengjun

Adolescent Nicotine Administration Effects on CNS Serotonergic Systems (E0709801)

- Objective(s):**
- 1) Determine whether adolescent nicotine administration elicits axonal/terminal damage in 5HT systems;
 - 2) Determine if adolescent nicotine administration alters 5HT presynaptic activity;
 - 3) Determine 5HT receptor and signaling activity and functions induced by adolescent nicotine exposure; and
 - 4) Determine if adolescent nicotine administration produces changes in cAMP-mediated signal transduction, 5HT metabolic enzymes and/or 5HT receptors.

Research Projects Completed in FY 2005

Slikker, William

Quantitative Procedures for Neurotoxicity Risk Assessment (E0310001)

Results:

Quantitative procedures for neurotoxicity risk assessment were developed and published in peer-reviewed journals. Quantitative methods to predict the toxicity for a series of organophosphate agents were developed and verified. Procedures to determine the impact of either measurement errors or animal variability on risk assessment outcome were published. And finally, recommendations were generated and published via a workshop focused on improving risk assessments based on pharmacokinetic and pharmacodynamic studies in children and developing animal models.

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Executive Summary

Introduction

Nearly 50 years ago, the renowned nutritional biochemist, Roger Williams, recognized that "... every individual has his own inborn metabolic characteristics," that "... every application of biochemistry to human beings must take these differences into account," and that "... all diseases, such as cancer, arthritis, heart disease, *etc.*, are related to biochemical individuality" (Roger J. Williams, "Biochemical Individuality," John Wiley & Sons, New York, 1956). Over the next few decades, biomedical research scientists focused their efforts largely on animal studies and model systems to delineate the mechanisms of carcinogenesis and therapeutic drug efficacy and whenever possible carried out comparative human studies. These efforts allowed the identification: of pathways involved in drug and carcinogen metabolism, of the mechanisms of DNA damage and repair, and of the role of genes that control cell growth and apoptosis.

Early on, however, it was recognized that there were genetic polymorphisms (*e.g.*, slow and rapid acetylators) in various populations and that these phenotypes affected not only drug metabolism and toxicity (*e.g.*, isoniazid) but also cancer risk (*e.g.* urinary bladder cancer). As biochemical and molecular biological techniques improved in the 1990s, it became possible to clone and sequence genes from a number of individuals, to discover single nucleotide polymorphisms (or combinations of these called haplotypes), and to develop high throughput genotyping methods and apply them to large numbers of individuals in population-based studies, thus giving rise to the field of molecular epidemiology. Now, we have a rich database of different alleles for the cytochromes P450 (CYP; see www.imm.ki.se/CYPalleles), acetyltransferases (NATs; see www.louisville.edu/medschool/pharmacology/NAT.html), flavin-containing monooxygenases (FMOs), sulfotransferase (SULTs), glucuronosyltransferases (UGTs), glutathione-S-transferases (GSTs), and other phase II enzymes, as well as most of the >130 genes involved in DNA repair and the approximately 100 genes that serve as positive or negative regulators of cell growth. In addition, cancers at various sites have now been found to be associated with specific genetic variants that often interact with specific exposures, known as gene-environmental interactions. Some examples include: *CYP1A1**4/*GSTM1*null-cigarette smoking and lung cancer; and *CYP1A2**1F or rapid phenotype/*NAT2**4-well done red meat and colon cancer.

The advent of molecular epidemiology and the outcome of the Human Genome Project gave the impetus to studies into how an individual's genetic inheritance affects the body's response to drugs. This included not only drug/carcinogen-metabolizing enzymes, but also drug targets, receptors, and transporters. This new field has come to be known as pharmacogenomics. The goal was to understand individual susceptibility for adverse drug reactions, cancer therapy; and to achieve the clinical practice of "personalized medicine." Thus far, this has been achieved only in a few medical centers with specific examples, notably thiopurine S-methyltransferase, which detoxifies 6-mercaptopurine and azathioprine. Some individuals homozygous for a defective allele are at high risk for a fatal toxicity and genotyping has been employed to adjust their treatment dose. However, until this year there had been no guidelines for submission of drug applications using pharmacogenomic data. In March, 2005, the U.S. FDA issued a document entitled "Guidance for Industry: Pharmacogenomic Data Submissions" (see www.fda.gov/cber/gdlns/pharmdtasub.htm). Now approved for clinical diagnostic use are the Roche/Affymetrix Amplichip for CYP2D6 and CYP2C19 (in the U.S. and E.U.), the TRUGENE and ViroSeq HIV-1 kits (in the U.S.) to detect HIV-1 variants that make the virus drug resistant, and the Invader UGT1A1 Molecular Assay (in the U.S.) that detects TA repeats in UGT1A1 and is useful for predicting Irinotecan metabolism/toxicity in colon cancer patients.

To the individual, genetic testing will eventually have a number of benefits. First, it will enhance patient choice on lifestyle decisions, including reproductive, environmental, and occupational. Moreover, clinical benefits will empower the individual to prevent, treat or cure their disease by controlling their diet and lifestyle through knowledge of metabolic differences, exposure, susceptibility, or other symptomatology. In addition, the use of individualized drugs will be made possible through "orphan drug discovery." (L. O. Gostin & J. G. Hodge, Jr., *Jurimetrics* 40: 21-58, 1999). It is perhaps not too unreasonable to comprehend the vision of the French philosopher Teilhard, who predicted: "With the discovery of genes, we shall soon be able to control... heredity, ... grasping the mainspring of evolution, seizing the tiller of the world." (Teilhard de Chardin, *The Phenomenon of Man*, Harper and Row, 1959).

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

FY 2005 Accomplishments

The intent is to better understand the mechanisms of human carcinogenesis and response to therapy; 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 foodborne heterocyclic amines, environmental aromatic amines and polycyclic aromatic hydrocarbons, on widely used drugs, as well as on tobacco usage. Projects on the etiology of human cancers of the colon/rectum, pancreas, urinary bladder, esophagus, breast, prostate, lung, and bone marrow are ongoing. These are outlined as follows:

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

Metabolic polymorphisms and individual cancer susceptibility: applications to personalized medicine.

- a) Polymorphisms of cytochromes P450, COX-2, oxidative stress genes, tissue-dependent expression, and hormone metabolism;
- b) Polymorphisms of sulfotransferases;
- c) Polymorphisms of glutathione S-transferases;
- d) Polymorphisms of glucuronosyl transferases;
- e) Pharmacogenomics of 5-fluorouracil and colorectal cancer efficacy; and
- f) Pharmacogenomics of drug transporters and chemotherapeutic drug efficacy.

Chemoprevention

- a) Modulation of gene expression by chemopreventive agents and identification of gene targets as surrogate biomarkers of effect (e.g., *NQO1*); and
- b) DNA methyltransferases, interindividual variation and cancer risk.

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

- a) Etiology of human breast and prostate cancers in African-Americans and Caucasians; and
- b) 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, and pancreatic cancers.

- a) DNA adduct detection in breast epithelial cells in relation to cancer risk and hair dye use;
- b) DNA adduct detection in prostate tissue in relation to dietary heterocyclic amine exposure; and
- c) DNA adduct detection in human pancreas by Mass Spectrometry.

Structural Genomics Center

- a) In an effort to develop genotype and haplotype markers of prostate, breast, colorectal, and esophageal cancer susceptibility, approximately 100 polymorphisms of drug/carcinogen metabolism and DNA repair enzymes have been genotyped and statistical analyses are underway;
- b) Genotyping of 72 polymorphisms of DNA repair enzymes in uniplex and multiplex in a 500-person esophageal cancer case-control study as a showcase for new technology, throughput and novel statistical methodology have been undertaken;
- c) Detection of aberrant tumor DNA in plasma of patients with prostate and breast cancer as biomarkers of cancer detection; and
- d) Retrospective pharmacogenomics studies of colorectal and breast cancer to better predict chemotherapy toxicity and cancer outcome are underway.

FY 2006 Plans

- Correlation of gene expression with activity of chemotherapeutic agents to identify the mechanism of resistance to geldanamycin analogs;
- Proteomic-base approach to determining gender differences in potential prognostic factors in pancreatic cancer in response gemcitabine treatment alone and in combination with dietary chemopreventive agents;
- Homeostasis of GSH and its impacts on etiology and chemotherapy outcome of colorectal cancer-functional genomics of γ -glutamylcysteine synthase and system x_c amino acid transporters;
- Evaluation of effects of genetic polymorphisms in oxidative-stress related genes HO-1, iNOS, and eNOS on the incidence and severity of colorectal cancer;
- Effect of genetic variation on efficacy of tamoxifen or toremifene adjuvant therapy in the NAFTA trial;
- The influence of preeclampsia on hormonal and anthropometric status in boys and girls at 10 and 13 years of age: Follow-up of the Stavenger Population-based nested case-control study;

- Early puberty, higher obesity, and lower energy expenditure in African-American than Caucasian girls: Can health disparities in the young lead to increased cancer burden; and
- Prostate cancer in high- and low-risk populations: role of food derived heterocyclic amine carcinogens and chemopreventive agents.

Contribution to FDA's Strategic Goals

The FDA is committed to increase access to innovative products and technologies to improve health. One of FDA's strategic goals is to increase the number of safe and effective new products by increasing the predictability, efficiency, and effectiveness of product development, including products for unmet medical and public health needs, emerging infectious diseases, and counterterrorism. This goal includes the Critical Path Initiative to personalized medicine.

The researchers of the Division of Pharmacogenomics and Molecular Epidemiology envision that applications utilizing gene diversity can be harvested to define disease susceptibility and predict drugs induced adverse events. The Division of Molecular Epidemiology and Pharmacogenomics has developed human study populations with treatment, toxicity and outcomes data for breast cancer, colorectal cancer and multiple myeloma to explore individualized approaches to cancer therapy. The hypothesis being tested in these human studies is that common genetic polymorphisms of enzymes involved in the metabolism of chemotherapy drugs modify toxicity, cancer survival and efficacy of treatment.

The premise of the breast cancer study is that the bio-available dose of drugs can be substantially modified by gene variation in enzymes that bio-transform drugs. Focused on enzyme pathways involved in the biotransformation and action of cyclophosphamide, 5-fluorouracil, methotrexate, doxorubicin and tamoxifen we are genotyping DNA isolated from tumor tissue sections. The outcome measure for this work is the association between toxicity rate, 5 year survival, 5 year recurrence and genetic polymorphisms. The anticipated benefit of this work is the identification of subgroups of people that respond favorably to specific drug therapies.

The colorectal cancer initiative is a collaborative study between the VA hospital in Little Rock, University of Arkansas for Medical Sciences and the NCTR. In this study we are currently determining the impact of selected genetic polymorphisms on colorectal cancer risk, prognosis and efficacy of treatment. In this study, we have obtained tumor tissue sections and records of treatment received and outcome from retrospectively identified colorectal cancer cases. We are genotyping DNA isolated from tumor tissue sections for polymorphisms of drug metabolizing enzymes and then evaluating the association between

toxicity, cancer survival and these polymorphisms. Special emphasis is being placed on enzymes involved in the biotransformation and action of chemotherapy drugs 5-fluorouracil and Leucovorin.

The multiple myeloma studies are conducted in collaboration with the Myeloma Institute for Research and Therapy (MIRT) in Arkansas, one of the leading myeloma referral centers. Myeloma is a very serious cancer with 5-year survival rates of less than 25%. Antineoplastic chemotherapy with a myeloablative dose of melphalan followed by stem cell transplantation is the cornerstone of myeloma treatment. The primary cause of stem cell transplantation failure is severe oral mucositis. NCTR investigators are working with MIRT to develop a predictive model for mucositis that includes clinical variables and variables of genetic polymorphisms of enzymes that metabolize melphalan and mediate inflammatory responses in the gastrointestinal tract. The initiatives in the Division are targeted to realize the goal of personalized medicine and to contribute to the regulatory mission of the FDA.

Applications utilizing gene diversity can be harvested to define disease susceptibility, predict adverse events, and to detect cancer and other diseases. The ongoing initiatives in the Division of Pharmacogenomics and Molecular Epidemiology are targeted to realize the goal of personalized medicine and to contribute to the regulatory mission of the FDA.

For example, we have developed human study populations with treatment, toxicity and outcomes data for breast cancer, colorectal cancer, prostate cancer, pancreatic cancer, and multiple myeloma to explore individualized approaches to cancer therapy. The hypothesis being tested in these human studies is that common genetic polymorphisms of enzymes involved in the metabolism of chemotherapy drugs modify toxicity, cancer survival and efficacy of treatment.

Ongoing Research Projects

Hammons, George J.

Assessment of Interindividual Variability in Expression of DNA Methyltransferases, DNMT 3a and DNMT 3b, in Liver and Identification of Factors Influencing Expressions (E0716701)

Objective(s):

- 1) To determine levels of expression of DNMT 3a and DNMT 3b in liver samples from a pool of donors selected according to smoking status, gender, and age;
- 2) Determine the effect of tobacco smoke on DNMT1, 3a, and 3b expression in liver cell systems; and
- 3) Assess the polymorphism frequency identified in *DNMT3b* in the sample pool included in the study and assess whether it is correlated with expression.

Kadlubar, Fred F.

A Case-Control Study of Pancreatic Cancer and Aromatic Amines (E0694601)

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.

Kadlubar, Fred F.

Role of Acetylation and N-Oxidation in Colorectal Cancer (E0694701)

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.

Kadlubar, Fred F.

Chemical Carcinogenesis: Epithelial Cells in Breast Milk (E0697801)

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; and
- 4) To evaluate the relationships between carcinogen-DNA adducts and smoking status and adduct levels with polymorphisms in *NAT1*, *NAT2*, *CYP1A1*, and *GSTM1*.

Kadlubar, Fred F.

Novel Recruitment Techniques for a Study of Culture-Specific Diet, Metabolic Variability and Breast Cancer in African-American Women—Formerly “Breast Cancer in African-American Women: Metabolic Modification of Dietary and Hormonal Risk Factors” (E0701501)

Objective(s): In this study, we intend to examine the role of interindividual variability in response to exogenous agents as it may relate to breast cancer risk in African-American women. By evaluating risk associated with exposure to oral contraceptives, hormone replacement therapy, and modification of that risk by genetic variability in their metabolism, the effects of substances regulated

by the FDA on breast cancer risk in African-American women may be further elucidated. Additionally, a successful model to increase African-American participation in research studies would greatly assist in future studies related to FDA-regulated substances in African-American populations.

Kadlubar, Fred

***In Vivo* Modeling of Steroid-mediated Gender Effects in Drug Metabolism**
(E0704301)

- 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 usage, menopause, and 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; and
 - 5) To statistically assess the impact of each of the measured variables on the *CYP1A2* phenotype.

Kadlubar, Fred F.

Somatic Alterations in Prostate Cancer and Its Precursor Lesions (E0711301)

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

Kadlubar, Fred F.

Detection of DNA Adducts in Buccal Cell DNA Before and After Consumption of Grilled Chicken (P00642)

- Objective(s):** Propose to analyze buccal cell DNA for adducts of BP, PhiP, and 2-AaC, known to be generated through high temperature cooking of meat, and also for DNA adducts of ABP, a ubiquitous environmental pollutant.

Lyn-Cook, Beverly A.	<p>The Effects of Nicotine and Other Cigarette Components on Normal and Neoplastic Human Pancreatic Cells: The Role of Low Zinc Levels on Ras, <i>mdr-1</i> Genes Activation, and Metabolizing Enzyme Activities as a Possible Risk Factor for Pancreatic Cancer (E0701701)</p> <p>Objective(s): The major objective of this proposal is to determine the effects of nicotine and other cigarette components on exocrine and endocrine human pancreatic cells <i>in vitro</i>. The final objective of this study is to examine <i>ras</i>, <i>mdr-1</i>, <i>CYP1A1</i>, and <i>CYP1A2</i> expression in normal and neoplastic human pancreatic tissue grouped according to race and sex obtained from a human tissue bank.</p>
Lyn-Cook, Beverly A.	<p>Mechanistic Actions of Chemopreventive Agents in Pancreatic Cancer (E0707601)</p> <p>Objective(s): Screen a number of agents found in natural products and establish mechanistic data on their potential as anticancer agents against pancreatic cancer.</p>
Lyn-Cook, Beverly A.	<p>CYP1 B1 Polymorphisms in Uterine Leiomyomas: Frequency in African-American Women and Response to Therapy (P00443)</p> <p>Objective(s): Determine the frequency of the polymorphic variant and others of cytochrome P450 1B1 in human uterine leiomyoma cases compared with the frequency in patient-matched controls.</p>
Ning, Baitang	<p>Regulatory Polymorphisms of SULT1A1 and its Impact on the Risk of Prostate Cancer in African-Americans and Caucasians (E0715801)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determination and mapping of polymorphisms in the promoter region of <i>SULT1A1</i> gene; 2) Association study between phenotype and haplotype of <i>SULT1A1</i>; 3) Case-control study to assess the high susceptible haplotype(s) of <i>SULT1A1</i> for prostate cancer; and 4) Functional characterization of high-risk haplotype(s) of <i>SULT1A1</i>.
Ratnasinghe, Luke D.	<p>Prostate Cancer: Exposure, Susceptibility and DNA Adducts (E0702101)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determine levels of carcinogen exposure in African-Americans and Caucasians with histologically confirmed prostate cancer using a case-control design; 2) Evaluate variability in hormone metabolism and susceptibility to carcinogen exposure, as measured by phenotypic and genotypic variability in carcinogen metabolism, and evaluate the interaction of these factors with the exposure data obtained in 1); and, 3) Characterize DNA adducts in prostate tissue from men with prostate cancer to identify mutagenic agents and evaluate levels of adducts in relation to carcinogen exposure data and susceptibility factors obtained in 1) and 2).

Ratnasinghe, Luke D.	<p>Pharmacogenomics of Colorectal Cancer Treatment and Outcome (E0714301)</p> <p>Objective(s): This proposal brings together a case-control study and a case-series follow-up study to determine the impact of selected genomic markers on colorectal cancer cases (CRC) risk, prognosis, and efficacy of treatment.</p>
Ratnasinghe, Luke D.	<p>Development of Lung, Colorectal, and Esophageal Cancer Genomic Signatures for Cancer Early Detection, Risk, and Outcome Assessment (E0715501)</p> <p>Objective(s): To develop lung, colorectal, and esophageal cancer genomic signatures for cancer early detection, risk, and outcome assessment.</p>
Ratnasinghe, Luke D.	<p>Association Between Aspirin Use and Chronic Disease in the NHANES I and II Cohorts (E0715601)</p>
Ratnasinghe, Luke D.	<p>Cancer Chemopreventive Effects of Thiazolidinediones: A Retrospective Study (E0722201)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Evaluate the association between use of Thiazolidinediones and other diabetes medications and risk of cancer in patients with type 2 diabetes; and 2) Evaluate the association between type, dose, and duration of Thiazolidinediones and other diabetes medications used and risk of cancer in patients with type 2 diabetes.
Ratnasinghe, Luke D.	<p>Molecular Genetics of NAT1 and NAT2 in Prostate Cancer (E0723301)</p> <p>Objective(s): Systematically investigate the genetic reasons responsible for high Human N-acetyl-transferase (NAT) activity that contributes to high incidence of prostate cancer.</p> <ol style="list-style-type: none"> 1) Evaluate genetic polymorphisms of carcinogen metabolism enzymes <i>NAT1</i> and <i>NAT2</i> and the enzymatic activity of <i>NAT2</i> as predictors of prostate cancer odds; 2) Evaluate haplotypes of genetic polymorphisms of <i>NAT1</i> and <i>NAT2</i> as predictors of prostate cancer odds; and 3) Evaluate polymorphisms of carcinogen metabolism enzymes <i>NAT1</i> and <i>NAT2</i> in relation to ethnicity.

Research Projects Completed in FY 2005

Methylation Status and Cancer Risk (E0704601)

Results:

Methods for measuring S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in red blood cells or whole blood were developed. Those metabolites, and also homocysteine (HCys) in plasma were measured in a number of experiments using samples from rats, mice, and human subjects. Studies indicate the erythrocyte levels of SAM and SAH, along with the corresponding SAM/SAH ratios, are reproducible, are quite characteristic for a given individual, and can be altered by relatively minor shifts in diet. Subsequently, the erythrocyte levels of SAM and SAH, the serum levels of HCys, and the lymphocyte activity of MTHFR were determined in patients with both Type I and Type II diabetes. The results were also arranged according to the degree of progression of the disease. Since no significant differences between the Type I and the Type II diabetics were observed in these studies, the results were combined.

A major finding from this study was that in control subjects the blood level of SAM was proportional to the activity of MTHFR; in diabetics it was not. The lack of proportionality between SAM and MTHFR was particularly marked in the early stages of the disease. Disease progression was accompanied by increased levels of HCys and of SAH, and decreased levels of SAM, of SAM/SAH ratios, and of MTHFR. SAH levels were proportional to those of HCys.

An additional finding in these and other collaborative studies is that the levels of SAM and HCys are lower in women than in men. SAM and SAH levels were also species specific. The results provide further evidence that alterations in the blood levels of SAM and related compounds may provide useful clues in examining the etiology and progression of disease.

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Division of Systems Toxicology

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Executive Summary

Introduction

The Division of Systems Toxicology (DST) supports the development of new technologies and works to facilitate integration of data from multiple technology platforms for application to questions associated with the FDA's Critical Path Initiative that are in direct support of the FDA mission. Six centers of excellence comprise the Division of Systems Toxicology including the Centers for Functional Genomics, Proteomics, Metabolomics, Hepatotoxicity, Toxicoinformatics, and Chemistry. The emphasis of this Division is to provide technical expertise and guidance for the inclusion of OMICs (genomic, transcriptomic, proteomic, and metabolomic) and *in silico* data into the review process. Our research focuses on novel technology development for Critical Path research that can lead to personalized medicine.

An important function of the FDA is to use risk management to provide the most health promotion and protection at the least cost to the public. Presently, this is accomplished by safety assessment of FDA-regulated products in surrogate organisms and to a lesser extent in humans. The Division of Systems Toxicology provides a unique and sophisticated analytical infrastructure that can be used to assess the safety of FDA-regulated products through the integration of genomics, proteomics, metabolomics, in conjunction with traditional biomarkers of safety, and toxicoinformatics. This systems approach is directed towards the creation of a more relevant and quantitative risk assessment paradigm. Presently, risk assessment depends greatly on statistical models that may not have biological relevance. The systems biology approach to toxicity testing can provide the FDA with data that are more easily extrapolated to the human, making data interpretation more facile and relevant. The move toward personalized medicine requires the more global analysis of the phenotypes of individuals on the health disease continuum that can be ascertained through an integrated systems biology approach using information from microarray analysis, metabolomics, and proteomics. Because these new technologies generate a great deal of data, a strong informatics underpinning is required. The Toxicoinformatics group provides state-of-the-art data analysis methods for the integration of OMICs data in assessing risk of adverse events and works to develop novel *in silico* tools for predictive toxicology. The Division also has a program in mass spectrometry-based analyses in counterterrorism, as well as a significant effort in sensor and nanotube technology. In addition, the Division

provides computational, including artificial intelligence, approaches to predictive toxicology.

The goal of the Division is to use proof-of-concept protocols to identify new disease markers and (adverse) drug targets that will aid in the design of biomedical products to prevent, diagnose, and treat disease that have a better efficacy, a lower risk of adverse events, and can be applied in the move toward personalized medicine.

FY 2005 Accomplishments

A major emphasis within the Division is to facilitate integration of data from multiple technology platforms for application to questions associated with Critical Path research. The Division performed an integrated study of acute valproic acid hepatotoxicity to demonstrate the feasibility of merging gene expression, proteomic, and metabolomic data, and performed an integrated microarray, proteomic, and metabolomic analysis of hepatic effects of subchronic tamoxifen and phenobarbital administration to further develop the tools for these types of analyses. Further progress in nanotube and sensor technology occurred during this time, and counterterrorism efforts increased.

Center for Metabolomics

A Metabolomics Research Team has been established at NCTR and has instigated active collaborations within NCTR, across the Agency, and with academic and pharmaceutical research groups. This program was developed to aid in the assessment of preclinical and clinical safety issues and as part of an Agency-wide biomarkers development effort. In addition, this research effort is an important component of the FDA's Critical Path Initiative for medical product development. Major accomplishments include:

- Developed a mass spectrometry (MS) analysis method to evaluate phospholipids.
- Received UPLC and LCT-Premiere MS from Waters under a "beta test" material transfer agreement (MTA).

Center of Proteomics

The Proteomics group has established methods to help elucidate the biological mechanisms associated with toxicity using mass spectrometry-based proteomics. The development of mechanisms to integrate proteomic data with that of other OMICs is underway. This work complements the development of software for large protein identification studies and is being extended to assist in quantitative comparisons. This work is necessary to permit a higher sample number to be examined to permit both dose and time studies in support of toxicology studies. Initial work focused on improvements in throughput and sensitivity of mass spectrometry-based proteomic experiments, as well as the development of

bioinformatics to handle the large volume of data that are generated.

Major accomplishments include:

- Developed ProteinTrack, software that allows confidence criteria to be applied to proteomics results and comparisons made between different proteomics experiments.
- Proteomic-based microbiology projects resulted in the identification of novel proteins produced by mycobacterium involved in the catabolism of poly-aromatic hydrocarbons and to identify virulence factors in antibiotic-resistant bacteria; and
- Proteomics are being used to identify serum biomarkers specific to liver cancer.

Center for Hepatotoxicology

The mission of the Center for Hepatotoxicology is to provide expertise in liver toxicology to the FDA. The focus of this group is two-fold and includes the mechanistic analysis of toxic responses and carcinogenesis in the liver. Both cross-cell and cross-species analyses will be performed. The vision for the Center is to develop and apply a systems toxicology approach to the analysis of questions in liver toxicity that is based on an integrated cell, molecular, transcriptomic, proteomic, and metabolomic platforms. Biomarker profiles will be generated for more effective assessment of risk for acute toxicity and liver cancer development. Biomarkers of liver injury and disease in mouse, rat, and human samples will be developed through an integrated OMICs analysis of biofluids and tissues from liver injury and disease. The integration of signaling pathway for cell proliferation, apoptosis, and differentiation provide the framework in which to assess these biomarkers. Our initial focus has been on PPAR alpha and PPAR gamma agonists, tamoxifen, and valproic acid. The systems biology approach of hypothesis, system perturbation, integrative analysis, and iterative perturbation is employed. Hepatotoxicity is the number one reason for drug recall. Development of new methods for identification and limiting the effect of agents to cause liver toxicity is of great importance in support of the Critical Path and the long-term impact on public health. Major accomplishments include:

- Comparative microarray analysis of hepatic effects of PPAR γ agonists;
- Cross-species microarray analysis of PPAR α agonist hepatic effects;
- Mechanism of action of (nongenotoxic) rodent carcinogens by systems biology;
- Development of biomarkers of liver disease and toxicity;
- OHSC proposal to institute prospective safety studies; and
- Systems toxicology of valproic acid effects in the liver.

Center for Functional Genomics

Microarray data shows great promise in drug safety evaluation and the FDA is actively encouraging this new technology. A major effort was made to identify sources of technical variability in microarray experiments and to develop QA/QC procedures to help ensure that microarray data submitted to the Agency is of sound quality. This effort included colleagues in the Division of Biometry and Risk Assessment and the Center for Toxicoinformatics and collaborations with CDER, CBER, and CDRH. In standardizing toxicogenomics experiments, it is also important to understand the potential sources of biological variability so that drug and nutrient effects will not be confounded with normal biological variation. To this end, studies on the impact of circadian rhythm and age- and sex-dependent variation in gene expression were examined. To examine the issue of age- and sex-specific susceptibility of drug toxicities, tissues from male and female rats throughout their life cycle have been collected. Eleven different tissues, including liver, brain, heart, kidney, muscle, lungs, spleen, bone marrow, thymus, adrenal gland, and testis or uterus, will be used to examine the age- and sex-related expression of genes, proteins, and metabolites.

Since the mitochondria is a target of many toxic responses and in many disease processes, a custom DNA microarray containing genes related to the structure and function of the mitochondria has been developed. More than 500 genes were identified that are associated with mitochondrial function, and gene-specific oligonucleotides were designed and synthesized for creation of the MitoChip.

The FDA is actively encouraging the new OMICs technology, including DNA gene expression microarrays as part of the FDA's Critical Path Initiative to new medical products. The Center for Functional Genomics has continued its efforts at identifying and reducing the technical variability in microarray experiments, and in developing quality control/quality assurance metrics. Major accomplishments include:

- Optimized all aspects of microarray use for mouse, rat, and human toxicogenomic studies;
- Developed a custom gene chip for examining mitochondrial structure and function;
- Played an essential role in the development of standards for microarray quality control and quality assessment;
- In collaboration with the Biometry and Risk Assessment Division, determined sources of variability in microarray analysis; and
- Defined time-of-day effects on gene expression in the liver as an important factor in animal experimentation.

Toxicoinformatics Center of Excellence

The mission of the Toxicoinformatics group is to conduct research in bioinformatics and chemoinformatics. As part of this initiative, the

Toxicoinformatics group acts to develop and coordinate informatics capabilities within NCTR, across FDA Centers, and in the larger toxicology community. The goals of the Toxicoinformatics group are to develop methods for the analysis and integration of OMICs datasets.

Major accomplishments include:

- Developed ArrayTrack™ to warehouse, visualize, analyze, and interpret microarray data. This integrated software solution is being extended to protein and metabolite data for systems biology questions;
- Provided ArrayTrack™ training to more than 80 FDA reviewers/scientists. Additional training courses have been offered at NCTR and UAMS;
- Integrated ArrayTrack™ into the Voluntary Genomic Data Submissions (VGDS) Program with training for reviewers, analysis of submitted data, and formal discussions between CDER/NCTR and pharmaceutical sponsors;
- Provided general support to the NCTR OMICs data management and analysis;
- Established an interagency agreement (IAG) with NIEHS to integrate ArrayTrack™ with the NIEHS CEBS system;
- Established a three-year cooperative research and development agreement (CRADA) with SAS to integrate ArrayTrack™ with SAS SDS so that the statistical functionalities in SAS can be accessed through ArrayTrack™;
- Developed several novel consensus methods, including Decision Forest, for diagnostic classifier and OMICs signature identification;
- Funded for an informatics grant proposal through collaboration with UMDNJ-RW Johnson Medical School;
- Hosted the informatics infrastructure (i.e. the VGDS server that is funded by CDER) at NCTR to support VGDS;
- Directly involved in data analysis of five VGDS submission;
- Provided ArrayTrack™ basic and advanced training course to the VGDS team;
- Participating in preparing the first manuscript on the “lesson-learn” from the VGDS data analysis; and
- Initiated and completed the MicroArray Quality Control (MAQC) project with inclusion of all of the FDA Centers, the primary microarray providers, and the major RNA standards providers in order to provide quality control throughout the microarray processing and analysis process. Significant milestone include:
 - Held the MAQC kickoff meeting at NCTR, and designed the Pilot-I on the selection/creation of two reference RNA samples;
 - Held the second face-to-face meeting at CDER. Two RNA samples (A and B) were selected for the main study based on Pilot-I datasets (160 arrays);

- Selected two titration points (samples C and D) in the ratios of 3:1 and 1:3 (A and B) for the main study (200 arrays);
- Completed main study data collection with over 600 arrays; and
- Held third face-to-face meeting in Palo Alto, California, with more than 100 participants. *Nature Biotechnology* will devote a supplemental issue to over 10 manuscripts from the MAQC project.

Center for Chemistry

The Counter Bioterrorism Research group has developed rapid, reliable, and cost-effective mass spectrometric methods to identify at the strain level pathogenic agents. These methods utilize pattern recognition-based biomarker methods to detect pathogenic agents and hoax counterterror materials.

Nanotechnology is an emerging research area within the Systems Toxicology Division. In collaboration with University of Arkansas at Little Rock (UALR), members of the Chemistry group have patented two nanotechnology-based cancer therapies, several large-scale nanoparticle production patents, and novel nanoparticle-based filter technology patents to protect the public from chemical and biological contaminants. This work complements ongoing sensor technology work for food quality assessment.

The Computational Chemistry group has a continuing collaborative effort with the University of Arkansas for Medical Sciences (UAMS) for development of noninvasive breast cancer detection methods and brain disease diagnostic markers. In addition, QSDAR models of predictive toxicity have been developed and experimentally validated for four dioxins previously believed to be nontoxic.

Major accomplishments include:

- Developed and patent applied for rapid bacterial detection and identification using flow cytometry, liquid handling robots, mass spectrometry, and computerized pattern recognition.
- Invented and patented novel computational methods for predicting biological, chemical, or physical properties of compounds.
- Developed colorimetric and electrical sensor systems for detecting food decomposition (volatile acids, bases, mercaptans, aldehydes).
- Developed methods for efficient, high purity carbon nanotube production and for monitoring and controlling quality issues associated with carbon nanotube production in continuous reactors.

Collaborations with Other Divisions

Scientists from the Division of Systems Toxicology participate in active collaborations with other Divisions at the Center. The Center for

Functional Genomics actively participates with each of the other Divisions at NCTR. For example, staff of the Functional Genomics Center act as collaborator or co-investigator on all microarray protocols that use this facility. The Proteomics group also has a number of cross-center collaborations including a very strong working relationship with the Division of Microbiology. One project was initiated with Neurotoxicology to examine the phosphoproteome. The Division of Biochemical Toxicology has a proteomic project looking at lymph nodes following tattoo ink exposure. The Metabolomics group is collaborating with the Neurotoxicology Division. This cross-center collaboration is epitomized by our Toxicoinformatics group with the use of ArrayTrack™ and the near completion of the MAQC project, both of which are essential to our FDA's Critical Path Initiative.

FY 2006 Plans

In FY 2006, the Division of Systems Toxicology will investigate the toxicity of selected liver, renal, and cardiovascular toxins using an integrated OMICs platform coupled with informatics and modeling analysis. This systems biology approach takes an integrative and iterative approach to test questions following perturbation of biological systems. To accomplish its mission, the Systems Toxicology group will:

- Develop an integrated, state of the art OMICs platform (consisting of microarray, NMR- and MS-based metabolomic, and proteomic signatures) that can perform analyses of compounds of interest to FDA and to provide the technical expertise and guidance to the agency in genomic, proteomic, and metabolomic interpretation;
- Utilize a series of liver, renal, and cardiovascular toxins to demonstrate the utility of integrated OMICs analyses;
- Develop a systems toxicology approach to the integrative analysis of OMICs data with conventional toxicology assessments;
- Develop computational models of toxicity and biomarker pattern identification; and
- Continue to develop nanotechnology and sensor technology efforts.

Contribution to FDA's Strategic Goals

The Division of Systems Toxicology contributes to the FDA strategic goal to increase innovative technology to advance health on several levels. First, we have developed a cadre of individuals with expertise in microarray performance and analysis, proteomic tool development and application, and metabolomics. Each of these has been applied to problems in preclinical development and is being assessed for use for clinical questions. The Metabolomics group members have been at the forefront in discussions on standards development in this rapidly evolving field. They are working diligently to develop biomarkers of hepatic, renal, and cardiovascular toxicity that might bridge preclinical and clinical tests.

The Proteomics group is developing a basic understanding of the field and its applicability to questions in toxicology. This work will lay the basis for future best practices. The existence of an internal OMICs expert panel at NCTR poises the NCTR to play a defining role in the integration of OMICs data into the review process. As a prelude to this, the members of the Systems Toxicology Division will be essential in training regulatory reviewers in how to assess OMICs data.

The Toxicoinformatics group has developed ArrayTrack™ as a reviewer tool for Microarray data and at least eighty reviewers have been trained thus far. This tool is under continuous development and is currently being developed to accept both proteomic and microarray data. NCTR staff have been essential in the initial VGDS data analyses and are an integral part of the IPRG. Their work on this initiative is in direct support of FDA's Critical Path Initiative. Other members of the team have been involved in other aspects of standardization of microarray technology. Specifically, the MAQC (MicroArray Quality Control) project catapulted NCTR/FDA into the front line discussions and contributions to standardization of microarray use. The Hepatotoxicology group is involved in three Critical Path Initiatives. In collaboration with CDER, they are working with a small company toward development of an *in silico* description of hepatotoxicity. In conjunction with CDER, they have proposed an innovative clinical trial design of a prospective safety study to develop biomarkers of liver toxicity should they arise in phase two. It is hypothesized that these biomarkers can then be applied prospectively to predict patients at risk. In addition, the Hepatotoxicity group is in the process of developing a CRADA with a company to jointly develop preclinical biomarkers of liver toxicity using pairs of compounds within a pharmacological (and preferably structural) class to determine OMICs biomarker signatures of compounds with a known clinical toxicity profile. Each of the activities of the Systems Toxicology Division is in support of both the Critical Path Initiative and the move toward personalized medicine goals of utmost importance to the mission of the FDA.

Ongoing Research Projects

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 NMR data and infuse three-dimensional atom-to-atom through-bond connectivity and atom-to-atom through-space intramolecular distance information into a three-dimensional pattern that can be used by pattern recognition software to build a model of a biological or toxicological endpoint. The results of the 3D-QSDAR models will be compared to the results of QSDAR and QSAR models from protocols E0706801, E0707701 and E0708301.

Beger, Richard

Case-Control Study of NMR Metabonomic Signatures for Prostate, Breast, and Colorectal Cancer (E0717601)

Objective(s): Determine if there are unique NMR spectral signatures in urine and/or serum obtained from prostate, breast, and colorectal cancer patients compared to controls.

Beger, Richard

Preclinical Metabonomic Biomarkers of Toxicity and Disease (E0720401)

Objective(s): Examine the utility of metabonomics as an approach to produce predictive models of cardiovascular, renal, neural, and hepatic toxicity. The models will be built using a variety of pattern recognition technologies to determine how temporal endogenous metabolic changes found in NMR and/or MS spectra of urine, serum, and tissue related to toxicity and disease state.

Beger, Richard

Clinical Metabonomic Biomarkers of Disease and Toxicity (S00643)

Objective(s): Characterize metabonomics signatures found in clinical urine and serum samples seen by ¹H NMR and mass spectrometry. The metabonomics approach will have important implications for medical, pharmaceutical, and regulatory agencies to assess efficacy and safety of emerging products.

Buzatu, Dan A.

The Development of Dynamic Mass Spectral/Pattern Recognition Based Methods for the Rapid Identification of Bioterror Agents (E0714601)

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

Buzatu, Dan A.	<p>Analysis of Proton MRS Data Using a Distributed Artificial Neural Network (E0719501)</p> <p>Objective(s): Evaluate whether a self-optimizing, parallel-distributed neural network can use the data from <i>in vivo</i> proton magnetic resonance spectroscopy (MRS) exams to provide additional information about a brain lesion. If so, this project will lead to improved brain tumor diagnoses from proton MR spectra.</p>
Buzatu, Dan A.	<p>The Development of Novel Nanotube-Based Technologies that Benefit Public Health, Protect the Public, Produce High Efficiency Separations and Filtration, and Improve Energetic Material Therapeutics (E0720501)</p> <p>Objective(s): Take advantage of the unique physical and electrical properties of nanotubes to develop:</p> <ol style="list-style-type: none"> 1) Novel technologies for the filtration of chemical and biological hazards from air, water, blood, and other media; 2) Technologies that protect public health or otherwise benefit the public; and 3) Novel nanotube/monoclonal antibody based cancer therapies.
Desai, Varsha G.	<p>Development of MitoChip, a Glass-based Oligonucleotide Microarray Containing Mitochondrial and Nuclear Genes Associated with Mitochondrial Function (E0718601)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) To develop a MitoChip containing genes associated with mitochondrial function such as oxidative phosphorylation, B-oxidation of free fatty acids, tricarboxylic acid cycle, apoptosis, as well as genes involved in the replication, transcription, translation of mitochondrial DNA, DNA repair, and regulation of DNA copy number; and 2) Validate the developed MitoChip by evaluating gene expression profiles of AZT, an anti-HIV drug, and 3-NPA, a neurotoxin that are known to alter mitochondrial function; and 3) Verify the relative expression levels of differentially expressed genes by real-time quantitative PCR.
Dragan, Yvonne	<p>Toxicological Effects of Ochratoxin A (E0709401)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Establish chemical and biological markers of oxidative stress to proteins using biochemical and mass spectrometry techniques; 2) Establish markers of oxidative damage to DNA by measurement of abasic site formation and oxidized DNA lesions by affinity detection and LC-MS methods; 3) Investigate changes in gene expression and protein expression in liver and kidney as a function of OTA treatment; and 4) Correlate differences in these above endpoints with <i>in vivo</i> mutagenesis using the Big Blue[®] Rat experimental model.
Dragan, Yvonne	<p>Biomarkers of Liver Disease and Toxicity (E0718801)</p> <p>Objective(s): Develop biomarker profiles for normal individuals and those with liver diseases or toxicity.</p>

Dragan, Yvonne	<p>Training in Hepatocyte Perfusion and Hepatic Cell Isolation (P00610)</p> <p>Objective(s): Train member(s) of the Hepatotoxicology Lab in primary liver cell isolation and culture. The long-term goals will be to obtain signature gene and protein expression patterns of each cell type for comparison to toxin-induced changes. Training must be provided to give confidence in the integrity of liver cells following perfusion, separation, and culture of the liver cells.</p>
Edmondson, Rick D.	<p>Methods for Support of a Functional Proteomics Facility at NCTR (E0713501)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Establish and standardize for routine use procedures for whole cell and subcellular organellar isolation for a variety of tissues; 2) Develop and standardize specific and sensitive markers of cell type and organellar purity and yield; 3) Identify, adapt, develop, and standardize appropriate 2-D protein separation techniques; and 4) Integrate results of specific aims 1-3 to provide “front-end” components of a functional proteomics facility.
Fuscoe, James	<p>Assessment of the Global Gene Expression Changes during the Life Cycle of Rats (E0712201)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Use the NCTR rat microarray chip to quantitate the relative expression of approximately 4000 genes in the liver of rats at the following ages: 2 wks, 5 wks, 6 wks, 8 wks, 15 wks, 21 wks, 52 wks, 78 wks, and 104 wks. These data will serve as a baseline measurement of gene expression that will be available for future studies on drug metabolism, toxicity, and susceptibility; and 2) Verify the relative expression levels by quantitative PCR or Northern analysis
Fuscoe, James	<p>Evaluation of Performance Standards and Statistical Software for Regulatory Toxicogenomic Studies (E0716801)</p> <p>Objective(s): Supply the experimental and statistical analyses necessary to help develop a consensus within FDA as to what performance standards would be beneficial for assessing the quality of microarray data submitted to the FDA on sponsor-selected platforms. The experimental results and conclusions from this intercenter project will be shared with other consortial microarray standardization efforts and made publicly available through publication.</p>
Fuscoe, James	<p>Prioritizing Sources of Variability in Genomic Profiling Data for Standards and Guidance Development (E0720601)</p> <p>Objective(s): Prioritize sources of variability in microarray data in order to determine how to focus additional experimental queries, guidance development, and experimental standards. The outcome should be an enhanced capability to address standards development and accept new technologies as they arise.</p>

Fuscoe, James	<p>Systems Biology Approach to Evaluate Sex Differences in the Heart of a Rat Model (E0723001)</p> <p>Objective(s): To produce a thorough and comprehensive knowledge base of biochemical and molecular sex differences in the hearts of a rat model system and to interpret these differences in light of sex-related health issues.</p>
Fuscoe, James	<p>General Support for Center for Functional Genomics (CFG) (S00616)</p> <p>Objective(s): The Center for Functional Genomics is a centralized facility to handle all aspects of microarray printing and processing. Its objectives are:</p> <ol style="list-style-type: none"> 1) To provide NCTR investigators with access to high quality microarray technology for the investigation of biological mechanisms of action underlying the toxicity of products regulated by the FDA, and related fundamental and applied research; 2) To create a validated toxicogenomics database that will be a resource for the scientific and regulatory community; 3) To be a focal point and scientific resource for issues in toxicogenomics, and 4) To utilize advances in genomics to address issues critical to the FDA mission. <p>In addition, the CFG will provide continual development of new and better approaches to microarray technologies, including larger gene collections, custom microarrays, validated gene expression databases, experimental design, and tools for handling and analyzing microarray data.</p>
Miller, Dwight W.	<p>Application of Solid Phase Detection Systems to Explosives in Airplane Cargo (E0708101)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Detection of ammonia—formulation, measurement of Am concentrations around container of ammonium nitrate, reformulation of FreshTag chemistry for label type detection, and development of PE or PVC film Shrink Rap detector; 2) Detection of acids, and 3) Detection of oxidizers such as peroxides and NO or No2.
Miller, Dwight W.	<p>Innovative, Static, and Dynamic Chemical Sensors (E0719901)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) To continue development of simple, inexpensive, field-compatible methods to monitor biochemical indicators of food quality; 2) To support development of manufacturing techniques that maintain food quality indicator (FQI) performance; and 3) Develop analytical laboratory procedures to confirm the colorimetric result and methods.

Shi, Leming	<p>The MAQC Project: MicroArray Quality Control (E0720701)</p> <p>Objective(s): The MicroArray Quality Control (MAQC) project aims to establish QC metrics and thresholds for objectively assessing the performance achievable by different microarray platforms and evaluating the merits and limitations of various data analysis methods. The MAQC project will help improve the microarray technology and foster its proper applications in discovery, development, and review of FDA-regulated products.</p>
Tong, Weida	<p>Development of a Novel Class Prediction Method, Decision Forest, for Analysis of Genomic and Proteomic Data (E0716901)</p> <p>Objective(s): 1) Develop the two-class Decision Forest method. The method will be developed on several publicly available gene expression and SELDI-TOF data sets, and the results will be compared with others that derived from traditional classification techniques; and</p> <p>2) The multiclass Decision Forest method will be also developed in this protocol. The method will be demonstrated on a gene expression data set to classify the pediatric acute lymphoblastic leukemia (ALL) subtypes.</p>
Tong, Weida	<p>Development of ArrayTrack™ Modules to Linking Functionality of ArrayTrack™ with SAS Scientific Discovery Solutions (SDS) (E0721401)</p> <p>Objective(s): Develop modules in ArrayTrack™ that integrate the functionalities of ArrayTrack™ with SAS Scientific Discovery Solutions (SDS) in order to provide the research community more comprehensive bioinformatics capabilities than each solution does alone.</p>
Tong, Weida	<p>Modification of a Version of ArrayTrack™ for Interoperability with the “Production” and “Research” Chemical Effects in Biological Systems (CEBS) Databases at NIEHS (S00652)</p> <p>Objective(s): Modify the NCTR ArrayTrack™ application to enable the importing of data files from the CEBS database and the processing of these files within the ArrayTrack™ application.</p>
Tong, Weida	<p>Use of ArrayTrack™ for CDER Drug Review Data Analysis (S00671)</p> <p>Objective(s): Data to be received from CDER drug review offices and ArrayTrack™ used to analyze data and send results back to CDER collaborators.</p>

Wilkes, Jon G.

Combining MAB/MS with Pattern Recognition to Sub-type Bacteria (E0707901)

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

Wilkes, Jon G.

Evaluation of Pyrolysis MAB/Tof MS and MALDI/Tof MS for Rapid Characterization of Presumptive Bioterror Agent Samples (E0714701)

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

Research Projects Completed in FY 2005

Buzatu, Dan A.

High Speed Parallel Distributed Neural Network Project (E0713101)

Results:

ANNs are extremely powerful pattern recognition tools. However, they are notoriously difficult to work with because of the amount of time it takes to properly develop a model (typically on one computer). Because this process is inherently tedious and inefficient and takes days to weeks to complete, a parallel distributed artificial neural network (PD-ANN) was developed in the summer of 2001. The PD-ANN produces accurate neural network models on many personal computers (PCs) simultaneously, cutting the ANN model development time down to a few hours or less. This was made possible through JGravity (a Java parallel distributed platform), which was obtained through a license agreement with Titan-LinCom Corp, and a computational backbone (an eight node PC cluster consisting of 8 2.4GHz P4s) that was developed in 2002.

The PD-ANN was first used to quickly develop the dioxin TEF predictive models. Following that success, the PD-ANN was used to develop two noninvasive brain tumor diagnostic models using abnormal and normal clinical magnetic resonance (MR) scans of human volunteers obtained from the UAMS in Little Rock. The scans were used to successfully teach the neural network the differences between the brain tissue chemical signatures of cancerous and healthy tissues. Leaving 10% of the data set out, the cross-validated models were able to achieve amazing $q_4^2=0.83$, and $q_4^2=0.88$ coefficients. Our goal is to automate the spectral interpretation process, enabling institutions that currently cannot interpret spectral exams because of lack of expertise to benefit from the added information one can get from an MRS exam. Additionally, if these techniques are implemented, their accuracy could significantly reduce the number of biopsies that have to be performed. The success of this study spawned another protocol-Analysis of proton MRS data using a distributed artificial network, which will make use of a greatly expanded brain MR data set to attempt to determine the type of cancer.

In a different project, the PD-ANN was used to create an accurate bacterial identification model using mass spectral data of salmonella. Pyrolysis mass spectra generated using metastable atom bombardment (a novel ionization method) were analyzed by the PD-ANN. The patterns of similarity among spectra in the *Salmonella* set were appropriately grouped and distinguished by genotype and phenotype. The PD-ANN was able to distinguish *Salmonella* strains by serovars, by PFGE patterns, and by antibiotic-resistance profiles. The study demonstrated that mass spectral methods in combination with ANNs can be used to build spectral databases capable of rapidly identifying bioterror agents is highlighted in a book chapter in *Identification of Microorganisms by Mass Spectrometry*. The development of this kind of accurate method is necessary for protecting the public, ensuring food safety, and improving food monitoring regulatory methods.

FY 2005 Publications

- Beger, R., Buzatu, D.A., and Wilkes, J.G., 2005. Combining NMR spectral information with associated structural features to form computationally nonintensive, rugged and objective models of biological activity, *DISCOVERY HANDBOOK Pharmaceutical Development and Research Handbook*, 1:227-286. (E0712601)
- Beger, R., Lehman-McKeeman, L.D., and Thomas, C.A., 2005. Standardisation of reporting methods for metabolic analyses: a draft policy document from the standard metabolic reporting structures (SMRS) group, *Nature Biotechnology*, 23:833-838. (E0717601)
- Brezna, B., Kweon, O., Stingley, R.L., Freeman, J.P., Khan, A.A., Polk, A.R., Jones, R.C., and Cerniglia, C.E., Molecular characterization of cytochrome P450 genes in the polycyclic aromatic hydrocarbon degrading *Mycobacterium vanbaalenii* PYR-1, *Applied Microbiology and Biotechnology*. (E0711801)
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Division of Veterinary Services

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Executive Summary

Introduction

The Division of Veterinary Services (DVS) provides professional and technical support for all animal-related research projects at NCTR. The Division administers the Center's Animal Care and Use Program, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). Included within the Division are contracted services for animal husbandry, veterinary care, diet preparation, and pathology. This workforce is stable, highly trained and skilled, and boasts a high percentage of certified employees in their respective disciplines.

The Division Director is a member of the Institutional Animal Care and Use Committee (IACUC), serving as Vice-Chair and Attending Veterinarian for NCTR. The liaison between DVS and the IACUC ensures maximum efficiency in protocol planning and review, provision of the highest quality of animal care and use, and delivery of superior service to the NCTR research community.

DVS oversees the operation of five animal facilities consisting of over 158,000 square feet of space dedicated to providing state-of-the-art housing and care of research animals. A variety of housing options are available for rodent models including ventilated rack systems and automatic watering systems. A barrier-maintained rodent breeding operation, established over thirty years ago, provides many of the strains used for on-site experiments.

Provision of veterinary services of the highest quality to NCTR's research animals is a Division priority. Two veterinarians certified by the American College of Laboratory Animal Medicine (ACLAM), a specialty board sanctioned by the American Veterinary Medical Association, are charged with ensuring that healthy animals are available for research projects, providing veterinary care if needed, training of research staff, and participating in projects requiring veterinary expertise. These veterinarians share emergency call duty during nonbusiness hours to ensure prompt attention to any animal in need of medical attention.

The Diet Preparation facility is a well-equipped, large-scale formulation services unit containing a specific pathogen free barrier work environment. The majority of dosed diets, water, and creams used in

experiments performed at the Center are prepared in this facility. Dosed feed production capability is 200,000 kg per year. Diets can be mixed with test articles in solution or solid state in concentrations as low as 0.1 ppb.

The Pathology Services group provides support to NCTR investigators to include necropsy and routine histopathology as well as molecular pathology, immunohistochemistry, clinical pathology, and other nonroutine services such as digital macrophotography, laser capture microdissection, and image archiving using digital storage of microscopic images at diagnostic resolution. The staff includes a professional team of pathologists and specialists in molecular and toxicologic pathology, medical technologists, and ASCP-certified technical support staff. Specific capabilities include: Laser Capture Microdissection (LCM); Tissue Microarrays; Real Time PCR, genetic monitoring of rodent strains; proliferation assays including BrdU, immunohistochemistry (IHC), Ki-67 (MIB-5) IHC, PCNA IHC, *in situ* hybridization for histone mRNA; Apoptosis assays including TUNEL, ISOL, Caspase-3 IHC, ssDNA monoclonal antibody IHC; immunoenzyme and immunofluorescent IHC on frozen sections, immunoenzyme and immunofluorescent IHC on paraffin-embedded sections, immunoenzyme and immunofluorescent IHC on cultured cells; *in situ* hybridization - nonradioactive ISH with RNA probes, nonradioactive ISH with oligonucleotide probes; PCR solution PCR, solution RT-PCR, *in situ* PCR, *in situ* RT-PCR; light and fluorescent microscopy, microphotography/digital and film. Digital macrophotography using either/or necropsy gross system or stereo microscope; Glycol Methacrylate and Methyl Methacrylate Plastic embedding and sectioning capability. Clinical Pathology - hematology and sperm analysis (rat/mouse) using Hamilton Thorn IVOS Sperm Analyzer.

FY 2005 Accomplishments

Immediate Office

The Division provided oversight and management of all laboratory animal facilities at NCTR. Divisional personnel were responsible for breeding, rearing, and/or acquiring and quarantining all experimental animals used on-site. Personnel submitted annual reports assuring compliance with federal regulations and NIH guidelines relative to our Animal Care and Use Program and participated in semi-annual program reviews, facility inspections, and experimental protocol reviews as part of the NCTR IACUC proceedings. All animal resource needs were managed for all research projects including ordering, quarantine, housing, and care of animals. Divisional personnel served as government project officers for the pathology services, animal care and diet preparation services, and rodent bedding contracts for the Center. The Veterinary Care Program was administered through this office and, in addition to providing veterinary care to NCTR's research animals, included oversight of policies

and procedures for animal procurement and transportation, preventive medicine, health and genetic monitoring, environmental enrichment, surgical protocols, anesthesia of laboratory animals, pain management, and euthanasia. In order to ensure state-of-the-art housing environments for research animals, members of this Division played an integral role in planning animal facility renovation projects conducted in FY 2005.

Animal Care/Diet Preparation Services

During FY 2005, contract personnel supported an average daily census of twenty-eight experiments. These experiments entailed at a minimum the daily husbandry services for an average 6730 rodents and 84 rhesus monkeys. A variety of technical procedures were performed on many experiments including tattooing, tumor palpations, biological sample collections, injections (SC, IM, and/or IV), oral gavage (including neonatal mice), behavior assessments on rats and rhesus monkeys, application of topical dosed creams, rodent breeding operations, quarantine of rodents and rhesus monkeys, physical and pregnancy examinations of rhesus monkeys, microchip implantations, anesthesia of animals, and humane euthanasia. An ongoing AALAS (American Association for Laboratory Animal Science) training program ensured the maintenance of a high percentage of certified staff. Currently 90% of animal care and diet preparation staffs are AALAS-certified, and eight members of the animal care management group are Certified Managers of Animal Resources (CMAR). In addition to processing standard rodent chow (autoclaving, packaging, and delivery), dosed diets, dosed water, and topical creams were prepared in a barrier facility to exacting specifications for National Toxicology Program (NTP) experiments. Quality control personnel performed monthly inspections of all animal housing and diet preparation units, performed hundreds of quality control audits of animal care and diet preparation procedures and maintained, updated, and created a large volume of SOPs (275 for animal care; 76 for diet preparation). Veterinary care for all animals was provided by two veterinarians who are board certified in laboratory animal medicine (American College of Laboratory Animal Medicine). An on-site barrier rodent production operation supplied animals for the majority of experiments. Extensive environmental and health monitoring activities were performed in cooperation with NCTR's microbiological surveillance and chemistry support groups to ensure pathogen exclusion from animal colonies, bedding, and feed. Veterinary surgical services were provided for rodent and nonhuman primate studies, and veterinarians served as Principal Investigators or Co-Investigators on several protocols.

Pathology and Pathology-related Services

During FY 2005, Pathology contract personnel implemented the newly developed Gross Pathology System for collecting and reporting of pathology data and tracking of specimens through the pathology process. The new system is designed to link gross pathology data with microscopic

data and all in-house processing tasks, including the archiving of specimens. A Virtual Microscopy/Pathology System (ScanScope) is being used to input, store, and retrieve the vast number of images collected for the phototoxicology studies. In addition, this system could be used to replace the traditional Pathology Working Group (PWG) process as it allows the sharing of slides/images via the internet with multiple off-center sites thus eliminating the need for the PWG members to meet in a single location for viewing the specimens of interest. The Molecular Pathology group developed the following immunohistochemical protocols: β -hydroxysteroid, dehydrogenase (marker for Leydig cells), polycystin-2, double immunofluorescent staining for histone H1 and β -catenin, Melan A, TRP-1, anti-fibroblast, pan-cytokeratin plus, p8, IRS-3, IRS-4, fatty acid synthase, apoA-IV, trimethyl-histone H4 (Lys20), anti-mouse Ki-67 (clone TEC 3), iNOS, nitrotyrosine, CD48, CD55. Pathology personnel assisted with RNA extraction from cultured cells, RNA extraction from brain tissue, and Gel Shift. The NTP quality assessment and peer review of pathology data for *aloe vera* and retinyl palmitate was accomplished. During 2004, pathology contract employees authored or co-authored multiple publications or presentations.

FY 2006 Plans

- Procure a new Animal Care and Diet Preparation Contract for the Center.
- Continue to support the research mission of NCTR through excellence in animal care, veterinary care, diet preparation, and pathology services.
- Continue supplying methods development and support, both technical and professional, needed to accomplish the NIEHS IAG work at NCTR.
- Continue a quality laboratory animal care and use program that is consistent with state and federal laws, regulations, and guidelines.
- Eradicate *Helicobacter hepaticus* from the NCTR rodent breeding colony.
- Expand and improve the environmental enrichment program for rodents.
- Continue active participation on research protocols as Principal Investigators and Co-Investigators.
- Expand and improve the disaster plan as it relates to the research animal program.
- Conduct quality assessment and PWG for *Aloe vera*.
- Add methodologies in pathology to support research proposed by personnel of the Division of Systems Toxicology at the Center.

Contribution to FDA's Strategic Goals

The Division of Veterinary Services (DVS) plays a critical support services role in NCTR's biomedical research program. DVS personnel

interact with individuals from every research division on a daily basis, providing expertise in animal care, diet preparation, laboratory animal medicine, and pathology. These services are provided by highly trained, skilled, and dedicated individuals whose contribution enhances the quality of the research conducted by NCTR scientists. In addition, DVS oversees the NCTR Laboratory Animal Care and Use Program, which has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) since 1977. This distinction assures Center scientists, the FDA, and the American consumer that data generated from animal experiments at NCTR are of the highest integrity.

DVS also contributes to NCTR's research program through participation in the projects of other divisions as Principal Investigators and Co-Investigators. Several DVS personnel are DVMs and/or Ph.D.s whose specialties in comparative medicine, veterinary pathology, and biochemistry complement the research teams in all other divisions. Each research division contributes to the FDA's Strategic Goals in its own unique way through the individual and collective talents of its personnel as described in this document. DVS, through its support services functions and research participation, is part of each division's contribution to these goals.

Ongoing Research Projects

Feuers, Ritchie J.

Evaluation of Calorically Restricted Human Surgical Samples Received from Department of Surgery University of Tennessee, Memphis (E0699801)

Objective(s): Determine whether rodents and humans behave biologically in the same manner when calorically deprived but nutritionally supplemented.

FY 2005 Publications

- Beland, F.A., Benson, R.W., Mellick, P.W., Kovatch, R.M., Roberts, D.W., Fang, J., and Doerge, D.R., 2005. Effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B₆C₃F₁ mice, *Food and Chemical Toxicology*, 43:1-19. (E0212001)
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- Boudreau, M.D., Olson, G.R., and Beland, F.A., 2005. Consumption of *Aloe Vera* induces hyperplasia in the colon of F344 Rats and B₆C₃F₁ Mice, *American Society of Experimental Biology*, 19:4. (E0214001)
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- Fu, X., Latendresse, J.R., Muskhelishvili, L., Blaydes, B.J., and Delclos, K.B., 2005. Dietary modulation of 7,12-dimethylbenz[a]anthracene (DMBA)-induced adrenal toxicity in female Sprague-Dawley rats, *Food and Chemical Toxicology*, 43:765-744. (E0702701)
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- Latendresse, J.R. 2004. Potentiation of carbon tetrachloride hepatotoxicity and lethality in type 2 diabetic rats, *Journal of Pharmacology and Experimental Therapeutics*, 308:694-704. (NA)
- Latendresse, J.R. 2005. Ovarian follicular counting in the assessment of rodent reproductive toxicity, *Toxicologic Pathology*, 33:408-412. (NA)
- Muskhelishvili, L., 2005. Proliferating cell nuclear antigen-a marker of ovarian follicle counts, *Toxicologic Pathology*, 33:3:365-368. (NA)
- Scallet, A.C., Muskhelishvili, L., Slikker, W., and Kadlubar, F.F., 2005. Sex Differences in cytochrome P450 1B1, as an estrogen-metabolizing enzyme in the rhesus monkey telencephalon, *Journal of Chemical Neuroanatomy*, 29:1:71-80. (NA)

- Tolleson, W.H., Doss, J.C., Latendresse, J.R., Warbritton, A.R., Melchior, W.B., Chin, L., Dubielzig, R.R., and Albert, D.M., 2005. Spontaneous uveal amelanotic melanoma in transgenic Tyr-RAS⁺ Ink4a/Arf^{-/-} mice, *Archives of Ophthalmology*, 123:1088-1094. (E0708901)
- Wang, C., Sadovova, N.V., Fu, X., Schmued, L.C., Scallet, A.C., Hanig, J.P., and Slikker, W., 2005. The role of N-methyl-D-aspartate receptor in ketamine-induced apoptosis in rat forebrain culture, *Neuroscience*, 132:967-977. (P00636)
- Warbritton, A.R. and Latendresse, J.R. 2004. Tolerance of aged Fischer 344 rats against chlordecone-amplified carbon tetrachloride toxicity, *Mechanisms of Aging and Development*, 125:421-435. (NA)
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- Young, J.F., Tsai, C., Chen, J.J., Latendresse, J.R., and Kodell, R.L., Database composition can affect the Structure-Activity Relationship prediction, *Journal of Toxicology and Environmental Health*. (E0708301)

NCTR Collaborative Activities

Throughout its history, NCTR has actively sought and participated in collaborative, cooperative partnerships with other scientific and regulatory organizations. These opportunities to leverage resources, both public and private, enable NCTR to address questions of common concern to both FDA and the collaborating agency. These partnerships have led to substantial research advances that have resulted in significant improvements in long-term public health, such as regulatory guidance, mechanistic understanding, and advanced methodology.

Interagency Agreements (IAGs)

Interagency Agreements are formal financial partnerships with other government agencies.

Environmental
Protection Agency
(EPA)

PI: Turturro, Angelo

Development of a Model for the Transmission Kinetics of Infection by *Cryptosporidium parvum* with Acquisition of Data on Key Parameters (E0708201)

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

Federal Aviation
Administration
(FAA)

PI: Miller, Dwight W.

Application of Solid Phase Detection Systems to Explosives (E0708101)

- Objective(s):**
- 1) Detection of ammonia—formulation, measurement of Am concentrations around container of ammonium nitrate, reformulation of FreshTag chemistry, originally developed for determining food decay, for label type detection, and development of PE or PVC film Shrink Rap detector;
 - 2) Detection of acids; and
 - 3) Detection of oxidizers such as peroxides and NO or NO₂ for use in biohazard identification.

National Institute of Environmental Health Science (NIEHS)
PI: Slikker, William

Assessment of Ketamine in the Developing Nonhuman Primate (E0718901)

- Objective(s):**
- 1) Determine, using neurohistochemical approaches, if, and at what developmental stages, ketamine exposure increases neuronal apoptosis/proliferation;
 - 2) Determine, using neurohistochemical approaches, the dose-response for ketamine to produce apoptosis at the most sensitive developmental stage;
 - 3) Determine the reversibility or permanence of the response using behavioral, imaging and neurohistochemical approaches; and
 - 4) Determine, at the most sensitive stage and dose, genomic and proteomic responses to ketamine treatment.

National Cancer Institute (NCI)
PI: Pogribny, Igor P.

Global and Locus-specific DNA Hypomethylation: A Common Mechanism Involved in Genotoxic and Nongenotoxic Rat Hepatocarcinogenesis (E0718101)

- Objective(s):**
- 1) Determine if the temporal alterations in genomic methylation profile in preneoplastic liver tissue observed in the folate/methyl deficient model of rat endogenous hepatocarcinogenesis also occur in other carcinogenesis model;
 - 2) Identify genes that are consistently up-regulated or down-regulated in target tissue during the promotion stage of carcinogenesis; and
 - 3) Evaluate whether or not the global and locus-specific DNA hypomethylation along with aberrant expression of related genes and changes in chromatin conformation is specific only to target tissues and may be used for early detection of chemicals with carcinogenic potential.

National Institutes of Health (NIH) / National Institute of Environmental Health Science (NIEHS)
PI: Tong, Weida

Modification of a Version of ArrayTrack™ for Interoperability with the “Production” and “Research” Chemical Effects in Biological Systems (CEBS) Databases at NIEHS (S00652)

- Objective(s):** Modify the NCTR ArrayTrack™ application to enable the importing of data files from the CEBS database and the processing of these files within the ArrayTrack™ application.

Environmental Protection Agency (EPA) / National Health and Environmental Effects Research Laboratory (NHEERL)
PI: Beland, Frederick A.

Detection of DNA Adducts in Mice Treated with Benzo[a]pyrene at Low Exposure Levels (E0723701)

- Objective(s):** Define dose-response curves for benzo[a]pyrene DNA adducts in the A/J mouse lung—will be utilizing the application of HPLC-ES-MS/MS methodologies developed at NCTR.

National Institutes of Health (NIH) / National Institute of Child Health and Human Development (NICHD)

PI: Morris, Suzanne M.

National Institutes of Health (NIH) / National Institute of Child Health and Human Development (NICHD)

PI: Morris, Suzanne M.

Evaluation of the Genetic Toxicity and Behavioral Effects of Chronic Methylphenidate Exposure in Juvenile Male Rhesus Monkeys (Macaca mulatta) (E0723401)

- Objective(s):**
- 1) Determine the baseline frequency of measures of genetic damage in a population of juvenile rhesus monkeys;
 - 2) Determine the frequency of these measures of genetic damage in a population of juvenile rhesus monkeys at defined intervals during a chronic exposure to methylphenidate;
 - 3) Determine if chronic exposure to methylphenidate results in measurable effects on the behavior of juvenile rhesus monkeys utilizing the NCTR Operant Test Battery; and
 - 4) Determine the plasma concentration of methylphenidate and its major metabolite, ritalinic acid, during the chronic exposure of juvenile rhesus monkeys to the drug.

Evaluation of the Genotoxicity and Pharmacokinetics of Methylphenidate in Male Big Blue[®] Mice (E0723501)

- Objective(s):**
- 1) Determine the metabolites of methylphenidate at early times after exposure in B₆C₃F₁ mice to compare the major metabolites in the human, monkey, and mouse;
 - 2) Determine the plasma levels of methylphenidate and its major metabolites in the B₆C₃F₁ mouse after 28 days of exposure;
 - 3) Determine the effect of exposure to methylphenidate on body and organ weights of the B₆C₃F₁ mouse after 28 days of exposure;
 - 4) Determine if long-term exposure to methylphenidate results in a dose-responsive increase in the liver *c11* gene mutant frequency of Big Blue[®] mouse; and
 - 5) Determine the pharmacokinetics of methylphenidate and its major metabolite, ritalinic acid, in B₆C₃F₁ mice.

***National Institute for Environmental Health Sciences
(NIEHS) / National Toxicology Program (NTP) Interagency
Agreement***

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In 1992 the Food and Drug Administration (FDA) entered into an Interagency Agreement (IAG) with the National Institute for Environmental Health Sciences (NIEHS). The design for this agreement concentrated on FDA's priority National Toxicology Program (NTP) nominations of chemicals/agents and utilized the unique resources and facilities at the National Center for Toxicological Research (NCTR). The research conducted under the IAG provided FDA the ability to better assess the safety of a number of FDA-regulated products.

The 1992 agreement provided support for five FDA priority chemical/agent NTP nominations. The agreement has expanded to include collaborative research on five commonly accepted endocrine disrupter compounds, which include three multigeneration studies and two chronic cancer studies. Currently the IAG includes the evaluation of AIDS therapeutic drugs, dietary supplements, mycotoxins, such as fumonisin, and acrylamide, a chemical produced when some food products, such as potatoes, are cooked at high temperatures. In 1998 NCTR opened a FDA/NIEHS Phototoxicity Research and Testing Laboratory. The facility is state-of-the-art, testing compounds applied to the skin in simulated solar light.

All research under the NIEHS/NTP IAG is designed with input from FDA regulatory scientists, NCTR and NIEHS scientists, experts from universities, and often experts from the regulated industry. The IAG utilizes resources from public funds and exceptional scientific expertise to provide the best possible assessment of product safety resulting in accomplishment of the missions of the FDA and NIH.

Beland, Frederick A.	<p>Perinatal Carcinogenicity of Drug Combinations used to Prevent Mother-to-Child Transmission of HIV (E0214111)</p> <p>Objective(s): To determine the carcinogenicity, genotoxicity and metabolism of antiretroviral drug combinations administered to mice transplacentally, perinatally, or neonatally.</p>
Beland, Frederick A.	<p>Genotoxicity and Carcinogenicity of Acrylamide and its Metabolite, Glycidamide, in Rodents—Range-Finding/Subchronic/Two-Year Chronic Carcinogenicity Studies (E0215001)</p> <p>Objective(s): To compare the carcinogenicity of acrylamide and its metabolite glycidamide in B₆C₃F₁ mice and F344 rats treated chronically for two years.³</p>
Boudreau, Mary D.	<p>Effects of <i>Aloe Vera</i> Components on Cell Proliferation and DNA Adduct Formation in SKH-1 Mice Following Simulated Solar Light Exposure (E0214001)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determine the dose-response and acute kinetics of topical exposure to <i>Aloe vera</i> 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 <i>Aloe vera</i> 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 <i>Aloe vera</i> 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 <i>Aloe vera</i> plant components following simulated solar light tumor initiation; and 5) Determine the influence of <i>Aloe vera</i> components on simulated solar light-induced tumor formations in mice.
Boudreau, Mary D.	<p>Bioassays in the F344 Rat and the B₆C₃F₁ Mouse Administered <i>Aloe Vera</i> Plant constituents in the Drinking Water (E0214201)</p> <p>Objective(s): The use of <i>Aloe vera</i> is not limited to over the counter dermal therapeutics and cosmetics. <i>Aloe vera</i> is also taken internally, and <i>Aloe vera</i> for internal consumption is also widely used as a prophylaxis and treatment for a variety of unrelated systemic conditions. In view of the complexities inherent in <i>Aloe vera</i> pharmacology and the inconsistencies reported in literature, the objective of these studies is to conduct bioassays in rats and mice using standardized preparations of <i>Aloe vera</i> to explore the limits of safety for the <i>Aloe vera</i> leaf constituents present in commercial products.</p>
Delclos, K. Barry	<p>Genistein: Evaluation of Reproductive Effects Over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages (E0213201)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 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

	<p>when administered to CD rats over multiple generations;</p> <ol style="list-style-type: none"> 2) To determine if subtle effects observed in the dose range-finding study are magnified through multiple generations; and 3) To evaluate the reversibility of any observed effects; and 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 (<i>in utero</i> through early adulthood, <i>in utero</i> and continuous for 2 years after birth, <i>in utero</i> and lactational only, and postweaning only).
Delclos, K. Barry	<p><i>p</i>-Nonylphenol: Evaluation of Reproductive Effects over Multiple Generations (E0213501)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Determine the effects of <i>p</i>-nonylphenol, an intermediate in the production of surfactants and other industrial products, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations; 2) Determine if subtle effects observed in the dose range-finding study are magnified through multiple generations; and 3) Evaluate the reversibility of any observed effects.
Delclos, K. Barry	<p>Ethinyl Estradiol: Evaluation of Reproductive Effects over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages (E0213801)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 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; and 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.
Delclos, K. Barry	<p>Assessment of Vehicles for Intravenous Delivery of di(2-ethylhexyl)phthalate (DEHP) and Methods of Development for Measurement of Serum and Urinary Metabolites of DEHP, and Hormonal and Male-specific Chromosomal Markers in the Blood of Rhesus Monkeys (P00650)</p> <p>Objective(s): Provide preliminary information for a study of the pharmacokinetics and biological effects of DEHP in a developing nonhuman primate model.</p>
Doerge, Daniel R.	<p>Genotoxicity, Mutagenicity and Exposure Biomarkers of Acrylamide and Its Metabolite, Glycidamide, in Rodents (E0214601)</p> <p>Objective(s):</p> <ol style="list-style-type: none"> 1) Synthesize chemically and characterize spectroscopically the major glycidamide-DNA adducts;

	<ol style="list-style-type: none"> 2) Develop and validate LC-ES/MS/MS assays to quantify the major glycidamide-DNA adducts; 3) Determine glycidamide-DNA adduct levels in rodent tissues following short-term exposures of rodents to acrylamide and to glycidamide; 4) Determine toxicokinetics and compare bioavailability of acrylamide and glycidamide following exposure by intravenous, oral gavage, and dietary administration; 5) Correlate the levels and kinetics of glycidamide-DNA adduct in target tissues and circulating lymphocytes with acrylamide- and glycidamide-hemoglobin adducts in rodent exposure studies for future use in monitoring human exposure through occupation, smoking, and the diet; and 6) Determine <i>in vivo</i> mutagenesis of acrylamide and glycidamide using transgenic mice (Big Blue[®]).
Doerge, Daniel R.	<p>Neuroendocrine Mechanisms For Acrylamide Carcinogenicity (E0214631)</p> <ol style="list-style-type: none"> 1) Dopamine and its metabolic turnover products will be measured in hypothalamus and pituitary to determine the effects of acrylamide on neurotransmitter release. In addition, circulating levels of pituitary-regulated hormones, the thyroid hormones, and sex steroid hormones (estradiol, progesterone, testosterone) will be determined. 2) Acrylamide-induced changes in expression of hormone-related genes will be determined, including those important for brain dopaminergic responses and the thyroid. 3) Acrylamide-induced changes in normal metabolites present in urine, serum, and brain will be investigated to identify a accessible biomarkers of effect for possible extrapolation to human biomonitoring studies. 4) Dose-dependent changes in testicular function (sperm motility and morphology) caused by acrylamide will be investigated.
Fu, Peter P.	<p>Effect of Topically Applied Skin Creams Containing Retinyl Palmitate on the Photocarcinogenicity of Simulated Solar Light in SKH-1 Mice (E0214301)</p> <p>Objective(s): Study the effects of topically applied skin cream containing retinyl palmitate on the photocarcinogenicity of simulated solar light in SKH-1 mice.</p>
Hansen, Deborah K.	<p>Developmental Toxicity of Bitter Orange in Rats (E0214701)</p> <p>Objective(s): To determine potential developmental toxicity of synthetic synephrine and citrus aurantium extract in rats.</p>

PI: Hansen, Deborah K.	Physiological Effects of Bitter Orange in Rats (E0214901) Objective(s): To determine potential physiological effects of synthetic synephrine as well as an extract from the botanical citrus aurantium alone and in combination with caffeine in rats.
Leakey, Julian E.	Toxicity Studies of Combination of AIDS Drugs in <i>p53</i>^(+/-) Transgenic Mice (E0215201) Objective(s): To evaluate the potential toxicity and carcinogenicity of perinatal and chronic exposures to AIDS drugs, Zidovudine (AZT) and Lamivudine (3TC) in C57BL/6(N5)trp53 (+/-) haplodeficient F1 transgenic mice.
Leakey, Julian E.	Development of Methods for the Analysis of Chondroitin Sulfate and Glucosamine in Dosing Solutions, Feed and Animal tissues (P00644) Objective(s): To develop methods for analyzing concentrations of chondroitin sulfate and glucosamine in dosing solutions, feed and animal tissues in preparation for a chronic toxicology and carcinogenicity studies of chondroitin sulfate and glucosamine.
Paule, Merle G.	Developmental Neurotoxicity Assessment of Acrylamide in Rats: Range-Finding Studies (E0214801) Objective(s): To determine acrylamide doses to be used in subsequent long-term developmental neurotoxicity studies by identifying those that will not result in overt toxicity as determined by alterations in body weight gain and a variety of physiological, developmental and behavioral parameters of either pups or dams.
Paule, Merle G.	Developmental Neurotoxicity Assessment of Acrylamide in Rats: Long-Term Studies (E0215101) Objective(s): To determine the consequences of long-term exposure to acrylamide on a variety of developmental milestones and measures of nervous system integrity throughout life.
Wang, Cheng	NMDA Antagonist/GABA Agonist-induced Cell Death in the Developing Rat Brain (E0215501) Objective(s): <ol style="list-style-type: none"> 1) To screen and evaluate pediatric anesthetic agents; 2) To determine if a one-time bolus dose or prolonged exposure of the developing rat to NMDA antagonist, GABA agonist alone, or their combinations will induce long-term behavioral deficits, as well as long-lasting pathological changes; 3) To determine the dose, temporal and pathophysiological relationships between MDA antagonist/GABA agonist-induced neurotoxicity and long-term behavioral changes; 4) To determine the neurotransmitter receptor mechanisms involved in the neuron degeneration and behavioral deficits caused by these agents, particularly the role of altered NMDA receptor function; 5) To determine by <i>in situ</i> hybridization and immunoblot the relative densities of NMD receptor NR1, NR2A and NR2B subunits following anesthetic drug administration; and 6) To identify mechanisms that could link altered NMDA

receptor function, elevation of superoxide free radicals to anesthetic drug-induced apoptosis, inhibitors will be added at various times to determine the contribution and temporal distribution of several elements of the proposed pathway leading to cell death.

Collaborative Research and Development Agreements (CRADAs)

Collaborative Research and Development Agreements are formal financial partnerships with nongovernmental organizations, nonprofit organizations and private companies.

Argus Research Laboratories, Primedica Corporation
PI: Howard, Paul C.

Historical Database of Skin Tumor Formation in SKH-1 Mice (S00213)

- Objective(s):**
- 1) Transfer photocarcinogenicity data from 12 studies at Argus Research Laboratories to NCTR, and enter into NCTR MultiGen database;
 - 2) Develop statistical methods for analysis of tumor incidence in these photocarcinogenesis studies;
 - 3) Share with Argus Research Laboratories methods that are developed for data analysis. NCTR and Argus Research Laboratories will also share information on the generation of simulated solar light.

AstraZeneca
PI: Paule, Merle G.

Evaluation of Changes in Gene Expression in the Brain Associated with Normal Development and the Behavioral Toxicity caused by Developmental Exposure to the N-methyl-D-aspartate (NMDA) Receptor Antagonists, Sodium Channel Blockers, and Combinations (E0716501)

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

Litmus, LLC
PI: Miller, Dwight W.

Innovative, Static, and Dynamic Chemical Sensors (E0719901)

- Objective(s):**
- 1) To continue development of simple, inexpensive, field-compatible methods to monitor biochemical indicators of food quality;
 - 2) To support development of manufacturing techniques that maintain food quality indicator (FQI) performance; and
 - 3) To develop analytical laboratory procedures to confirm the colorimetric result and methods.

Pfizer, Inc.
PI: Paule, Merle G.

Cognitive Assessments of Several Psychotropic Compounds using the NCTR Operant Test Battery (OTB) (E0721101)

Objective(s):

- 1) Determine the acute dose-effect relationships of several psychotropic drugs on a battery of operant behavioral tasks in rhesus monkeys;
- 2) Characterize the relative sensitivities of the various behavioral end-points in NCTR's Operant Test Battery (OTB) to these agents; and
- 3) Compare the behavioral profiles of these agents to those of a variety of reference compounds with well-characterized mechanisms of action.

RxGen, Inc.
PI: Beger, Richard

Preclinical Metabonomic Biomarkers of Toxicity and Disease (E0720401)

Objective(s): Examine the utility of metabonomics as an approach to produce predictive models of cardiovascular, renal, neural and hepatic toxicity. The models will be built using a variety of pattern recognition technologies to determine how temporal endogenous metabolic changes found in NMR and/or MS spectra of urine, serum, and tissue related to toxicity and disease state.

SAS Institutes, Inc.
PI: Tong, Weida

Development of ArrayTrack™ Modules to Linking Functionality of ArrayTrack™ with SAS Scientific Discovery Solutions (SDS) (E0721401)

Objective(s): Develop modules in ArrayTrack™ that integrate the functionalities of ArrayTrack™ with SAS Scientific Discovery Solutions (SDS) in order to provide the research community more comprehensive bioinformatics capabilities than each solution does alone.

Sigma Tau Research, Inc.
PI: Binienda, Zbigniew K

Development of Biomarkers for Early Detection of Mitochondrial Dysfunction (E0711021)

Objective(s):

- 1) Develop the 3-NPA *in vitro* model to characterize the early genomic biomarkers for the mitochondrial dysfunction using the microarray technique; and
- 2) Provide information on a standardized microarray system to allow the screening of agents with the potential to affect mitochondrial function and, therefore, predict brain injury.

University of Arkansas at Little Rock
PI: Paule, Merle G.

Effects of Prenatal Cocaine on Behavioral Plasticity (E0663307)

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

University of
Arkansas for
Medical Sciences
PI: Paule, Merle G.

Novel Studies on Sites of Action and Mechanisms in Chronic Balance Dysfunction (E0722301)

Objective(s): Develop and implement a comprehensive assessment of all levels of the neuraxis in an effort to determine CNS deficits due to balance disorder and vertigo and develop and assess strategies to restore those deficits.

University of Illinois
PI: Doerge, Daniel R.

Phytoestrogens and Aging: Dose, Timing, and Tissue (E0721001)

Objective(s): Evaluate the potential benefits or detrimental effects of dietary phytoestrogens on breast cancer progression, adipose tissue, and the brain, using well-established laboratory animal models.

Office of Science
and Health
Coordination
(OSHC)
PI: Fuscoe, James

Office of Women's
Health (OWH)
PI: Fuscoe, James

Intra-agency Agreements

Consistent with its role within the FDA, NCTR actively participates in collaborative research with the other FDA Centers. For some high priority research projects, NCTR receives financial resources from the other Centers to facilitate the timely conduct and completion of important studies.

Prioritizing Sources of Variability in Genomic Profiling Data for Standards and Guidance Development (E0720601)

Objective(s): Prioritize sources of variability in microarray data in order to determine how to focus additional experimental queries, guidance development and experimental standards. The outcome should be an enhanced capability to address standards development and accept new technologies as they arise.

Systems Biology Approach to Evaluate Sex Differences in the Heart of a Rat Model (E0723001)

Objective(s): To produce a thorough and comprehensive knowledge base of biochemical and molecular sex differences in the hearts of a rat model system and to interpret these differences in light of sex-related health issues.

Memorandum of Understanding (MOUs)

A Memorandum of Understanding is a formal agreement for collaborative research or other partnership activities. NCTR has a work sharing agreement with the State of Arkansas Department of Health to share expertise and laboratory infrastructure in support of the state's public health preparedness and response to bioterrorism. NCTR also has a Letter of Understanding with BG Medicine to develop a CRADA for Development of Liver Toxicity Biomarkers using an integrated OMICs approach. The CRADA is under review within the Agency.

Material Transfer Agreements (MTAs)

Material Transfer Agreements provide a mechanism whereby materials can be exchanged between government and nongovernmental organizations. A number of MTAs are in place for NCTR including:

ACD Software	Lipomics
ABI	MD Anderson
Agilent	Pfizer
Eppendorf	Schering Plough
GeneExpress	St Louis University
GeneGO	Waters
Ingenuity Systems, Inc.	

Informal Collaborations

NCTR scientists are active collaborators with investigators at numerous national and international organizations in a wide variety of research projects. Organizations with which NCTR scientists collaborate include:

Agilent	North Carolina State University
Arkansas Cancer Research Center	Patterson Institute for Cancer Research (Great Britain)
Arkansas Children's Hospital	Penn State
ARS Biosciences Research Laboratory (Fargo, ND)	St. Jude Children's Research Hospital
Central Arkansas Veterans Health Care Systems	SAS Institute
Environ, International	State University of New York (Stony Brook)
Environmental Protection Agency	U.S. Department of Agriculture
Eppendorf	University of Alabama (Birmingham)
GeneGo	University of Arizona
Harding University	University of Arkansas (Fayetteville)
Harvard University	University of Arkansas (Little Rock)
Health Canada	University of Arkansas (Pine Bluff)
Icoria	University of Arkansas for Medical Sciences
Imperial College London	University of California (Berkeley)
INSERM, Strasbourg (France)	University of California (Irvine)
Institute of Cancer Research	University of California (San Luis Obispo)
Institute of Statistical Science (Taiwan)	University of Connecticut
Instituto Superior Técnico	University of Coimbra (Portugal)
József Fodor National Center for Public Health	University of Georgia
KeyMolnet	University of Kiel (Germany)
Litron Laboratories	University of Maryland
Lovelace Inhalation Toxicology Research Institute	University of Massachusetts (Amherst)
Loyola University (Chicago)	University of Miami (Florida)
Marshfield Clinic Research Foundation	University of Mississippi
Massachusetts Institute of Technology	University of Missouri
MD Anderson	University of Montreal
NASA Johnson Space Center	University of Rochester
National Cancer Institute	University of South Carolina
National Institute on Drug Abuse	University of Utah
National Institute of Health Sciences (Tokyo, Japan)	University of Wisconsin (Madison)
National Institute for Occupational Health Safety	University of Wisconsin School of Medicine
National Institute of Standard Technology (NIST)	Uppsala University (Sweden)
National Institutes of Health	Utah University
New Mexico Tech	Vanderbilt University
New York Department of Health	Veterans Affairs Medical Center (Minneapolis)
	Wake Forest
	Washington School of Medicine
	Yale University

Acronyms

3-NPA	3-Nitropropionic Acid
AA	Aristolochic Acid
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care, International
AALAS	American Association for Laboratory Animal Science
ACB-PCR	Allele-specific Competitive Blocker-Polymerase Chain Reactionn (ACB-PCR)
AIDS	Acquired Immune Deficiency Syndrome
AMPH	Amphetamine
ARL	Arkansas Regional Laboratory
CBER	Center for Biologics Evaluation and Research
CD	Sprague-Dawley
CDC	Centers for Disease Control and Preventions
CDER	Center for Drug Evaluation and Research
cDNA	Complementary DNA
CDRH	Center for Devices and Radiological Health
CE	Competitive Exclusion
CEBS	Chemical Effects in Biological Systems
CFSAN	Center for Food Safety and Applied Nutrition
CNS	Central Nervous System
CR	Caloric Restriction
CRADA	Cooperative Research and Development Agreement
CVM	Center for Veterinary Medicine
CYP	Cytochrome P450
DBRA	Division of Biometry and Risk Assessment
DBT	Division of Biochemical Toxicology
ddC	2',3'-Dideoxycytidine
DEHP	Di(2-ethylhexyl)phthalate
DGRT	Division of Genetic and Reproductive Toxicology
DHHS	Department of Health and Human Services
DHP	6, 7-Dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine
DNA	Deoxyribonucleic Acid
DNT	Division of Neurotoxicology
DM	Division of Microbiology
DPME	Division of Pharmacogenomics and Molecular Epidemiology
DST	Division of Systems Toxicology
DVS	Division of Veterinary Services
ENU	N-Ethyl-N-nitrosourea
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FAO/WHO	Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO)

GD	Gestational Day
GI	Gastrointestinal
HCys	Homocysteine
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HPLC-ES/MS	HPLC combined with Electrospray Tandem Mass Spectrometry
IACUC	Institutional Animal Care and Use Committee
IAG	Interagency Agreement
JECFA	Joint Expert Committee on Food Additives
LCM	Laser Capture Microdissection
MAB	Metastable Atom Bombardment
MALDI	Matrix-assisted Laser Desorption/ionization
MAQC	MicroArray Quality Control Project
MDR	Multidrug Resistant
MLST	Multilocus Sequence Typing
MPP ⁺	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	Messenger RNA
MS	Mass Spectrometry
NAT	N-Acetyl-transferase
NCTR	National Center for Toxicological Research
NIEHS	National Institute of Environmental Health Sciences,
NIH	National Institutes of Health
NMDA	N-Methyl-D-aspartate
NMR	Nuclear Magnetic Resonance
NO _x	Nitrogen oxides
NTP	National Toxicology Program
OMICs	Genomics, transcriptomic, proteomic, and metabolomic data
ORA	Office of Regulatory Affairs
OTB	Operant Test Battery
PAHs	Polycyclic Aromatic Hydrocarbons
PBPK	Physiologically Based Pharmacokinetic (PBPK)
PCR	Polymerase Chain Reaction
PD	Pharmacodynamic
PFGE	Pulsed-field Gel Electrophoresis
PhIST	Phosphoprotein Isotope-coded Solid-phase Tag
NMR	Nuclear Magnetic Resonance (NMR)
NP	<i>p</i> -Nonylphenol
QA/QC	Quality Assurance/Quality Control
QRT-PCR	Quantitative Real-time Polymerase Chain Reaction
QSAR	Quantitative Structure Activity Relationship
RFLP	Restriction Fragment Length Polymorphism

RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAB	Science Advisory Board
SAH	S-Adenosylhomocysteine
SAM	S-Adenosylmethionine
SAR	Structure Activity Relationship
SDS	Scientific Discovery Solutions
SELDI	Surface-Enhanced Laser Desorption/Ionization
SSL	Simulated Solar Light
SNPs	Single Nucleotide Polymorphisms
TOF	Time-of-flight
UVA/UVB	Ultraviolet-A or Ultraviolet-B
VRE	Vancomycin-Resistant Enterococci

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