National Center for Toxicological Research

Annual Report

FY 2010 – FY 2011

3900 NCTR Road, Jefferson, AR 72079
(870) 543-7000
http://www.fda.gov/AboutFDA/CentersOffices/NCTR/ResearchAccomplishmentsPlans/default.htm

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 FDA's and the Department of Health and Human Services' (DHHS) mission to promote and protect public health. NCTR is an FDA research center that provides global leadership and innovative scientific solutions in support of FDA's mission to improve public health. NCTR—in partnership with researchers from government, academia, and industry—develops, refines, and applies current and emerging technologies to improve safety evaluations of FDA-regulated products. NCTR fosters national and international collaborations to improve and protect public health and enhance the quality of life for the American people. Through the training of scientists from around the world, as well as FDA staff, NCTR researchers propagate the principles of regulatory science globally. The Center—located in Jefferson, Arkansas, approximately 30 miles south of Little Rock—is co-located with the Office of Regulatory Affairs (ORA) Arkansas Regional Laboratory (ARL).

NCTR conducts FDA mission-critical, peer-reviewed, critical path (translational) research targeted to developing a scientifically sound basis for regulatory decisions and reducing 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. NCTR's research efforts are primarily directed at supporting FDA's Strategic Goal framework by implementing the objectives of FDA's Strategic Goal 2.1 (Cross-Cutting Research to Advance Regulatory Science and Innovation), FDA's Strategic Goal 3.1 (Advance Food Safety and Nutrition), and FDA's Strategic Goal 3.2 (Promote Public Health by Advancing the Safety and Effectiveness of Medical Products).

Customized bioassessments of chemicals of vital interest to FDA involves the coordination of expertise in the areas of biochemical and molecular markers of safety and toxicity, neurotoxicology, microbiology, chemistry, genetic or molecular toxicology, and systems-biology assessments for characterizing biomarkers for an individual's susceptibility to toxicants, disease risk, and health status. Using its strengths in methods development, statistics, analytical chemistry, and spectroscopy, NCTR has developed and is standardizing technologies, such as genomics, proteomics, metabolomics, and nanotechnology, to identify and characterize early biomarkers of toxicity using quantitative risk-assessment methods. The NCTR/ORA Nanotechnology Core Facility was established to address the current needs of NCTR/FDA to characterize nanoscale materials used in toxicology studies.

NCTR is using toxicoinformatics (data collection, interpretation, and storage of information about gene, protein, and metabolite 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 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 including a focus on women's health issues. The training of scientists within and outside FDA concerning these principles of regulatory science including cutting-edge concepts, approaches, and techniques is a major objective of NCTR.

A significant contribution to our research accomplishments is the benefit gained by sharing knowledge through collaborations with scientific staff in all disciplines from 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 NCTR to store, analyze, and interpret DNA microarray data. This tool is being used by several FDA regulatory Centers in assessing pharmacogenomic and other omics data voluntarily submitted by the regulated industry. In addition, the recent publication of a dozen papers in high caliber, peer-reviewed journals has improved the basis for standards and approaches for the routine use of genomic data in the safety assessment paradigm. These collaborations document FDA as a catalyst in the development of new standards that will facilitate product development for the promotion and protection of public health and provide a pathway to Personalized Nutrition and Medicine. To facilitate the accomplishment of these goals, a new NCTR/FDA Bio-Imaging Center has been developed to provide noninvasive, translatable biomarkers for safety assessment. In addition to methods and standards development, NCTR conducts safety assessment of compounds nominated by FDA reviewers to provide integrated, multidisciplinary scientific solutions.

/s/

William Slikker, Jr., Ph.D.

Director, NCT

NCTR Overview

Vision

The National Center for Toxicological Research (NCTR) is an FDA research center that provides global leadership and innovative scientific solutions in support of FDA's mission to improve public health.

Mission

NCTR conducts peer-reviewed research and develops new scientific tools for FDA to improve public health. This research produces innovative tools to solve complex health issues and anticipated toxicological problems, thus enhancing the science of regulatory decision making. NCTR provides multidisciplinary training and fosters national and international collaborations with scientists from government, academia, and industry.

NCTR is dedicated to supporting FDA's mission to protect and promote public health by:

- Providing innovative and interdisciplinary research that promotes personal and public health.
- Developing novel translational research approaches to provide FDA/DHHS with sound scientific infrastructure and multidisciplinary scientific expertise targeted towards addressing critical Agency, Department, and public-health needs.
- Engaging with scientists across FDA and other government agencies, industry, and academia in cooperative learning to strengthen the scientific foundations vital to developing sound regulatory policy and leveraging resources in order to promote the international standardization and global harmonization of regulatory science.
- Participating in or leading national and international consortia for the development of harmonized standards for technologies and risk-assessment methods vital to FDA's regulatory and public health mission.

Strategic Plan

NCTR's Strategic Plan sets forth our long-term strategic goals and objectives. The plan also details specific actions we are committed to taking as we carry out our mission to provide global leadership and innovative scientific solutions in support of FDA's mission to improve public health. This Strategic Plan charts NCTR's course for the future, focusing on three strategic goals. The three strategic goals NCTR established to accomplish its mission include:

Goal 1: Advance scientific approaches and tools necessary to support public health.

Goal 2: Develop new and innovative ways to engage and inform NCTR's internal and external communications and outreach

Goal 3: Strengthen and modernize administrative management to support FDA/HHS science goals

The NCTR Strategic Plan can be found on the FDA website at: http://www.fda.gov/AboutFDA/CentersOffices/NCTR/NCTRStrategicPlan/default.htm.

Research Structure

Established by executive order in 1971, NCTR is internationally recognized for the conduct of scientific research that supports the FDA mission to bring safe and efficacious products to market and reduce the use of adverse-health effects.

The research staffs include interdisciplinary teams of scientific experts that conduct fundamental and innovative laboratory research that translates knowledge and technology into processes that improve the safety assessment of FDA-regulated products and reduces the risk of adverse effects from products on the market. NCTR science is structured into divisions having specific disciplines that work as cross-functional teams on projects in three research programs: 1) Personalized Nutrition and Medicine, 2) Strengthen Surveillance and Risk Analysis, and 3) Enhancing Medical Product Safety.

NCTR research staffs include:

- Biochemical Toxicology
- Genetic and Molecular Toxicology
- Microbiology
- Neurotoxicology
- Personalized Nutrition and Medicine
- Systems Biology
- Veterinary Services

Science Advisory Board

Function

The Science Advisory Board (SAB) advises the NCTR Director in establishing, implementing, and evaluating the scientific-research programs conducted at NCTR. NCTR conducts innovative scientific research that assists the FDA Commissioner in fulfilling the FDA's regulatory responsibilities. Through site-visit reviews and annual meetings, NCTR's SAB provides an extra-agency scientific program review of the research programs at NCTR. The recommendations of the SAB are critical to the scientific rigor of the studies conducted at NCTR. Members of the SAB and the SAB Chair are selected by the FDA Commissioner, or designee, from among leading authorities in fields related to toxicological research.

FY 2010 Accomplishments

The NCTR SAB held a subcommittee site visit in 2010 to perform an in-depth review of the research programs within the Division of Neurotoxicology . The NCTR Division of Neurotoxicology is responsible for characterizing neuropathic and neurobehavioral toxicity as functional outcomes and the mechanisms underlying these effects. The Division's work is essential to the mission of both NCTR and FDA since neurotoxicity is one of the possible side effects of several therapeutic agents, food contaminants, and cosmetics under the FDA's regulatory purview.

The Site-Visit Team responded positively to the programs presented by the Division of Neurotoxicity's leadership, noting that the Division has an impressive breadth in the areas of imaging, molecular, behavioral, and physiology-based assessments that are complementary in nature, and that this expertise is being applied in a strategic manner toward integrating and validating the detection and understanding of compound-induced neurotoxicity. The Subcommittee report was presented and accepted at the NCTR SAB meeting October 19 and 20, 2010.

The annual meeting of the NCTR SAB started on October 19, 2010 with the NCTR Center Director providing a center-wide update on scientific endeavors at NCTR. This overview included a discussion of the current research programs, the organizational changes designed to support the research programs, and the strategies NCTR uses to prioritize its research programs. The meeting attendees next heard updates from each of the NCTR divisions, their programs, their individual accomplishments, and future research plans based on the items identified in their last subcommittee review. The SAB meeting continued with a presentation by the Director of the Office of Science at FDA's Center for Tobacco Products outlining the new regulatory mandate, the research needs, and opportunities for collaboration.

Following the lunch break, the NCTR Center Director discussed NCTR's strategy on communication and training, which is designed to extend the reach and impact of the research conducted at the Center. The SAB attendees next heard presentations from the other government representatives at the meeting, including FDA colleagues. These presentations focused on the upcoming regulatory challenges faced by their respective organization and the potential role of research collaborations in meeting these challenges.

On October 20, 2010, the Director of the Division of Neurotoxicology presented an overview of the Division and was followed by a presentation of the Report of the Subcommittee Review of the Division of Neurotoxicology. Following a discussion on some of the technical elements of the report, it was adopted by the full Board. The open public meeting concluded with a discussion on the organization of the SAB site visits and suggestions on how to improve the process.

SAB Membership Roster

CHAIR:

Cynthia A. Afshari, Ph.D., DABT
Term: 08/01/08 – 06/30/12
Expertise: Molecular Toxicology & Molecular Carcinogenesis
Director, Investigative Toxicology Dept.
Amgen, Inc.
One Amgen Center Drive, MS 25-0-A
Thousand Oaks, CA 91320-1799

DESIGNATED FEDERAL OFFICER:

Margaret A. Miller, Ph.D.
Nat'l Center for Toxicological Research
10903 New Hampshire Avenue
Building 32 Room 2208
Silver Spring, MD 20993-0002
Tel: 301-796-8890
Fax: 301-847-8600
E-mail: margaret.miller@fda.hhs.gov

MEMBERS:

John D. Baker, Ph.D.
Term: 07/19/10 – 06/30/14
Expertise: Clinical Research/ Molecular Medicine
Pfizer, Inc.
MS 9126-120
Eastern Point Road
Groton, CT 06340

Scott W. Burchiel, Ph.D.
Term: 10/11/10 - 06/30/14
Expertise: Immunology/Nanotoxicology
Professor & DeSantis Chair, Pharmacogenomics
Department of Pharmaceutical Sciences
Associate Dean for Research
Director, NM Center for Isotopes in Medicine
UNM HSC College of Pharmacy
Albuquerque, NM 87131-0001

Diana Dow-Edwards, Ph.D.
Term: 10/18/10 - 06/30/13
Expertise: Neurochemistry/Developmental Neurotoxicology
Professor of Physiology/Pharmacology
SUNY Downstate Medical Center
450 Clarkson Ave
Brooklyn, NY 11203

Jose M. Ordovas, Ph.D.
Term: 08/01/08 – 06/30/12
Expertise: Nutrition Director, Nutrition & Genomics
Professor Nutrition & Genetics
JM-USDA-HNRCA at Tufts University
711 Washington St
Boston, MA 02111

Janice W. Yager, Ph.D., MPH
Term: 11/03/09 – 11/03/13
Expertise: Genetic Toxicology, Epidemiology
University of New Mexico Health Sciences Center
Department of Internal Medicine,
Epidemiology MSC 105550
University of New Mexico
Albuquerque, NM 87131-0001

Ronald Hines, Ph.D.
Term: 11/03/09 – 11/03/13
Expertise: Pediatric Clinical Pharmacology
Medical College of Wisconsin
Department of Pediatrics
Clinical Pharmacology
TBRC/CRI Building
8701 Watertown Building
Milwaukee, WI 53226

Paul B. Watkins, MD.
Term: 07/19/10 – 06/30/14
Expertise: Liver Toxicity/Drug Interactions
The Hamner–UNC Institute for Drug Safety Sciences
Six Davis Drive
PO Box 12137
Research Triangle Park, NC 27709

Consumer Representative—Vacant

NCTR Advances Research Through Outreach and Collaboration

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

An Interagency Agreements (IAG) is a formal financial partnership with another government agency. NCTR has been fortunate in establishing IAGs with other government agencies to conduct research on problems of common interest to FDA and the collaborating agency. The most significant, in terms of size, is the IAG between NCTR/FDA and the National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program (NTP) .

National Institute of Environmental Heath Sciences/National Toxicology Program (NIEHS/NTP)

Paul C. Howard, Ph.D., Associate Director of Scientific Coordination
FDA Liaison to National Toxicology Program
Phone: 870-543-7672
E-mail: Paul.Howard@fda.hhs.gov

In 1992, FDA entered into an IAG with NIEHS. The NIEHS/NTP conducts toxicology studies at the request of federal agencies, including FDA. The IAG is an instrument that allows chemicals nominated to the NTP to be studied for toxicity using the unique resources and facilities at NCTR. The research, conducted under the IAG, provides FDA the ability to better assess study design and initial data on the safety of FDA-regulated products.

The 1992 agreement provided support for five FDA priority chemical/agent NTP nominations. The agreement has expanded to allow continued collaborative toxicity testing on compounds of interest to FDA and NTP. The IAG has led to the investigation of the mechanism-of-action and toxicity assessment of many classes of chemicals including cosmetics, endocrine-disruptor compounds, food contaminants, food cooking by-products, dietary supplements, drugs, and anesthetics. In response to experimental design needs for compounds studied under the IAG, the IAG supported the development of the Phototoxicity Research and Testing Laboratory (NTP Center for Phototoxicology) and NCTR/ORA Nanotechnology Core Facility.

All toxicology studies conducted under the IAG are designed with input from FDA regulatory scientists, NCTR and NIEHS scientists, scientists from other agencies, and invited subject-matter experts. The IAG utilizes resources from public funds and exceptional scientific expertise to provide the best-possible assessment of product safety through toxicological studies.

Toxicological studies on numerous compounds have been supported since 1992. Many of the compounds are listed below with the nominating Center in parenthesis.

- α and β hydroxy acids dermal (CFSAN)
- Acrylamide (CFSAN)
- AIDS therapeutics (Zidovudine, Nelfinavir, Nevirapine, Lamivudine)
- *Aloe vera* oral
- *Aloe vera* dermal
- Bisphenol A (CFSAN)
- Bitter orange, *Citrus aurantium* (CFSAN)
- Chloral hydrate (CFSAN)
- Di-(2-ethylhexyl)phthalate (CBER, CDRH)
- Ethinyl estradiol (CDER)
- Fumonisin B_1 (CFSAN)
- Furan (CFSAN)
- Genistein (CFSAN)
- Glucosamine/Chondroitin (CFSAN)
- Ketamine (CDER)
- Malachite green (CVM)
- Melamine + Cyanuric acid (CVM)
- Nanoscale silver (FDA)
- Nonylphenol (CDER)
- Permanent makeup pigments (CFSAN)
- Retinyl palmitate dermal (CFSAN)
- Riddelliine (CFSAN)
- Urethane/Ethanol (CFSAN)
- Usnic acid, *Usnea* lichen (CFSAN)

The NIEHS/NTP IAG currently supports the NCTR research projects listed below.

- **Acrylamide**—Genotoxicity and carcinogenicity of acrylamide and its metabolite, glycidamide, in rodents (range-finding, subchronic, two-year chronic carcinogenicity studies); developmental neurotoxicity in rats
- **AIDS therapeutics**—Perinatal carcinogenicity of drug combinations used to prevent mother-to-child transmission of HIV; Toxicity studies of combinations in p53 (+/-) haploinsufficient transgenic mice
- **Berberine**—pharmacokinetics and photoactivation
- **Bisphenol A**—Determination of the pharmacokinetics in rats and nonhuman primates, physiologically based pharmacokinetics (PBPK) modeling, and subchronic toxicity in rodents
- **Bitter Orange (*Citrus aurantium*)**—Developmental and physiological toxicity in rats
- **Cellular telephone radiation**—Histopathological studies on brains from rodents exposed *in vivo* and *in vitro* effects on rodent blood-brain barrier epithelial cells
- **Di(2-ethylhexyl)phthalate (DEHP)**—Toxicokinetics in neonatal male rhesus monkeys following intravenous and oral dosing
- **Furan**—Determination of carcinogenic mechanisms and low-dose carcinogenesis in rats
- **Glucosamine and chondroitin sulfate**—Subchronic toxicity in Fischer 344 rats and diabetic Zucker rats
- **Melamine and cyanuric acid**—Acute and subchronic toxicity studies and biomarker identification in rodents
- **Nanoscale oxides**—Skin penetration and phototoxicity of nanoscale oxides of titanium and zinc, and quantum dots
- **Nanoscale silver**—Pharmacokinetics, tissue distribution, and subchronic toxicity in rats
- **Permanent makeup inks**—Determination of the immunogenicity of inks and their components
- **Retinyl palmitate**—Effect of topically applied skin creams containing retinyl palmitate on the photocarcinogenicity of simulated-solar light (SSL) in SKH-1 mice
- **Triclosan**—toxicokinetics and dermal carcinogenicity studies
- **Usnic acid, *Usnea* lichen**—Toxicity studies in Fischer 344 rats and B6C3F$_1$ mice

Collaborative Research and Development Agreements

NCTR actively pursues and maintains partnerships with nongovernmental organizations, nonprofit organizations, and private companies through Collaborative Research and Development Agreements (CRADAs). The FY 2010 or FY 2011 CRADAs supporting NCTR research projects include those listed below.

BG Medicine, Inc.

Liver Toxicity Biomarkers Study: Phase 1, Entacapone and Tolcapone (E0726601)

Boehringer Ingelheim Pharmaceuticals, Inc.

Pramipexole: Use of a Nonhuman Primate Model for Studying the Consequences of Long-Term Dopaminergic Receptor Stimulation on Complex Brain Functions Using the NCTR Operant Test Battery (E0725201)

The Hamner Institutes for Health Sciences

Evaluating the Utility of ACB-PCR in Dose-Response Assessment and Mode-of-Action Evaluation (E0726901)

Pfizer, Inc.

Evaluation of the Mechanisms of Inactivation and Degradation of Third-Generation Cephalosporins by the Bovine Intestinal Microflora (E0721901)

University of Arkansas for Medical Sciences

Ketamine Pharmacokinetics in Children (E0726201)

University of Arkansas for Medical Sciences

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

University of Illinois

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

Office of Women's Health

FDA's Office of Women's Health (OWH) Science Program was started in 1994 to fund research projects that provide a foundation for the development of sound policies and regulations that enhance women's health. This program has provided support for approximately 35 women's health research studies at NCTR. These studies have investigated several important women's health issues including:

1) Importance of sex differences in drug metabolism and detoxification.

2) Comparative effectiveness of chemotherapeutic medicines.

3) Identification of biomarkers for diseases that disproportionately affect women, including lupus.

In 2008, NCTR formed a Women's Health Research Group and Seminar Series to promote and coordinate research in women's health within the Center. The Women's Health Research Group runs an active and innovative research program that focuses on understanding: 1) the molecular basis of drug efficacy and safety, and 2) how genetics, sex, diet, and other environmental factors influence drug efficacy and safety.

This group also coordinates women's health-research projects that are funded by NCTR, FDA's Office of Women's Health, FDA's Fellow program, and extramural grants and partnerships to ensure that the research conducted fills critical knowledge gaps in the safety and efficacy of FDA-regulated products as they relate to sex differences in improving women's health. However, research results are translated into improved health for both men and women. In September 2010, the NCTR Women's Health Research Group hosted a one-day workshop titled, "Sex Differences in FDA-regulated Products: Research for the Future." The workshop brought together clinical practitioners and basic researchers to identify research needs in four critical areas of women's health: autoimmune diseases, mental health, cardiovascular diseases/devices, and drug-drug interactions.

The outcome of the workshop discussions:

1) Provided NCTR scientists a greater understanding and awareness of critical issues in women's health as they relate to regulated products.

2) Served as a basis for the development of collaborative research projects between clinicians and NCTR.

3) Promoted the development of a woman's health research agenda at NCTR and FDA.

The NCTR projects listed below were supported by OWH in FY 2010 or have been approved for support in FY 2011.

Application of Co-Culture and Simulated-Vaginal Models to Elucidate the Inhibitory Properties of Naturally Occurring and Bioengineered Strains of *Lactobacillus* Toward Toxic-Shock Syndrome Toxin-1 Producing Strains of *Staphylococcus aureus* (E0728601)

Effects of Phytoestrogens on Gene-Expression Responses of Vaginal Epithelial Cells After Contact With *Candida albicans* (E0740601)

Genotyping of Transporter Genes Associated with Gender Differences and Promoter Methylation of UGT1A1 in Human Liver: A Means of Assessing Safety and Toxicity of Chemotherapeutic Drugs (E0729801)

Inactivation of UDP-Glucuronosyltransferases in Human-Breast Tissues: Assessing Cancer Risk, Tamoxifen Safety, and Toxicity (E0734001)

Neurotoxicity Assessment of Silver (Ag) Nanoparticles in PC-12 Cells and in Rats (E0728201)

Quantum Mechanical and NMR Spectral Approaches for the Rapid Prediction of Estrogen Activity of FDA-Regulated Chemicals (E0740701)

Office of Minority Health

FDA's Office of Minority Health (OMH) Program was started in 2010 as required by the Affordable Care Act. OMH will work to support FDA's mission to protect the public health by assuring the safety, efficacy, and security of human and veterinary drugs, the food supply, biological products, medical devices, cosmetics, radiation-emitting products, and by regulating tobacco. OMH will also work to support the HHS Office of Minority Health's efforts to eliminate racial and ethnic disparities, and to improve minority health and the quality of health care that minorities receive.

Moving forward, NCTR will promote and coordinate research studies within NCTR to improve minority health.

Division of Biochemical Toxicology Summary of Activities

Frederick A. Beland, Ph.D., Director
870-543-7205
Frederick.Beland@fda.hhs.gov

Introduction

The Division of Biochemical Toxicology conducts fundamental and applied research designed specifically to define the biological mechanisms-of-action underlying the toxicity of products regulated by, or of interest to, FDA. This research centers on quantifying the toxicities and carcinogenic risks associated with specific chemicals and introducing new risk assessment techniques. 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 risk-assessments. Research within the Division capitalizes on scientific knowledge in the areas of biochemistry, organic chemistry, analytical chemistry, cellular and molecular biology, nutritional biochemistry, toxicology, phototoxicology, and pharmacology.

FY 2010 Accomplishments

A major emphasis within the Division continues to be conducting research on compounds nominated by FDA for evaluation by the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP). This focus reflects NCTR's superb animal facilities supported by a multidisciplinary staff of scientists with strong mechanistic-research experience, which allows subchronic and chronic toxicological assessments to be conducted in a rigorous manner. These studies currently serve as the benchmark by which toxicological assessments are made by FDA and other federal agencies. 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 2010, a draft final report was completed for rodent chronic bioassays on acrylamide, a carcinogen found in many baked and fried foods, which was nominated to NTP by the Center for Food Safety and Applied Nutrition (CFSAN). These data were used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to establish the risk of dietary exposures to acrylamide. The risk estimate included consideration of data from two-year chronic rodent bioassays of glycidamide, a genotoxic metabolite of acrylamide.

In further investigations of foodborne carcinogens, experiments continued to characterize the risks associated with dietary exposure to furan. These experiments include a large-scale bioassay encompassing a very wide range of doses and ancillary mechanistic studies. The mechanistic studies emphasize dose-response relationships for the formation of furan-DNA adducts in the liver, the target organ for furan's

carcinogenicity. These data will be combined with other biomarker data to develop PBPK models for furan.

During FY 2010, a final NCTR report was completed for rodent bioassays on *Aloe vera,* a widely used dietary supplement. The bioassay results indicate that whole-leaf preparations of *Aloe vera* induce a high-incidence of colonic tumors in rats.

A major focus within the Division is elucidating potential toxicities associated with endocrine-disrupting chemicals. During FY 2010, much emphasis was placed on bisphenol A (BPA), a suspected endocrine disruptor to which there is ubiquitous environmental exposure. This research effort is in response to an increasing concern over BPA due to a large and growing body of research reporting effects of BPA at doses approaching human-exposure levels. The Division's research program included a subchronic study in rats that is evaluating a broad range of BPA doses administered orally during pregnancy and to the pups after birth. Endpoints related to reproductive development, control of energy utilization, and general toxicity are being evaluated. In addition to conducting subchronic studies, manuscripts were published describing the pharmacokinetics of BPA administered orally to rodents and nonhuman primates.

During FY 2010, Division investigators conducted studies with di(2-ethylhexyl)phthalate (DEHP), a suspected endrocrine disruptor and plasticizer present in a variety of polyvinyl chloride medical devices. These experiments are intended to model the exposure of male infants in neonatal intensive care units, the human population identified as being at the highest risk of DEHP-induced reproductive toxicity. The results indicate that, in neonatal rats, intravenous exposure to DEHP is more toxic to the lung than an equivalent oral dose, and that the lung may be more sensitive than the testes to the toxicities of intravenous DEHP.

An area of concern to FDA, in particular CFSAN, is the potential toxicity of cosmetic ingredients due to their interaction with light. During FY 2010, a draft NTP report on photocarcinogenesis studies of retinyl palmitate was completed. In addition, studies were initiated to investigate the toxicities of topically applied triclosan, a broad-spectrum antimicrobial agent present in a wide variety of antibacterial soaps, deodorants, toothpastes, cosmetics, fabrics, plastics, and other products. Initial experiments have included pharmacokinetic studies to measure the dermal absorption of triclosan and to characterize the metabolites formed.

Antiretroviral drugs are being used to prevent the mother-to-child transmission of human immunodeficiency virus type-1, the virus responsible for AIDS. While effective in preventing viral transmission, the long-term consequences of perinatal exposure to these drugs are presently unknown. During FY 2010, a draft NTP report was completed on rodent bioassays in which antiretroviral drugs were administered transplacentally to mice. In addition, manuscripts were published characterizing the amino acid and protein adducts of the antiretroviral drug, nevirapine.

The intentional adulteration of pet food with melamine and derivatives, including cyanuric acid, has been implicated in the kidney failure and death of a large number of

cats and dogs in the U.S. While individually these compounds present low-toxicity, co-exposure can lead to the formation of melamine-cyanurate crystals in the nephrons of the kidney and eventual kidney failure. During FY 2010, investigators in the Division, in collaboration with colleagues at FDA's Center for Food Safety and Nutrition (CFSAN) and Center for Veterinary Medicine (CVM) , conducted experiments in rats to determine the dose-response for nephrotoxicity upon co-administration of melamine and cyanuric acid. The results from the study suggest that the Tolerable Daily Intake values considered in current risk assessments may need to be lowered to provide an adequate safety margin from dietary exposure to melamine and its analogues.

Infants and very young children are considered to represent a more vulnerable target population for foodborne bioterrorism agents for a number of reasons, including the fact that they are prone to consume a single type of food as a complete meal (e.g., baby foods or infant formulas). Investigators in the Division compared the thermal stability of the toxin ricin in milk, milk-based infant formula, soy-based infant formula, four types of fruit juices, a yogurt-fruit blend marketed for toddlers, and plain yogurt. The observed stability of ricin was similar in most of the dairy or fruit products tested, but blended yogurt-fruit drink enhanced the thermal stability of ricin, an effect that was partially duplicated by plain yogurt. In further experiments, investigators in the Division addressed a strategic goal of FDA by developing an improved method for detecting the biochemical activity of ricin, abrin, and the shiga-like toxins in dairy products.

Silver nanoparticles are highly effective antibacterial agents, and this property of silver nanoparticles is being exploited in an expanding number of commercial and consumer products. Human exposure to silver nanoparticles continues to increase with every new application, which has led to public concerns regarding their safety. During FY 2010, Division investigators examined the effect of the size of silver nanoparticles on the bioavailability, tissue distribution, metabolism, and clearance in rats. The results indicated limited absorption after oral administration, with the extent of absorption increasing as the size of the particle decreased.

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 2009, Division investigators expanded their investigations to study the underlying molecular mechanisms involved in breast carcinogenesis in the ACI female-rat model. The results demonstrate that early stages of estrogen-induced breast carcinogenesis are characterized by epigenetic alterations, including altered global DNA- methylation, aberrant expression of proteins responsible for the proper maintenance of DNA methylation patterns, and epigenetic silencing of the critical *Rassf1a* tumor-suppressor gene.

FY 2011 Plans

Division of Biochemical Toxicology investigators will continue research in FY 2011 to:

- Evaluate various measures of cytotoxicity and other assay parameters for the *in*

vitro micronucleus.

- Complete NTP reports on the transplacental carcinogenicity of antiretroviral drugs.
- Complete research on chronic two-year rodent bioassays of acrylamide.
- Conduct chronic and mechanistic studies on the food contaminant, furan.
- Determine the toxicities associated with exposure to silver nanoparticles. These investigations, which have initially focused on pharmacokinetic measurements, will be expanded to include subchronic studies.
- Investigate the toxicities of topically applied triclosan. These experiments will include toxicokinetic measurements and also characterize the products formed during the photodecomposition of triclosan.
- Characterize the toxicities of BPA in rodent models, especially with regard to developmental exposures.
- Characterize the toxicities associated with intravenous administration of DEHP in neonatal rats.
- Evaluate the toxicities of melamine in combination with cyanuric acid, with an emphasis on increasing the length of exposure. In addition, experiments will be expanded to include newborn rats, in order to address concerns raised by the adulteration of baby formula with melamine and its derivatives.
- Measure thermodynamic constants for the thermal inactivation of bioterrorism agents, such as ricin and abrin, under conditions found in foods and to compare the potencies of detergents and chemical-sanitizing agents to inactivate or eliminate these bioterrorism agents that contaminate food-contact surfaces.

Contribution to FDA's Strategic Priorities/Goals

The research conducted by the Division of Biochemical Toxicology contributes to NCTR Strategic Goal 1 (Advance scientific approaches and tools necessary to support public health) and to FDA Strategic Goals:

2.1: Cross-Cutting Research to Advance Regulatory Science and Innovation

- **3.1: Advance Food Safety and Nutrition**
- **3.2: Promote Public Health by Advancing the Safety and Effectiveness of Medical Products**

Goal 2.1 (Cross-Cutting Research to Advance Regulatory Science and Innovation)

The Division provides expert advice and innovative research to the other FDA Centers—thus contributing to FDA's mission of advancing public health. Research projects involve

new and innovative technologies and approaches that support FDA's regulatory Centers.

Goal 3.1 (Advance Food Safety and Nutrition)

A major emphasis of the Division's research is to ensure the safety of food products, for example, ongoing assessments of acrylamide, a known rodent carcinogen and neurotoxicant that was recently identified in baked and fried starchy foods—notably french fries, potato chips, bread, coffee, and many other consumer food products. A similar research strategy is being applied to furan, another contaminant in food. Evaluations are also being conducted on BPA, a chemical to which there is ubiquitous oral-environmental exposure, and on *Aloe vera*, a natural product incorporated into dietary supplements. As part of the Division's efforts to ensure the safety of foods, assays are being developed and applied to detect the biological activities of potential bioterrorism agents, for example ricin and abrin, in various food products. Division investigators are conducting studies to assess the toxicities associated with exposure to melamine and cyanuric acid, contaminants that have been found in certain food products.

Goal 3.2 (Promote Public Health by Advancing the Safety and Effectiveness of Medical Products)

Division investigators are evaluating the toxicities associated with intravenous exposure to DEHP, a scenario designed to mimic infant exposures in neonatal intensive-care units. Other experiments are focused on investigating the toxicities of perinatal exposure to antiretroviral drugs, studies that are designed to model treatments to prevent mother-to-child transmission of human immunodeficiency virus type-1.

Division of Genetic & Molecular Toxicology Summary of Activities

Martha M. Moore, Ph.D., Director
870-543-7050
Martha.Moore@fda.hhs.gov

Introduction

The Division of Genetic and Molecular Toxicology conducts basic and applied research to address specific high-priority issues related to the induction of genetic damage. Division research is directed toward developing and validating new methods or improving existing methods for the identification of potentially hazardous food additives, human and animal drugs, biological therapies, and medical devices. In collaboration with other FDA scientists, the Division utilizes the methodologies it develops to understand the potential toxicity of specific high-priority drugs, dietary supplements, and other agents.

As experts in the field of genetic toxicology, scientists in the Division 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. Division scientists frequently provide expert advice to FDA Centers, other government agencies, academia, and industry. They also are active participants in the FDA Genetic Toxicology Network, the CDER Genetic Toxicology Network, and other interagency workgroups.

The Division's research is divided into four themes:

1) Research Involving Current Regulatory Genetic-Toxicology Assays

2) Chemical-Specific Research

3) Development of New Assays and Approaches

4) Research To Improve Risk Assessment

Division activities provide both direct support for, and the generation of, new approaches used by FDA Centers and, in particular, provide research and expertise directly related to the FDA Critical Path Initiative.

FY 2010 Accomplishments

In FY 2010, Division of Genetic and Molecular Toxicology scientists actively participated in providing genetic-toxicology advice to FDA Centers. These consultations included general advice concerning the conduct and interpretation of data from specific assays, as well as evaluation of data from FDA submissions. Division scientists participated in various Genetic Toxicology Working Groups of several organizations, including the International Workshop for Genotoxicity Testing (IWGT) and International Life Sciences Institute/ Health and Environmental Sciences Institute (ILSI/HESI). Division scientists

were, and will continue to be, involved in discussions concerning the appropriate strategies for evaluating chemicals for product safety.

Specific 2010 research accomplishments involving the current regulatory assays include:

- In direct response to an FDA need, a project was continued to evaluate various measures of cytotoxicity and other assay parameters for the *in vitro* micronucleus assay. The project also compared two different cell lines and also gained experience with the flow-cytometric method to evaluate micronucleus formation.
- Continued a study to gain expertise in the comet assay and conduct research to evaluate the important parameter of this technique. The ultimate goal of this project is to provide information and expertise that can be used to assist with the development of guidance documents for the conduct of this assay.
- Expanded research to understand the mechanistic basis of the *in vitro* regulatory assay—the mouse lymphoma assay (MLA).
- Continued a study to evaluate whether the current genetic-toxicology assays are appropriate for evaluating the potential toxicity of nanomaterials.

The Division was actively involved in research addressing specific chemicals and generating data that can be used by the FDA Regulatory Centers.

- Completed a comprehensive study to assess methylphenidate-induced genetic damage. This study, funded by the National Institute for Child Health and Development (NICHD), aims to characterize both behavioral changes in methylphenidate-exposed nonhuman primates and the metabolism of the drug in young rodents. Methylphenidate is a drug often prescribed to children to control Attention Deficit Hyperactivity Disorder (ADHD).
- Completed a study in collaboration with Centers for Disease Control and Prevention (CDC) to evaluate the relative mutagenic potential for a series of cigarette condensates.
- In collaboration with the Division of Biochemical Toxicology, completed a study evaluating the potential for acrylamide to induce mutation through a mutagenic mode-of-action.
- Continued a study to evaluate the cancer mode-of-action for furan.
- At the request of a CDER reviewer, initiated research to investigate the potential for Tempo to induce mutations.
- At the request of a CFSAN reviewer, initiated research to investigate whether the samples of *Aloe vera* used as a part of the National Toxicology Program (NTP) can induce mutations.

Substantial progress was made in 2010 in the development of new methods and in bringing new methodologies to NCTR.

- Continued a project to evaluate whether microRNA expression analysis can be used to detect carcinogens from noncarcinogens.
- Continued a project to establish and evaluate the gpt delta transgenic mouse model for use at NCTR.
- Continued a project to establish chromosome-painting technology at NCTR.
- Continued the validation of a new approach for directly analyzing mutations. This assay uses fluorescent probes to detect mutation in the endogenous X-linked *PIG-A* gene. The 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-throughput analyses in humans and animal models. These properties make *PIG-A* an attractive reporter-gene for *in vivo* mutation studies.

The Division conducted research to improve risk assessment in 2010.

- Continued two studies evaluating the presence of *p53* mutations in colon cancer in both mice and humans.
- Initiated a project to use *in vivo* mutation analysis to inform cancer mode-of-action.
- Continued a project to investigate whether mutagens can have thresholds.
- Continued research using an allele-specific competitive blocker-polymerase chain reaction (ACB-PCR) technology and progress 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. This appears to be a promising biomarker that may provide a strategy that might ultimately lead to the replacement of the traditional two-year cancer bioassay and hasten the development, safety assessment, and approval of new drugs.
- Initiated research to investigate whether the ACB-PCR methodology can be applied to detecting subpopulations of specific mutations in tumors and thereby be a useful tool in distinguishing individuals who will benefit from particular cancer therapies from those that will not. This project has the potential to advance personalized medicine.

FY 2011 Plans

The Division will continue research in all four theme areas. Specific plans include:

- Project to evaluate various measures of cytotoxicity and other assay parameters for the *in vitro* micronucleus assay.
- Project to gain expertise in the comet assay and conduct research to evaluate the important parameter of this technique. The ultimate goal of this project is to provide information and expertise that can be used to assist with the development of guidance documents for the conduct of this assay.

- Research to understand the mechanistic basis of the *in vitro* regulatory assay—the mouse lymphoma assay (MLA).
- Research to evaluate whether the current genetic-toxicology assays are appropriate for evaluating the potential toxicity of nanomaterials.
- Initiate a new study in collaboration with CDC and the newly formed FDA Center for Tobacco Products to evaluate the relative mutagenic potential for tobacco products.
- Evaluate the cancer mode-of-action for furan.
- Validate a new approach for directly analyzing mutations. This assay uses fluorescent probes to detect mutation in the endogenous X-linked *PIG-A* gene.
- Evaluate whether microRNA expression analysis can be used to detect carcinogens from noncarcinogens.
- Establish and evaluate the gpt delta transgenic-mouse model for use at NCTR.
- Establish chromosome painting technology at NCTR.
- Research using an ACB-PCR (allele-specific competitive blocker-polymerase chain reaction)-technology. Progress 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.
- Direct a new research effort toward understanding the background frequency of these cancer mutations in "normal" individuals. This will include the potential impact of rodent strain and age of the rodents. In addition, efforts will be made to make the technology more rapid and easy to conduct.
- Initiate a project to use *in vivo* mutation analysis to inform cancer mode-of-action. This will be done under a newly approved CRADA with Toxicology for Excellence in Risk Assessment (TERA) and utilize the model chemical ethylene oxide.
- Investigate whether mutagens can have thresholds.

Contribution to FDA's Strategic Priorities/Goals

The research conducted by the Division of Genetic and Molecular Toxicology contributes to NCTR Strategic Goal 1 (Advance scientific approaches and tools necessary to support public health) and to FDA Strategic Goals:

- **2.1: Cross-Cutting Research to Advance Regulatory Science and Innovation**
- **3.1: Advance Food Safety and Nutrition**
- **3.2: Promote Public Health by Advancing the Safety and Effectiveness of Medical Products**

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

Genetic toxicology is concerned with the ability of chemicals to alter genetic material. 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 focuses 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 that have an increased ability to detect genetic damage, will provide 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.

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 toxicology generally evaluate single endpoints, the new genomic technologies are providing the opportunity to detect alterations in a number of endpoints. In the future, these new approaches to evaluate 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.

Division of Microbiology Summary of Activities

Carl E. Cerniglia, Ph.D., Director
870-543-7341
Carl.Cerniglia@fda.hhs.gov

Introduction

The Division of Microbiology goals are to perform fundamental and applied research to address critical issues in support of the FDA mission, as well as provide technical-service support in responding to microbial surveillance and diagnostic needs for research using experimental animals at NCTR. The Division of Microbiology research projects are based on Division staff expertise and consultation with scientists from academia, industry, and the FDA Regulatory Centers. The Microbiology research program is divided into four focus areas:

1. Food Safety, Food Biosecurity, and Methods Development
2. Antimicrobial Resistance
3. Microbes and Host Interactions
4. Environmental Biotechnology

During FY 2010, the Division of Microbiology scientists engaged in research addressing a variety of FDA issues with special emphasis on:

1. Developing rapid technologies to detect, identify, and characterize foodborne pathogens.
2. Determining antimicrobial resistance and virulence mechanisms of microbial pathogens that may enter the food supply.
3. Using current molecular biological approaches to monitor interactions between human-intestinal microbiota, antimicrobial agents, food contaminants, food additives, and probiotics.
4. Conducting studies impacting women's health.
5. Improving environmental risk assessments of priority pollutants, including polycyclic aromatic hydrocarbons and drugs, by integrating systems biology approaches.

Selected accomplishments are listed below.

FY 2010 Accomplishments

Food Safety, Food Biosecurity, and Methods Development

- Research under an Interagency Agreement (IAG) with the U.S. Department of Agriculture (USDA) and the Department of Homeland Security on the survival of *Bacillus anthracis* in processed liquid-egg *anthracis* media was completed. At 20°C, viability was unchanged in all liquid-egg media, whereas from 5°C to 15°C, it declined by 60%. At moderate temperatures, robust growth of *B. anthracis* was observed in whole egg and egg yolk, but the lysozyme in egg white inactivated it. These data can be used for risk assessment for food biosecurity.
- In collaboration with NCTR's Division of Systems Biology and Division of Personalized Nutrition and Medicine, scientists in the Division of Microbiology populated ArrayTrack™ with bioinformatics data from public domains to facilitate foodborne-pathogen detection. This new ArrayTrack™ microbial analysis tool will support the FDA's Food Protection Plan by aiding rapid identification of bacteria in outbreaks.
- Division investigators are examining RNA and infectivity of coronaviruses in unprocessed vegetables. Viral RNA levels were stable up to 30 days on romaine lettuce and infectious virus was recovered up to day 14. Because coronaviruses are stable during the shelf-life of lettuce, they may be transmitted to humans.
- A rapid multiplex real-time PCR method was developed for *Salmonella* detection in foods. Different serovars were screened for antimicrobial-resistance genes; some were resistant to several antibiotics. The method is being validated in collaboration with the Office of Regulatory Affairs (ORA) Pacific Regional Laboratory-SW and other ORA laboratories.

Antimicrobial Resistance

- Division staff characterized plasmid-associated antimicrobial resistance in *Salmonella enterica* from poultry and human infections. Typhimurium, Enteritidis and Heidelberg are the first, second, and fourth most common serovars in human disease. Resistance to multiple antimicrobial agents was demonstrated in some *Salmonella* isolates, which were selected for plasmid sequencing to evaluate the impact of antimicrobials on transfer of drug-resistance plasmids.
- Use of antibiotics in foreign aquaculture may select for resistant bacteria, so imported aquaculture products may contain antibiotic residues and drug-resistant bacteria. Division scientists isolated fluoroquinolone-resistant *Aeromonas* mutants from imported shrimp and screened them by PCR. Most of the strains contained aerolysin genes and some contained enterotoxin genes. Imported shrimp may be a reservoir of virulent drug-resistant aeromonads.
- *Salmonella* Javiana infections comprise the fifth most common serovar in the CDC's PulseNet database. Clinical *S*. Javiana isolates were analyzed for drug susceptibility, plasmid profiles, and virulence genes. Several resistant strains with multiple plasmids were analyzed with microarrays. Multiple drug resistance, plasmids, replicons, and genes for transferring resistance were found; both

resistant and susceptible isolates harbored multiple virulence genes.

- *Clostridium perfringens* is a potential foodborne pathogen. Microarrays were used to detect changes in gene expression after exposure to fluoroquinolones, which results in development of resistant mutants. Other alterations, shown by microarray analysis, were in gene expression and enzyme production for pathogenicity. Some strains produced substances to inhibit other bacteria.
- Division scientists, in collaboration with USDA and West Virginia University, used microarrays to detect genes for drug resistance in *Salmonella*. The biochip detected resistance genes for β-lactams, aminoglycosides, fluoroquinolones, phenicols, tetracyclines, and sulfa drugs. *Salmonella* isolates from poultry raised without antibiotics harbored genes for resistance to several antimicrobials used in poultry and humans. The ability of microarrays to detect thousands of genes can help FDA during outbreaks.

Microbes and Host Interactions

- A mouse model was used to study microbial interaction with the mucosal immune system. Probiotic *Lactobacilli* and bifidobacteria modified the immune response to *Salmonella enterica*, enhancing the proliferative response and reducing caspase activity in lymphocytes. They suppressed pro-inflammatory genes and induced immune-cell differentiation and apoptosis-repression genes in lymphoid tissues of mice challenged orally with *S. enterica*. Probiotic *Lactobacilli*, associated with increased T cells in the ileum, restored immunity.
- Division scientists completed research under a CRADA with Pfizer to study degradation of the veterinary antimicrobial ceftiofur by bovine-intestinal microflora. Bacteria from the bovine intestinal tract that inactivated or degraded ceftiofur included *Bacillus* and *Bacteroides* strains producing β-lactamases.
- Dietary supplements made from soybeans may contain daidzein, a weak estrogen. Ciprofloxacin was investigated for its effect on the metabolism of daidzein by colonic microfloras of nonhuman primates. Low concentrations of ciprofloxacin enhanced daidzein metabolism by eliminating ciprofloxacin-sensitive bacteria and allowing more growth of daidzein-metabolizing strains. High concentrations of ciprofloxacin, however, also eliminated the daidzein-metabolizing bacteria.
- A new study was initiated on the seroepidemiology of coronaviruses; real-time Polymerase Chain Reaction (PCR) showed 10% incidence of coronaviruses in domestic animals and wildlife. Partial sequencing of positive bovine samples revealed isolates belonging to coronavirus group 1a, whereas most bovine coronaviruses belong to group 2a. A group 1a coronavirus found in a dog may represent a feline coronavirus.
- The use of antimicrobial agents in food-producing animals may select for resistant bacteria, which could endanger human health. A culture of intestinal

bacteria was used to mimic the gastrointestinal tract and changes in bacterial populations in response to enrofloxacin were monitored. PCR, denaturing gradient-gel electrophoresis, terminal-restriction fragment length polymorphism, and pyrosequencing detected community changes in the intestinal microbiota.

- Division staff participated in the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medical Products (VICH) Expert Working Group organized by FDA's Center for Veterinary Medicine (CVM) to evaluate the VICH GL36 guideline on the human safety of veterinary antimicrobial drugs, including assessments of the impact of drug residues in meat on human intestinal microbiota.
- Scientists in the Division demonstrated that some Sudan dyes are metabolized by intestinal microbiota; azoreductases to genotoxic compounds. Biochemical and molecular characterization and crystal structure studies of azoreductases were completed. Site-directed mutagenesis of the enzyme-active site revealed the amino-acid residues involved in cofactor binding and activity, improving understanding of the risks of food contamination with Sudan dyes.

Women's Health

- Some strains of *Lactobacillus* inhibit toxic-shock syndrome toxin-1 (TSST-1) producing strains of *Staphylococcus aureus.* Strains producing TSST-1 and/or enterotoxins B or C are responsible for most toxic-shock syndrome cases. *Lactobacillus* species in the healthy vaginal tract produce lactic acid. However, during menstruation the vaginal pH increases to neutral, and if *S. aureus* is present, it proliferates. Proteins expressed by *L. plantarum* in a modified genital tract secretion medium at neutral pH includes lysostaphin, which kills *S. aureus*. Lysostaphin-producing *L. plantarum* could be administered as a probiotic to inhibit staphylococci during menstruation when the vaginal pH becomes neutral.
- The effects of phytoestrogens in soy products on vaginal epithelial-cell gene expression were studied. These cells respond to *Candida albicans* yeast infection by pro-inflammatory and pre-apoptotic changes in gene expression, which may be affected by phytoestrogens. In the absence of estrogens, probiotic *Lactobacilli* and epithelial cells produce hydrogen peroxide to inhibit *C. albicans*, but 17β-estradiol enhanced peroxide resistance in the yeast.
- Division microbiologists have demonstrated that human-skin bacteria convert azo dyes in cosmetics to DNA-damaging metabolites that are readily absorbed through the skin. Several bacterial azoreductases metabolize azo dyes; research is continuing to characterize the range of dyes metabolized. The data should assist FDA regulation of the safety of cosmetics.

Environmental Biotechnology

- Fluoroquinolone residues in wastewater increase pressure for the development

of resistance genes by bacteria, so inactivation of fluoroquinolones may prevent resistance. Studies with mixed microbial cultures derived from wastewater showed that some bacteria inactivate fluoroquinolones; a *Microbacterium* isolate from wastewater metabolized norfloxacin to four less active metabolites.

- Division of Microbiology scientists have elucidated the genes and enzymes for microbial PAH degradation. *Mycobacterium vanbaalenii*, a metabolically versatile bacterium from oil-contaminated marine sediments, degrades high molecular-weight PAHs. Determination of the complete genome sequence has allowed through proteomics and functional genomics, insights into the molecular mechanisms. Division scientists have constructed a complete pathway for high molecular-weight PAH degradation for use in rational oil-spill bioremediation.

Surveillance/Diagnostic Program

- The primary mission of the Surveillance and Diagnostic program is to provide assurance that NCTR research data is not compromised by infected or unhealthy experimental animals. During FY 2010, program personnel worked to prevent the introduction of microbial pathogens into NCTR animal colonies. Routine monitoring of the animals, environment, food, and water from the breeder colonies was conducted. Quarantine and sentinel mice and rats were screened for potentially pathogenic microorganisms.

FY 2011 Plans

Food Safety, Food Biosecurity, and Methods Development

- Culture human epithelial cells (cutaneous, gastrointestinal, and pulmonary) to determine gene-expression profiles after infection by *Bacillus anthracis* and compare with gene expression in uninfected cells. The data will identify markers unique to the three forms of anthrax.
- Characterize toxin-producing enterohemorrhagic *Escherichia coli* O157:H7 strains and their antigenic variants. The genetic diversity/clonal spread, antimicrobial resistance, pathogenicity, and cytotoxicity will be assessed.
- Initiate quantitative proteomic, transcriptomic, and phenotypic microarray analyses of *Campylobacter jejuni* to identify colonization factors for poultry.

Antimicrobial Resistance

- Characterize plasmid-associated antimicrobial resistance in *Salmonella enterica* serovars associated with poultry and human infections, to assist CVM's Office of New Drug Evaluation in evaluation of antimicrobials for food animals.
- Characterize the genes likely involved in antimicrobial resistance and virulence among *Salmonella* isolates by in-depth plasmid sequencing.
- Quantify the impact of antimicrobial exposure on the efficiency of multidrug resistance and virulence-plasmid transfer among *Salmonella* isolates.

- Begin to evaluate the impact of plasmid-associated genes on pathogenicity of bacterial strains.
- Characterize fluoroquinolone-resistant *Vibrio* species from imported shrimp and determine the antimicrobial profiles of the isolates.
- Screen *Vibrio* isolates by PCR for fluoroquinolone-resistance and hemolysin-related virulence genes.
- Evaluate the association between fluoroquinolone-resistance selection and virulence of *Clostridium perfringens* strains to humans and other animals.
- Analyze gene-expression changes associated with fluoroquinolone resistance in *Clostridium perfringens*.
- Study resistance to fluoroquinolones and extended-spectrum β-lactamases in *E. coli* isolates from companion animals and identify mechanisms of resistance and pathogenicity.

Microbes and Host Interactions

- Use Liquid Chromatography with Nuclear Magnetic Resonance Spectroscopy (LC-NMR) analysis to characterize the metabolites of ceftiofur degradation by *Bacillus cereus* and related strains that contain metallo-β-lactamases.
- Critically examine the degradation of cephalosporins by the intestinal microbiota and analyze the distribution of resistance genes in untreated populations.
- Complete the determination of the multiple types of β-lactamases involved in ceftiofur degradation.
- Continue studies on the effect of cosmetics containing azo dyes on microbiota of the human skin.
- Determine the toxicogenomic response of *Staphylococcus aureus* to cosmetics containing azo dyes, using proteomic analyses.
- Probe the substrate-binding site of an FMN-dependent azoreductase from *Enterococcus faecalis.*
- Elucidate the dose-response relationships of azo dyes and their metabolites on intestinal bacterial-cell toxicity, using cell biology and proteomic approaches.
- Determine the effect of Sudan azo dyes in food on human intestinal-microbial ecology, using metagenomics.
- Survey the molecular seroepidemiology of coronaviruses and the diseases produced in adults, children, domestic animals, and wildlife.
- Continue the detection of coronaviruses in animals and begin detection in human nasal and fecal samples.

- Determine the seroepidemiology of coronaviruses in serum from local residents and characterize new strains.
- Assess methods for the premarket evaluation of diagnostic devices for seasonal and pandemic influenza, avian H5N1 influenza, and H1N1 influenza.
- Evaluate the bioavailability of antimicrobials and the impact of antimicrobial residues on the human-intestinal microbiota.
- Determine the inhibitory capabilities of *Lactobacillus* species, including an *L. plantarum* strain expressing lysostaphin, for staphylococci in a vaginal-tract model under various conditions.
- Identify factors produced by *Lactobacillus* species that inhibit expression of staphylococcal-extracellular proteins when grown with *S. aureus* in a buffered (neutral) medium.
- Challenge tissue cultures of phytoestrogen-treated vaginal epithelial cells with *Candida albicans* with qRT-PCR analysis of differential gene expression.
- Evaluate the effects of inhibitory peptides on the NF-κB signal transduction pathway to assess how the cellular response to estrogens affects cytokine expression by vaginal-epithelial cells.
- Evaluate the estrogen-receptor dependence of phytoestrogens by using the specific modulator ICI 182,780 to stop pro-inflammatory cytokine production.
- Determine the effect of nanomaterials on the permeability of intestinal epithelial cells and correlate the interaction of nanoparticles with epithelial cells.
- Initiate studies to detect *Mycobacterium paratuberculosis* in foods by real-time PCR and monitor the effect of electron-beam irradiation on survival.

Environmental Biotechnology

- Identify bacteria in wastewater that degrade fluoroquinolones to products without antimicrobial activity.
- Identify fluoroquinolone metabolites produced by bacteria with LC-MS/MS and NMR analysis.
- Conduct metabolic and proteomic analyses of *Mycobacterium vanbaalenii* mutants defective in dioxygenase genes to understand mechanisms of PAH degradation.
- Examine the impact of BP Deepwater Horizon crude oil on biodegradation pathways and microbial physiology, as well as the toxicity of crude oil and mixtures of PAHs to edible fish, oysters, and shrimp.

Microbiological Surveillance and Diagnostic Support of Research

- Continue working to ensure that the research-animal population remains healthy

and disease-free.

- Develop and use real-time PCR-based assays, conventional PCR assays, and additional new assays.
- Update and use the Microbial Identification system (MIDI) for the surveillance program.
- Train lab personnel for new technologies and methods in microbial diagnostics.
- Study the feasibility of participation in a national surveillance program's surge capacity.

Contribution to FDA's Strategic Priorities/ Goals

The research conducted by the Division of Microbiology contributes to NCTR Strategic Goal 1 (Advance scientific approaches and tools necessary to support public health) and to FDA Strategic Goals:

- **2.1: Cross-Cutting Research to Advance Regulatory Science and Innovation**
- **3.1: Advance Food Safety and Nutrition**
- **3.2: Promote Public Health by Advancing the Safety and Effectiveness of Medical Products**

Goal 2.1 (Cross-Cutting Research to Advance Regulatory Science and Innovation)

The Division provides expert advice and innovative research to the other FDA Centers—thus contributing to FDA's mission of advancing public health. Research projects involve new and innovative technologies and approaches that support FDA's regulatory Centers.

Goal 3.1 (Advance Food Safety and Nutrition)

- Epidemiological, drug-resistance, and pathogenicity data from food-safety studies should be useful in creating integrated baseline data to assess the threat of accidental or deliberate outbreaks by foodborne pathogens and help prevent the spread of resistance in the food chain.
- Factors contributing to antimicrobial resistance and virulence in foodborne bacteria help to develop intervention strategies to improve the safety of food.
- Metabolism of veterinary antimicrobials by the normal intestinal microbiota; may affect development of resistance. Since FDA sets drug-residue limits for animal products, this will assist the agency's strategies for antibiotic use.
- Drug-resistance data obtained using state-of-the-art technologies will provide FDA with knowledge to make regulatory decisions.
- Molecular characterization of multidrug-resistant *Salmonella* isolates from imported foods will allow better understanding of the transfer of virulence and drug-resistance plasmids. This will explain the genetic basis of resistance, which

can be used to assess risk of the use of antibiotics in food production.

- Studies on *Salmonella* Javiana and non-0157:H7 toxin-producing *E. coli* will provide data for developing an FDA risk-assessment model for the microbial genetics of foodborne pathogens.
- Responses of cutaneous and intestinal epithelial cells to *Bacillus anthracis* should identify markers to enhance product safety.
- Because several recent outbreaks of *Salmonella* have required quick response by FDA to source-track the organisms, research will help improve detection methods for rapid response to outbreaks.
- Rapid and sensitive molecular-biology tools are essential for tracking antibiotic-resistant pathogens in imported seafood and to aid FDA in regulating imports.

Goal 3.2 (Promote Public Health by Advancing the Safety and Effectiveness of Medical Products)

- Research on coronaviruses will increase FDA's preparedness for future outbreaks and for the review of vaccines and diagnostic tests for use in organ transplantation and blood transfusions.
- Research on influenza viruses will accelerate the availability of effective and rapid influenza diagnosis.
- FDA will gain a clearer understanding of how drug residues, probiotic products, dietary supplements, and xenobiotic substances affect the intestinal microflora and human health.
- The ArrayTrack™ bioinformatics database for enteric pathogens will link data analysis with biological relevance of pathogens for all FDA staff. It can serve as a valuable tool to share information about bacterial isolates and outbreak threats.
- Studies examining vaginal bacterial interactions with probiotic supplements could be extended to other commercial probiotics.
- Environmental biotechnology research at NCTR provides examples to drug manufacturers for documenting environmental fate and toxicity before FDA approval of human or veterinary medical products. According to FDA's Center for Drug Evaluation and Research (CDER) Guidance for Industry "Environmental Assessment of Human Drug and Biologics Applications" and CVM Guidance for Industry #89 (2001, Docket No. 99D-2975) and #166 (2006, Docket No. 2004D-0156), degradation is important in environmental impact assessments of drugs. It may also be useful for mitigating environmental impacts.
- Research on the effects of dietary supplements on resistance to pathogens can influence regulatory decisions by FDA. Supplements, including probiotics, are regulated by the Dietary Supplement Health and Education Act of 1994, which

focuses on adulteration or health risks. Our research looks at the effects of dietary supplement ingredients on microbial infections. If lowered disease resistance seems likely in the consumer, the supplement should be evaluated for safety. Additionally, products that are effective for treating diseases should be regulated as drugs or biologics, instead of as dietary supplements.

- The use of probiotic supplements to promote human health is an emerging issue. Knowledge of how a vaginal probiotic could be established and maintained may be utilized by FDA to assess the efficacy of other probiotics.

Division of Neurotoxicology Summary of Activities

Merle G. Paule, Ph.D., Director
870-543-7147
Merle.Paule@fda.hhs.gov

Introduction

Fifty million Americans have a permanent neurological disability that limits their daily activities, and one in three Americans will experience some form of mental disorder during their lifetime. Health care, lost productivity, and other economic costs associated with brain-related diseases are estimated to exceed $500 billion annually. Disability from depression alone exceeds that of diabetes, hypertension, gastrointestinal, and lung diseases, costing over $43 billion annually. The number of persons with Alzheimer's and other age-related neurological disorders will increase dramatically as our population ages. Known and suspected causes of brain-related disorders include exposures to chemicals, such as therapeutic drugs, food additives, food products, cosmetic ingredients, pesticides, drugs of abuse, and naturally occurring substances. Recent advances in technology have provided a variety of new tools with which to better study and understand the etiology of brain-related disorders and the time-course and mechanisms associated with chemically induced neurotoxicity and to further reduce the risks associated with neurotoxic events.

The number of neuroactive chemicals that require FDA regulation is estimated to be in the thousands. Thus, identifying methods and mechanisms is critical for the development of guidelines for the assessment of neurotoxic risk. Chemicals that are known or suspected causes of brain-related disorders are vital to the national economy and our quality of life. However, the challenge is to determine at what doses or exposure levels, and under what conditions, these compounds can be used effectively while minimizing the likelihood that they will cause adverse effects on the nervous system.

The overall goals of the Division of Neurotoxicology are to develop and validate quantitative biomarkers and identify biological pathways associated with the expression of neurotoxicity. Specific focus areas of fundamental research are employed to broadly examine the involvement of specific systems in the expression of neurotoxicity. These include:

- Monoamine-neurotransmitter system.
- Mitochondrial function and oxidative stress.
- The N-methyl-D-aspartic acid (NMDA) receptor complex and associated glutamate metabolism.
- The role of amyloid ß-peptide aggregation.

- The role of the blood-brain barrier.

An increased understanding of the processes associated with neurotoxic outcomes will provide opportunities for improved assessments of risk and identification of potential therapeutic approaches. The strategies employed for achieving these goals involve multidisciplinary approaches that capitalize on the neurochemistry, molecular neurobiology, neuropathology, neurophysiology, and behavioral expertise of Division personnel. The Division is expanding its imaging capabilities by adding microPET, magnetic resonance imaging (MRI), and computerized tomography (CT) efforts to increase approaches that translate into the clinic. In addition, efforts to develop sensitive, high-throughput systems (stem cells; zebrafish) for screening potential neurotoxicants are underway. Other unique features of the Division's research capabilities include the ability to:

- Determine chemical concentrations and cellular-level interactions in target tissue.
- Determine changes in gene and protein expression associated with chemical exposures.
- Effect high-throughput, comprehensive cognitive and behavioral assessments.
- 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.
- Develop novel histochemical tracers to aid in the evaluation of chemical-induced pathologies.

FY 2010 Accomplishments

The Division continued to provide data important for the regulatory needs of the agency with respect to pediatric anesthetic agents, central nervous system stimulants, and nanoparticles. In addition, analyses of data from a life-time rodent study on the neurotoxic effects of the food contaminant acrylamide and from a study in juvenile nonhuman primates on the effects of chronic treatment with pramipexole (PPX), a type of dopamine receptor agonist, continued. Rodent studies on the developmental toxicity of the ubiquitous plasticizer, bisphenol A (BPA), were begun.

In partnership with FDA's CDER colleagues, Division staff broadened their study of the neurotoxicity associated with pediatric anesthetics utilizing both *in vitro* (rodent and nonhuman primate neuronal cell cultures) and *in vivo* (rat and nonhuman primate) approaches to include inhalation anesthetics. Importantly, the use of nonhuman primate and rodent models are beginning to help identify compounds that may be able to prevent or ameliorate anesthetic-induced neurotoxicity. In addition, Division scientists continued their work with CDER reviewers to assist in the development of guidelines for the assessment of developmental neurotoxicity, neuropathology, and seizures.

Approaches to assessing damage to the brain vasculature and meninges after exposure to the amphetamines (AMPs) included evaluation of changes in the expression of vascular-related genes. The meninges and surface vasculature of the brain were shown to be susceptible to damage when pronounced hyperthermia accompanied exposure to AMPs.

Studies on the assessment of human brain/cognitive function using the NCTR Operant Test Battery—the same instrument used in the Division of Neurotoxicity's Nonhuman Primate Research Center—continued at our laboratory at nearby Arkansas Children's Hospital (ACH), primarily in children with depression or anxiety disorder. Studies were also begun to explore the long-term effects of ketamine in a pediatric population that directly parallels the work conducted in nonhuman primates at NCTR. These studies are exemplary of translational neuroscience and highlight the cross-species comparison capabilities within the Division.

In collaboration with Wright-Patterson Air Force Base (WPAFB) and FDA colleagues at CDER and CDRH, Division staff demonstrated that metal oxide-based nanoparticles (manganese, silver, copper, and aluminum) produce free radicals and induce oxidative stress in both cell culture and animal models, effects that are associated with selective alterations in the expression of genes associated with apoptosis and oxidative stress. These data are providing mechanistic information that will help researchers understand the potential risk of nanoparticles to human health.

Utilizing the 3-NPA-induced rat model of peripheral neuropathy, morphometric (variation and change in the size and shape of organisms) and electrophysiological (electrical activities of biological cells and tissues) analyses were undertaken to examine the protective potential of resveratrol. Resveratrol is a naturally occurring neuroprotectant found in the skins of grapes and in certain other plants, fruits, and seeds. Preliminary findings suggest that resveratrol is a good candidate for the treatment of metabolic neuropathy—a nerve disorder that occurs with diseases that disrupt the chemical processes in the body.

Studies of the neurobehavioral toxicity associated with adolescent methylphenidate (MPH) exposure in rats and nonhuman primates indicated that, at serum levels matching or near human-therapeutic levels, there were few detectable alterations. Given the controversy surrounding the use of MPH to treat ADHD and its potential to affect risk of later substance use, these studies were reassuring of the safety of this drug.

In support of many of our areas of research, genomic, proteomic, and bioinformatics approaches were employed to allow for the identification of gene and protein-expression profiles associated with neurotoxic events, such as chemically induced mitochondrial dysfunction. Importantly, a zebrafish breeding and maintenance facility was created to support higher-throughput evaluations of potential neurotoxicants. Identification of specific toxic events during zebrafish development can serve to elucidate mechanisms and provide markers of toxicity. MicroPET imaging provided new data on the time-course of ketamine-induced apoptosis in our rodent model and is

critical for the development of radioligands for clinical use. The search for radioligands applicable to primates continued. A newly acquired preclinical MRI scanner was successfully installed and configured. New MR spectroscopic and morphometric data were collected *in vivo* from rat brains during early development. These kinds of data will be important in translational developmental neuroscience and the development of imaging biomarkers. Several other MRI projects were begun including the development of biomarkers for the glutamate cycle, pediatric anesthetic-induced neurotoxicity, and Alzheimer's disease

FY 2011 Plans

Much of the work in FY 2011 will involve continuation of the efforts mentioned above, focusing on specific agency regulatory needs. These include:

- Publication of data from studies on acrylamide, pediatric anesthetics, nanoparticles, AMPs, and related compounds (e.g., PPX).
- Data on the developmental toxicity of BPA and on the efficacy and toxicity of a variety of potential therapeutic agents in a transgenic-mouse model of Alzheimer's disease will become available.
- Renovations will begin to expand the capacity of the Nonhuman Primate Research Center, and the MRI and CT will be fully integrated into our multi-modal research programs, dramatically increasing our imaging capabilities and enhancing efforts to describe and define neurotoxic events as they occur over time in living-animal models.
- Studies to determine the placental transfer of BPA will be carried out in near-term rhesus macaques. The similarities of nonhuman primates to humans will provide a foundation for subsequent estimation of the potential exposure of the developing fetus to this agent after maternal exposure.
- Attempts will be made to identify biologically significant changes in gene expression that accompany mitochondrial dysfunction and oxidative stress. Further utilization of omics techniques and the zebrafish developmental-neurotoxicity model should allow for the identification of specific genes and pathways involved in the expression of neurotoxicity. The addition of our state-of-the-art imaging capabilities will provide a new dimension to our abilities to understand adverse neural events.
- New projects will be implemented to develop novel histochemical tracers for the localization of various elements of the brain vasculature and to use these tracers to illuminate the effects of neurotoxicants.
- In collaboration with WPAFB and FDA colleagues at CDER and CDRH, Division staff will explore the effects of nanoparticles and carbon nanotubes on the integrity of the blood-brain barrier (BBB) . A microvascular endothelial-cell culture will be used to model the BBB and allow the study of its permeability to

nanoparticles, *in vitro*. The toxicity of selected nanoparticles will then be studied *in vivo*.

- Novel MRI approaches will be utilized in regulatory science projects to develop standards for neuropathology protocols: MRI signals suggesting neurotoxicity will be used to direct follow-up traditional neuropathological assessments.
- As part of a continuing validation process, studies at our ACH Laboratory will be conducted to determine the relationship between the tasks that comprise the NCTR Operant Test Battery and standard psychological tests that are used clinically.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by the Division of Neurotoxicology contributes to NCTR Strategic Goal 1 (Advance scientific approaches and tools necessary to support public health) and to FDA Strategic Goals:

- **2.1: Cross-Cutting Research to Advance Regulatory Science and Innovation**
- **3.2: Promote Public Health by Advancing the Safety and Effectiveness of Medical Products**

Goal 2.1 (Cross-Cutting Research to Advance Regulatory Science and Innovation)

The Division provides expert advice and innovative research to the other FDA Centers—thus contributing to FDA's mission of advancing public health. Research projects involve new and innovative technologies and approaches that support FDA's regulatory Centers.

To assist the agency with the rapid determination of the neurotoxicity of a vast array of regulated chemicals and food contaminants, the Division has begun using a high-throughput, *in vitro* (zebrafish) system. This approach to identifying potential vertebrate toxicants will have broad applicability and be relevant for a variety of life stages—from fertilization throughout development. Coupled with our primary, organotypic, and stem-cell culture efforts, this approach will help direct subsequent resource utilization in further defining the risks associated with the use of regulated products.

The establishment of state-of-the-art imaging capabilities is providing opportunities to monitor the onset of toxic responses and to delve further into their mechanisms and time-course. These imaging resources provide the capabilities to get maximal information from invaluable preclinical models while minimizing the number of animals needed. It is hoped that the development of the new MRI techniques to guide follow-up histopathological analyses will improve the safety of new drugs by decreasing the incidence of false-negative findings. Not only do these and similar efforts serve to strengthen FDA's base of operations, they also strengthen the scientific foundation of FDA's regulatory mission and the science that supports product safety. Many of these efforts involve partnerships within the agency, with industry, and with academic centers. In addition, Division staff continue to provide training for undergraduate and graduate students, postdoctoral fellows, visiting scientists, and FDA Commissioner's

Fellows—many of whom will go on to serve the agency as employees endowed with the knowledge and expertise needed to preserve its science base.

Research at our ACH laboratory contributes to this priority by identifying appropriate, translatable biomarkers of brain function and their validity for assessing toxicity of the various foods and drugs that are regulated by FDA.

Goal 3.2 (Promote Public Health by Advancing the Safety and Effectiveness of Medical Products)

NCTR research to elucidate the mechanisms underlying the neurotoxicity associated with the pediatric use of anesthetic agents, define sensitive periods of development, explore critical dose-response relationships, and develop protective therapeutic strategies continue to provide the agency and clinicians with the knowledge needed to minimize risk and protect children's health. Studies employing exposures early in development (BPA) or in juvenile animal models (PPX, AMP, MPH) are providing data relevant to the pediatric use of these agents. Our laboratory at ACH is also serving as one of several sites for the ASK Children Study initiated by colleagues at CDRH in an effort to better regulate medical devices by developing methods for measuring quality-of-life for children who utilize these devices.

By developing effective methods for elucidating the biochemical pathways that underlie the expression of toxicity, it should be possible to use those methods to assess the toxic or beneficial effects of new medical and nutritive products. Utilization of *in vitro* brain-cell cultures, as well as the zebrafish model in our studies on the toxicity of anesthetic compounds, are providing new information for understanding toxic mechanisms and should provide insight into possible rescue or protective approaches. Utilization of a transgenic mouse model of Alzheimer's plaque deposition is being used in efforts to help delineate toxic mechanisms and illuminate the potential efficacy of different therapeutic strategies. Mechanistically based approaches, including a zebrafish developmental toxicity screen and neural stem-cell cultures that are currently being established, will be applied to define and understand the potential of a broad range of drugs and other chemicals to produce neurotoxic effects during all stages of development and senescence. This kind of information will be invaluable in the development of safe, new products.

Division of Personalized Nutrition and Medicine Summary of Activities

James A. Kaput, Ph.D., Director
870-543-7997
James.Kaput@fda.hhs.gov

Introduction

The Division of Personalized Nutrition and Medicine is charged with developing strategies, methods, and resources for improving individual and public health. The need for this Division and research paradigm resulted from data generated by the human genome and Haplotype Map (HapMap) projects. These international efforts laid the foundation for one of the most significant scientific contributions to humankind—an evidence-based understanding that while humans are genetically similar, each retains a unique genetic identity that contributes to the wide array of biochemical, physiological, and morphological phenotypes in human populations. Parallel molecular genetic studies have demonstrated that nutrient and environmental chemicals directly or indirectly regulate the expression of one's genetic makeup.

While the research strategies of the 20th century yielded data and knowledge that extended our average lifespan and improved personal and public health, much of that knowledge was based on the average response of a population to a food, nutrient, or environmental chemical, or the average risk for carrying a specific allele of a gene involved in disease. Such knowledge may or may not be applicable to an individual with different genotypes or environmental exposures.

The overall goals of the Division are to develop and implement research strategies that account for genetic, environmental, and cultural diversity that influence expression of genetic makeups and produce knowledge for improving personal and public health. These overarching goals will be met with four parallel efforts that develop:

- Integration of omics methodologies to assess an individual's health status and, as importantly, susceptibility to specific chronic conditions influenced by environmental factors, including diet.
- Means to capture and assess an individual's nutritional, environmental, and activity exposures.
- Classification algorithms that integrate data from omics and environmental assessments that will result in evidence-based and validated biomedical-decision making.

- A novel pathogen knowledge base for the Food Protection Plan that will become the foundation for a metagenomic (human microbiota) program within the division.

The Division has two areas—Biometry and Biology. The main function of the Biometry area is to develop biometrical methods for all aspects of FDA's mission, goals, and objectives. A subgroup within the area analyzes all data from the National Toxicology Program (NTP). The Biology area is focusing on the broad areas of pharmacogenomics and nutrigenomics—how individuals respond to drugs and nutrients in foods.

FY 2010 Accomplishments

The Division of Personalized Nutrition and Medicine met major milestones in FY 2010 and laid the foundation for future programs in FY 2011 and beyond.

The National Toxicology Program (NTP) statistician subgroup of the Biometry area completed 56 statistical reports for NTP protocols. Members of this team also provided statistical support to other protocols, including protocol review for a number of additional NTP and non-NTP studies, reviewed protocols for the Institutional Animal Care and Use Committee (IACUC), and maintained correspondence with the FDA Statistical Association in Washington.

The statisticians in the Biometry area continue to contribute to multiple Division of Personalized Nutrition and Medicine, NCTR, and FDA research projects and continue to maintain communications with the scientists on risk-assessment methodology in the Interagency Risk Assessment Consortium. The research efforts focused on statistical and data-mining methods for the analyses of high-dimensional genomic, proteomic, metabolomic, and toxicoinformatic data. Projects related to microarray data analysis include:

- Normalization methods
- Analysis of sources of variation
- Sample-size estimation
- Testing for differentially expressed genes
- Multiplicity adjustment gene-set enrichment analysis.

Projects related to data mining and bioinformatics include:

- Feature selection
- Ensemble classification algorithms
- Imbalanced class-size prediction
- Integrated analysis of genomic, proteomic, and metabolomic data
- Genomics knowledge base for detection and characterization of microbiological pathogens

These efforts are leading to improved ability to develop classification algorithms to facilitate the use of high-dimensional genomic biomarkers, contributing to the development of statistical methods to analyze individual genes and biological pathways, and investigating hierarchical-probabilistic models for characterization of uncertainty in risk/safety assessment.

The Biometry area is also leading a cross-division program to develop a food-pathogen knowledge base as a part of the agency's Food Protection Plan,. Division researchers and statisticians published a collaborative study testing new algorithms to assess relatedness of the foodborne pathogen, *Salmonella*.

The area participated in a biomedical-focused, community-based participatory research program (CBPR) in collaboration with the U.S. Department of Agriculture (USDA)/Agricultural Research Service Delta Obesity Prevention Research Unit in Little Rock, Arkansas, and the Boys, Girls, and Adults Community Development Center (BGACDC) in Marvell, Arkansas, for a third year. The study is analyzing the levels of selected vitamins and metabolites in the serum of children attending a five-week summer day camp at BGACDC. Interns from the Washington and Lee University Shepard Poverty Program contributed to this effort. Their primary focus was to help assess the physical activity of children in the summer day camp using a commercially available accelerometer device. Metabolomic analyses of 2008 and 2009 samples indicated that many children and adults had low levels of vitamin D and metabolites involved in one-carbon metabolism. The Delta Vitamin Project forms one of the components of a national and international effort to develop a micronutrient genomics program and knowledge base. The international coordinating team consists of the Division Director and five other scientists from Canada, Australia, and Europe. This program received funding from the Marie Curie Foundation to support the travel of European scientists and scientists from developing countries to participate in the creation of a micronutrient genomics knowledge base.

The Division has completed the development of a genomic laboratory, including the acquisition of a next generation sequencer. The first laboratory results of whole genome genotyping arrays for the Delta Vitamin Project were completed in FY 2009, and novel statistical analyses are underway that will eventually enable the analyses of epistatic (gene-gene) interactions that could provide insights into individual responses to drugs, dietary chemicals, and toxicants.

The Division's stem-cell program is examining the effects of toxins on development and the influence of nutrients on metabolism. This new program is being vertically integrated into new programs in mouse-epigenetics (the changes in RNA expression information without a change in DNA sequence) studies being developed in the Division in collaboration with scientists in NCTR's Division of Biochemical Toxicology. Integrating data and results from the model systems of stem cells, laboratory animals, and humans will provide results ranging from mechanisms to applications in humans. The Division co-hosted a workshop on Stem Cell Biology in collaboration with scientists at the University of Arkansas for Medical Sciences (UAMS) and the Arkansas Biosciences

Institute in April 2010. Over 80 scientists met in Little Rock to hear seven distinguished researchers in stem-cell biology, including a colleague from FDA's Center for Biologics Evaluation and Research (CBER) involved in regulatory activities. As a result of the program, UAMS initiated the development of a stem-cell biology program.

Members of the Division are co-leading the national and international development of web-based nutrient- and physical-activity assessment tools, software, and databases for biomedical research. NCTR's Division of Personalized Nutrition and Medicine and USDA co-sponsored an interagency (USDA, FDA, and National Institutes of Health) meeting held in Spring 2009, which resulted in a publication in the *Journal of Nutrition* that calls for the development of national programs to further develop tools and databases for research. In collaboration with European scientists, three reports were published on national and global needs for the nutrition and nutrigenomics communities.

FY 2011 Plans

Division of Personalized Nutrition and Medicine investigators will conduct the following research studies in FY 2011.

- The Division is extending its CBPR program to include a dietary intervention study to improve nutrient intakes of micronutrients, particularly of vitamin D. The CBPR program will enter its fourth year and continue the collaboration with BGACDC, USDA-ARS, and the Washington and Lee University Shepard Poverty Program.
- The Division also is developing a broader community-based effort to analyze micronutrient levels and genetic makeup of adults in Phillips County, Ark.
- Division scientists are also developing a research protocol based on the concept of analyzing and understanding the "healthy state." Health is often considered the absence of disease, and disease biomarkers may not be useful in predicting susceptibility to chronic diseases. The Healthy Challenge Study is being planned as a collaborative project with scientists and physicians at UAMS and their Center for Clinical and Translational Research; as well as other NCTR scientists in the Divisions of Systems Biology, Biochemical Toxicology, Neurotoxicology, Microbiology, and Genetic and Molecular Toxicology, along with colleagues in CDER and CDRH who are experts in transcriptomic, proteomic, and metabolomic concepts and instrumentation.
- Scientists from academic institutions and companies are contributing expertise to studies of responses to the oral-glucose and the oral-fructose challenges. An individual's response to these challenges may be unique to their genetic makeup and provide information for long-term health outcomes.
- The Division is continuing the development of using mouse stem cells for toxicology research and the effects of nutrient metabolism in stem cells. Preliminary data show that fructose alters how glucose is metabolized in

adipocytes in culture. These results add to a growing body of literature indicating that intake of high levels of fructose may be detrimental to health.

- The Biometry area will continue to focus on the development of:
    a. Decision models for clinical assignments of patients based on the patient's genomic features and disease phenotypes.
    b. Methods to identify genomic, proteomic, and metabolomic liver-toxicity biomarkers.
    c. Computational algorithms that will efficiently compute adjusted p-values for the large numbers of subsets defined through gene ontology.
- The Biometry staff will investigate methods for integrating the associations between the genomic-predictor variables and phenotype-class variables (such as tumor types or treatment efficacy), predictive models, and computational methods for quantitative assessment of benefit/risk models for regulatory decisions in personalized medicine, and initiate research on biostatistical approach for relative-risk ranking for food protection.
- The NTP staff will continue its critical mission of analyzing data from NTP studies.
- The Division's Biometry area is the lead in developing a protocol for an integrated genomics knowledge base for rapid-threat assessment of enteric foodborne pathogens. This project is in collaboration with the Divisions of Microbiology and Systems Biology. Milestones for FY 2010 include the development of a manuscript describing needs of the agency, resources available in FDA and outside agencies, and the design of the knowledge base.

Contribution to FDA's Strategic Priorities/Goals

The research conducted by the Division of Personalized Nutrition and Medicine contributes to NCTR Strategic Goal 1 (Advance scientific approaches and tools necessary to support public health) and to FDA Strategic Goals:

- **2.1: Cross-Cutting Research to Advance Regulatory Science and Innovation**
- **3.1: Advance Food Safety and Nutrition**
- **3.2: Promote Public Health by Advancing the Safety and Effectiveness of Medical Products**

The Division provides expert advice and innovative research to the other FDA Centers—thus contributing to FDA's mission of advancing public health. Research projects involve new and innovative technologies and approaches that support FDA's regulatory Centers.

Research in the Biology area contributes primarily to improve patient and consumer safety by increasing access to new medical and food products and by developing methods and knowledge for understanding differences based on individual genetic makeup. While the methods and knowledge are just beginning to be developed, the

consumer and public are already exposed to genetic testing and products designed for individuals, even though the science to support those products is somewhat lacking.

The Biometry area collaborates with scientists at NCTR and other FDA Centers by analyzing data with novel risk-assessment algorithms. Specifically, the Biometry area estimates risks associated with toxic substances and helps set safe-exposure levels that correctly reflect underlying uncertainties. FDA relies on NCTR's Division of Personalized Nutrition and Medicine to:

- Conduct risk assessments for the regulation of specific products and investigating generic risk-assessment issues.
- Develop mathematical models and computer systems for analyzing pharmacokinetic and pharmacodynamic components of toxic mechanisms.
- Develop classification algorithms for biomedical decision making, including identifying food hazards and assigning patients to drug therapies.
- Develop statistical methods for analyzing genomic, proteomic, metabolomic, and toxicoinformatic data.
- Apply statistical methods to evaluate toxicological, pharmacological, and nutritional concerns.
- Provide expertise to NCTR scientists on the design, conduct, and analyses of research studies to evaluate the toxicity of regulated products. The Biometry area is also contributing to FDA's responsibilities to protect the food system in the United States.

Division of Systems Biology Summary of Activities

Donna L. Mendrick, Ph.D., Director
870-543-7718
Donna.Mendrick@fda.hhs.gov

Introduction

The Division of Systems Biology supports the development of new technologies and identification of new biomarkers to facilitate the integration of scientific data for application to questions that are in direct support of the FDA regulatory mission. Six Centers of Excellence comprise the Division of Systems Biology: Bioinformatics, Functional Genomics, Hepatotoxicity, Innovative Technologies, Metabolomics, and Proteomics. This multidisciplinary group is comprised of microbiologists, biologists, analytical chemists, applied statisticians, physical chemists, physicists, bioinformaticians, etc. The goals of this Division are to convert emerging science and technology into:

1. Finding new translational biomarkers to improve detection of drug and food supplement safety issues, disease onset, and progression.
2. Developing innovative tools to improve detection of food contaminants, identification of infectious diseases, and diagnostic procedures.
3. Building bioinformatic solutions that facilitate the regulatory process.

The **Center for Bioinformatics** conducts research in bioinformatics and chemoinformatics and develops and coordinates informatics capabilities within NCTR, across FDA Centers, and in the larger scientific community. A goal of this group is to develop methods for the analysis and integration of omics (genomics, proteomics, and metabolomics) datasets with classical in-life parameters. This Center is taking an active role in supporting FDA's bioinformatics modernization plan, including the e-submission process.

The **Center for Functional Genomics** uses high-information content microarrays in the identification of biomarkers for improved safety assessments and disease management. Major efforts include the development of preclinical predictive-toxicology biomarkers, understanding the mechanistic links between mitochondrial dysfunction with toxicity and disease processes, and continuing to serve as an FDA resource for genomic sissues.

The **Center for Hepatotoxicity** addresses critical issues related to liver injury by applying a systems-biology approach to find biomarkers. The goals include the identification of biomarkers that will improve the preclinical identification of drugs and dietary supplements that prove to be hepatotoxic in humans and to augment the detection of early signs of liver injury in humans induced by drugs, chemicals, and disease processes.

The **Center for Innovative Technologies** uses multifaceted approaches to address important issues of human health. Examples include programs in mass spectrometry - and flow cytometric-based analyses for rapid detection of bacteria in food and clinical samples and significant computational efforts in modeling to improve diagnosis and toxicity-risk assessment.

The **Center for Metabolomics** employs open, focused, and flux-based metabolomic analytical profiles to improve the detection of toxicity and disease in preclinical and human studies. The Center is focused in connecting the results of these metabolomics studies to magnetic resonance spectroscopy imaging studies

The **Center for Proteomics** conducts proteomic research to address FDA critical issues related to drug safety and efficacy and early-disease detection. The Center continues to develop and evaluate novel proteomic technologies with the aim of facilitating the translation of basic science to medical products.

FY 2010 Accomplishments

During FY 2010, Division scientists engaged in research addressing a variety of agency issues with special emphasis on areas in biomarker identification, food safety, improvement of diagnostic procedures, and bioinformatics. Accomplishments include the following:

1. Identification of appropriate methodologies to identify and statistically validate genomic biomarkers
    - Completed Phase II of the MicroArray Quality Control (MAQC) project, which involved 200 participants from 86 organizations. Results were reported in 13 publications (2 papers in *Nature Biotechnology* and 11 papers in *The Pharmacogenomics Journal)*.
2. Advances in our understanding of hepatotoxicity using omics approaches
    - Collected biological samples from two pivotal studies to find new hepatotoxicity biomarkers and started processing the samples for multiple-omics analyses. Started a study of the potential additive effects of dietary supplements to acetaminophen-induced hepatotoxicity.
    - Completed additional work on the Liver Toxicity Knowledge Base (LTKB) project, including performance of a high-content assay on drugs for drug-induced liver injury potential, finished the first gene-expression assays using primary rat hepatocytes and collected diverse datasets from the public domain on >220 drugs (including histopathology findings, identification of targets and side effects, etc.). In collaboration with the Tox21 interagency program, a high-throughput assay was carried out on 280 drugs for three mechanistic endpoints.
    - Examined the liver mitochondrial toxicity of acrylamide. Profiled gene expression and DNA methylation to study the potential effects of a methyl-deficient diet on liver injury.

3. Studies of biomarkers relevant to other organ and cellular injury
    - Initiated a study that will aid in the identification of potential biomarkers of drug-induced cardiotoxicity.
    - Completed analyses of energy-related metabolites in preclinical studies of an agent that induces Parkinson's-like symptoms in humans in an effort to identify new biomarkers of neuronal injury.
    - Conducted stable isotope labeling-based quantitative proteome analysis for the identification of kidney-injury biomarkers as a result of toxicant exposure.
    - Characterized histone modifications for understanding of the mechanisms of diabetes and obesity.
4. Development of *in silico* models to predict adverse events
    - Completed a collaborative interagency project of *in silico* computational toxicology modeling to predict adverse polypharmacy interactions with Cytochrome p450 enzymes.
    - Conducted the first successful modeling of diverse and flexible endocrine-disrupting compounds using a patented 3D-QSDAR approach.
    - Started a pilot project to examine the ability of an *in silico* approach to predict the binding of drugs that induce idiosyncratic hepatotoxicity to specific Human Leukocyte Antigen (HLA) haplotypes.
5. Improvements to software and new database development
    - Released a new version of ArrayTrack™, a data management and warehousing system, which contains new and improved analysis functionality including: 1) tools for microarray-based genomic biomarker development, and 2) the ability to import proteomics and metabolomics data.
    - Two new modules in ArrayTrack™ were developed to support genetic research for personalized medicine and nutrition.
    - Developed two separate databases to support CDER scientists in their study of Hepatitis-C virus and animal models for pediatric application.
    - Completed the initial phases of the pilot studies to develop Janus models for pharmacogenomics and preclinical data submission.
6. Imaging
    - Published novel data-analysis methods that allow development of accurate computational models for brain-cancer diagnosis using MRS data. Continued collaboration with CDRH and Johns Hopkins University Medical School to extend the method for additional diagnostic tasks.
    - Begin preliminary bio-imaging work in rats in an effort to identify biomarkers of liver impairment.

7. Improvements in food safety

    - Conducted validation studies in collaboration with ORA/ARL for rapid detection of very low levels of *E. coli* H7:0157 in raw spinach. The RAPID-B™ technology proved to be much faster than the standard FDA bacterial analytical manual (BAM) method. Additional studies demonstrated the ability to utilize this technology in cookie dough, salami, etc.
    - In concert with assays completed previously, developed additional RAPID-B™ assays to span most common aerobic foodborne pathogens. Developed new sample preparation and treatment techniques that can be applied to many difficult food matrices to enable their use with any of the pathogen-specific RAPID-B™ assays.

8. Methods development

    - In collaboration with CDRH, initial studies were conducted to characterize whole genome amplified DNA for its suitability for genetic device validation
    - Discovered a novel and superior way to volatilize and ionize organic solids for mass-spectrometric analysis and named it Direct Impact Corona Ionization (DICI) Mass Spectrometry (MS). This process increases analytical sensitivity by a factor up to 40,000.
    - Performed investigative studies to improve proteomics technology. This included the study of phosphopeptide fragmentation characteristics in tandem mass spectrometry for robust identification of phosphoproteins, development of N-terminal peptide enrichment from complex protein samples for mass spectrometry analysis, and evaluation of the reliability and reproducibility of different sample preparation methods, protein assays, and mass spectrometry-operation conditions for quantitative proteomics.

FY 2011 Plans

In FY 2011, the Division of Systems Biology will continue to emphasize a systems biology approach for development of biomarkers and mechanistic information for safety and efficacy assessments of medical products and foods. Additional studies will focus on the development of improved *in silico* modeling approaches for drug and food supplement safety and medical imaging. To accomplish its mission, the Division of Systems Biology will continue to: study toxicity, efficacy, and disease-utilizing systems biological approaches and other methods to identify new biomarkers that will improve the safety, efficacy, review, and usefulness of FDA-regulated products.

- Continue a multi-year, systems-biology study to identify potential biomarkers to improve early detection of idiosyncratic hepatotoxicants and to explore dietary-supplement interaction with acetaminophen-induced hepatotoxicity.
- Identify biomarkers of drug-induced cardiotoxicity.

- Continue studies on age- and sex-related gene-expression profiles in rat organs and relate findings to potential age- and sex-related susceptibilities to drug toxicities and effectiveness in the rat model, as well as in humans.
- Begin to connect systems-biology data in preclinical studies with imaging biomarkers.
- Develop mitochondria-specific gene expression arrays for multiple species for better safety evaluation of drugs and disease that target mitochondria.
- Continue development of open profiling and focused metabolomic analyses of biofluids and tissues from preclinical and clinical studies and utilize flux analyses to identify early biomarkers of tissue injury or dysfunction.
- Continue developing intra-lab and inter-lab quality-control standards for metabolomics analyses.
- Continue to use proteomic approaches to identify biomarkers and mechanisms of diseases and drug-induced organ toxicity including liver, kidney, and heart.
- Establish targeted proteomic analysis pipelines to accelerate biomarker verification.
- Work to improve food safety and infectious disease diagnosis.
- Explore the potential of the RAPID-B™ technology to detect anaerobic foodborne pathogens and foodborne viruses. Investigate its potential application for infectious public-health applications in tissues or body fluids.
- Maintain efforts to build data repositories and improve *in silico* methods and analysis tools.
- Continue development of the Liver Toxicity Knowledge Base.
- Continue evaluation of *in silico* technology related to protein-drug interaction for personalized medicine.
- Continue the development of ArrayTrack™ to warehouse, visualize, analyze, and interpret data from diverse omics technologies, as well as clinical and nonclinical data.
- Continue to support FDA's regulatory mission
- Continue providing technical expertise to FDA in genomic, proteomic, and metabolomic interpretation and guidance.
- Continue the MAQC-III project to evaluate the technical performance and practical utility of next-generation sequencing technology.
- Continue participation in the review of the pharmacogenomics data submitted through the Voluntary Exploratory Data Submission (VXDS) program.

Contribution to FDA's Strategic Priorities/Goals

The research conducted by the Division of Neurotoxicology contributes to NCTR Strategic Goal 1 (Advance scientific approaches and tools necessary to support public health) and to FDA Strategic Goals:

- **2.1: Cross-Cutting Research to Advance Regulatory Science and Innovation**
- **3.2: Promote Public Health by Advancing the Safety and Effectiveness of Medical Products**

The Division provides expert advice and innovative research to the other FDA Centers—thus contributing to FDA's mission of advancing public health. Research projects involve new and innovative technologies and approaches that support FDA's regulatory Centers.

The Division of Systems Biology will continue to utilize multifaceted approaches (including genomic, proteomic, and metabolomic tools) to discover predictive and diagnostic biomarkers to improve drug safety and efficacy and disease prevention and management. Efforts will continue to be made to utilize *in silico* (computer simulated) approaches for predictive toxicology to enhance product safety. Continued development of RAPID-B™ will support food safety and augment the diagnosis of infectious diseases.

The MAQC-III consortium aims to determine and identify the issues and challenges associated with the next generation of sequencing technology. It anticipates that the review of such data as a part of an Investigational New Drug (IND) and New Drug Application (NDA) submission will soon become an FDA responsibility.

Innovation will be continue to be a hallmark of this Division and will include the development of new proteomic methods to enable discovery of new types of biomarkers, the merging of metabolomics and bio-imaging to find translational biomarkers, and improvements in mass-spectrometric technologies. The continued development of new bioinformatics tools will allow reviewers to easily access information from both private and public domain—thus enhancing the FDA review process. Novel computational models will continue to be developed that predict drug safety and efficacy. These new methods will increase the number of safe and effective medical products.

Veterinary Services Summary of Activities

Jefferson H. Carraway, D.V.M., MS, DACLAM, Director
870-543-7347
Jeff.Carraway@fda.hhs.gov

Introduction

The Veterinary Services staff provides professional and technical support for all animal-related research projects at NCTR. The staff administers NCTR'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 staff are contracted services for animal husbandry, veterinary care, and diet preparation. This workforce is stable, highly trained and skilled, and boasts a high-percentage of certified employees in their respective disciplines.

The staff Director is a member of NCTR's Institutional Animal Care and Use Committee (IACUC), serving as Vice Chair and Attending Veterinarian. The liaison between the Veterinary Services staff 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 services to the NCTR research community.

The staff oversees the operation of five animal facilities consisting of over 112,000 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 rodent-breeding operation established over thirty years ago provides many of the strains used for on-site experiments. A highly trained and American Association for Laboratory Animal Science (AALAS)-certified animal care staff provides a wide variety of husbandry and technical services in support of NCTR's AAALAC-accredited Animal Care and Use Program.

Provision of veterinary services of the highest quality to NCTR's research animals is a staff priority. Three veterinarians, two certified by the American College of Laboratory Animal Medicine (ACLAM) , one certified by the American Board of Toxicology, and all of whom hold research degrees in addition to DVMs, are charged with ensuring that healthy animals are available for research projects, providing veterinary care as needed, training research staff, and participating in projects requiring veterinary expertise. These veterinarians share emergency-call duty during non-business hours to ensure prompt delivery of veterinary care to any animal in need of medical attention.

The Diet Preparation Facility is a well-equipped, large-scale formulation-services unit. All animal diets received at NCTR are processed through the Diet Preparation Facility. The majority of dosed diets, dosed water, gavage solutions, 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 parts-per-billion. In addition, test articles can be mixed in the animals' drinking water to exacting standards in concentrations as low as one microgram per milliliter.

FY 2010 Accomplishments

Immediate Office

The Veterinary Services staff provided oversight and management of all NCTR laboratory animal facilities. Staff personnel were responsible for breeding, rearing, acquiring, and quarantining all experimental animals used on-site. Personnel submitted annual reports assuring compliance with federal regulations and National Institutes of Health 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. The staff Director is NCTR's Attending Veterinarian, IACUC Vice Chair, and the government project COTR (contracting officer's technical representative) for animal care and diet-preparation services, rodent bedding, and rodent-diet contracts. This arrangement ensured coordination of activities and provided essential input associated with IAG and CRADA development, initiation, and completion.

The Veterinary Care program was administered through the staff and, in addition to providing veterinary care and surgical services 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. Veterinarians also served as principal investigators or co-investigators on several protocols, including rodent breeding operations, animal procedures training, and a dose response study comparing two heparin products in swine and primates. All staff veterinarians were voting members of the IACUC. To ensure state-of-the-art housing environments for research animals, members of this staff continued to play an integral role in planning animal-facility renovation projects, especially the renovation and expansion of the Nonhuman Primate Research Center and the Building 5A Processing Area.

The heparin study ("A dose-response study to evaluate the potential clinical effect due to a change in the analytical methodology used to assay heparin in pig and monkey") was conducted at the request of and in collaboration with Center for Drug Evaluation and Research (CDER). NCTR was the only FDA organization with the facilities, animals, and appropriate personnel immediately available to conduct this critical study under urgent conditions. Staff personnel developed the protocol and conducted the study within two months of the original request. The quality data produced were the basis for FDA recommendations for the clinical use of "old" and "new" heparin.

The addition of a new zebrafish facility this year expands NCTR's expertise in the care and use of an additional laboratory animal species and provides new research

opportunities, especially in the area of neurotoxicology.

A major accomplishment this year was preparing for and hosting the AAALAC site visit July 13-15, 2010. Although official notification will not be received until late 2010, all indicators point to being granted full-accreditation for another three years. Site visitors especially noted excellence in documentation, research and support staff training, the occupational safety and health program, the zebrafish facility, quality animal care and veterinary staffs, sanitation program, animal husbandry, and facility management.

Animal Care/Diet Preparation/Veterinary Care Services

During FY 2010, contract personnel supported a daily average of 32 experiments. These experiments entailed the daily husbandry services for an average census of 4989 rodents, 139 rhesus monkeys, 3 mini-pigs, and 192 zebrafish. A variety of technical procedures were performed on many experiments, including tattooing, tumor palpations, biological sample collections, injections, oral gavage, behavior assessments on rats and rhesus monkeys, application of topical-dosed creams, rodent breeding operations, quarantine of rodents, physical and pregnancy examinations of rhesus monkeys, microchip implantations, and humane euthanasia. An ongoing AALAS 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 five members of the animal care management group are Certified Managers of Animal Resources (CMAR). Other training activities were conducted in technical procedures, occupational safety and health, standard operating procedures, and good laboratory practices. In addition to processing standard irradiated rodent chow (receipt, storage, and delivery), dosed diets, dosed water, and topical creams were prepared to exacting specifications for National Toxicology Program (NTP) experiments. Quality-control personnel performed monthly inspections of all animal housing and diet-preparation units, performed thousands of quality-control audits of animal care and diet-preparation procedures, and maintained, updated, and created a large volume of SOPs. An on-site 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. Prompt and state-of-the-art veterinary care was provided to all NCTR laboratory animals as needed

FY 2011 Plans

- Continue to support the research mission of NCTR through excellence in animal care, veterinary care, and diet preparation services.
- Continue a quality Laboratory Animal Care and Use Program that is consistent with state and federal laws, regulations, and guidelines.
- Continue to monitor the Animal Care/Diet Preparation Services Contract

- Play a lead role in the recompetition of the Animal Care/Diet Preparation/Veterinary Care Services Contract, including participation in planning, preparation of Request for Proposal, preproposal conference, proposal review, and negotiations.
- Play an active role in animal-facility improvement projects, including: 1) phases 2 and 3 of the expansion and renovation of the nonhuman primate facility, and 2) completion of the new cage-processing rooms in Building 5A.
- Continue active participation in IACUC endeavors.
- Continue active participation on research protocols as principal investigators and co-investigators.
- Continue supplying methods development and support, both technical and professional, needed to accomplish the NTP work at NCTR.

Contribution to FDA's Strategic Priorities/Goals

Each research division contributes to FDA's Strategic Goals in its own unique way through the individual and collective talents of its personnel as described in this document. The staff, through its support-services functions and research participation, is part of each division's contribution to these goals. The staff also contributes to NCTR's research program through participation in the projects of other divisions as principal investigators and co-investigators. Several staff personnel are DVMs or PhDs whose specialties in comparative medicine, veterinary pathology, toxicology, and genetics complement the research teams in all other divisions.

The Veterinary Services staff plays a critical support-services role in NCTR's biomedical research program. Staff 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 contributions enhance the quality of the research conducted by NCTR scientists. In addition, the staff oversees the NCTR Laboratory Animal Care and Use Program, which has been accredited by the AAALAC since 1977. This distinction assures FDA and American consumers that data generated from animal experiments at NCTR are of the highest integrity.

FY 2010 Ongoing Research Projects

FDA is responsible for protecting the public health by assuring the safety, efficacy, and security of human and veterinary drugs, biological products, medical devices, cosmetics, radiation-emitting products, tobacco products, and the food supply. FDA also advances public health by helping to speed innovations that make medicines and foods more effective, safer, and more affordable; and helping the public get the accurate, science-based information they need to use medicines and foods to improve their health. All NCTR research is grouped by NCTR Research Program area as shown below:

NCTR Research Programs:

- Personalized Nutrition and Medicine
- Strengthen Surveillance and Risk Analysis
- Enhancing Medical Product Safety

Personalized Nutrition and Medicine

PI: Ali, Syed F., Ph.D.

Wireless Deep-Brain Stimulation in Nonhuman Primates with MPTP-Induced Parkinson's Disease (E0723801)

Responsible Division: Neurotoxicology

Collaborating Division(s): Biochemical Toxicology, Office of the Director, Regulatory Compliance & Risk Management

Objective(s):

1) Develop a primate model of Parkinson's disease (PD) using the chemical neurotoxin, MPTP.

2) Implant microelectrodes within the subthalamus through stereotaxic guidance for Deep-Brain Stimulation (DBS) to:

a) Monitor and analyze patterns of tremor and dyskinesia in the PD/MPTP animals wirelessly using smart wireless sensors developed by the University of Arkansas at Fayetteville, Arkansas (UAF). Data gathered in this phase will be compared with data from controls.

b) Study patterns of tremor and dyskinesia after DBS treatment in a PD/MPTP animal model. Data will be compared in this phase within each animal as its own control.

3) Evaluate brain neurochemistry, which includes the neurotransmitters dopamine, serotonin, and their metabolites, oxidative stress markers such as reactive oxygen species (ROS), formation of 3-nitrotyrosine (3-NT), antioxidant-enzyme activities, gene-expression, transcription factors associated with dopaminergic neurodegeneration, and post-mortem brain pathology using histochemical

techniques.

PI: Azevedo, Marli, Ph.D.

Molecular and Seroepidemiology of Coronavirus and Disease Spectrum in Adults, Children, Domestic Animals, and Wildlife in the United States (E0738001)

Responsible Division: Microbiology

Objective(s):

1) Investigate the molecular epidemiology of circulating enteric and respiratory human CoV (HCoV) and nonhuman CoV strains.

2) Determine whether there is substantial genetic variability of HCoV in our community and examine geographical-genetic variation by comparisons with published findings.

3) Determine the zoonotic potential and health-safety threat of newly emerging CoV by comparing to strains currently circulating in domestic animals and wildlife.

4) Define the seroepidemiology and cross-reactivity of circulating HCoV with known human and nonhuman CoV; for new strains with high antigenic variation, we will generate immunobiologicals to develop an immunoassay to detect HCoV antibodies.

5) Use the HCoV-specific antibody ELISA or virus-neutralization assays to define the seroepidemiology of the newly detected viruses in adults and children and estimate their prevalence.

PI: Beland, Frederick, Ph.D.

DNA Adducts of Tamoxifen (E0701101)

External Funding: Office of Women's Health (OWH)

Responsible Division: Biochemical Toxicology

Objective(s):

The nonsteroidal antiestrogen tamoxifen, which is currently being used in clinical trials as a chemoprotective agent against breast cancer, has been associated with the induction of certain malignancies. In order to determine if tamoxifen is acting through a genotoxic mechanism, this project will characterize DNA adducts from suspected tamoxifen metabolites, and develop methods for their detection and quantitation.

PI: Beland, Frederick, Ph.D.

Liver Toxicity Biomarkers Study: Phase I, Entacapone and Tolcapone (E0726601)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Systems Biology

Collaborating FDA Center(s): CDER

Objective(s):

Establish liver-toxicity biomarkers and associated algorithms for use in preclinical drug development that will predict the probability of occurrence of hepatocellular injury at any subsequent phase of drug development or following approval of the drug for marketing. Emphasis will be placed upon drugs that do not demonstrate "classical" signs of liver toxicity during preclinical stages

of drug development.

PI: Beger, Richard, Ph.D.

Natural History of Acute Kidney Injury at Central Arkansas Veterans Healthcare System (E0726801)

Responsible Division: Systems Biology

Objective(s):

Acute kidney injury (AKI) is a complication caused by some currently used drug compounds and other medical issues, such as ischemic changes due to blood loss, etc. The current clinic markers of AKI are serum creatinine and blood-urea nitrogen (BUN) that monitor renal function. Thus, these serum biomarkers arise only when significant renal damage has occurred. In acute situations where renal function is compromised, failure to have more sensitive biomarkers results in the inability to prioritize patients that require aggressive treatment. This results in increased medical costs and patients requiring kidney dialysis or transplant. AKI is a significant healthcare burden that affects 5-10% of all hospitalized individuals and one third of those in the intensive care unit. The mortality rate for individuals with AKI is substantial and exceeds 40%. There is currently no proven treatment for AKI other than supportive renal- replacement therapy with dialysis and/or transplantation. Therefore, the identification of more sensitive and specific markers of renal injury may alert the physicians to institute appropriate therapy with the goal of preventing irreversible kidney damage. Additionally, such biomarkers may provide the means to move more drugs into clinical trials as one can monitor their potential toxicity more effectively. This project will test clinical biomarkers of AKI identified from previous studies that precede the rise in serum creatinine and BUN and can be measured in easily obtained urine samples from patients. This project addresses the need for more clinical biomarkers of toxicity outlined in the Critical Path Initiative.

PI: Beger, Richard, Ph.D.

Identification of New Mechanistic Biomarkers of Adverse Responses to Acetaminophen (E0731301)

Responsible Division: Systems Biology

Objective(s):

The following proposal addresses the examination of APAP-ADDUCTS in hospitalized children/adolescents receiving therapeutic exposures of APAP and the development of second generation, mechanism-based APAP-protein adduct biomarkers.

PI: Beger, Richard, Ph.D.

Collaborative Interagency Development of In Silico Computational Toxicology Modeling To Predict Adverse Drug/Chemical Interactions with Cytochrome P450 Enzymes (E0734501)

Responsible Division: Systems Biology

Collaborating Division(s): Office of Management

Objective(s):

The objective of this proposal is to develop *in silico* computational models to predict chemical interactions that can inhibit CYP3A4 and CYP2D6 enzymes.

PI: Bowyer, John, Ph.D.

Further Studies on the Effects of Afmid/TK Deficiencies and Brain, Liver, and Kidney Function (E0726101)

Responsible Division: Neurotoxicology
Collaborating Division(s): Genetic and Molecular Toxicology, Biochemical Toxicology
Objective(s):

Supplement and extend the preliminary histological studies that initially evaluated the changes in the BBB in the Afmid/TK mice that occur during the development of pathology.

PI: Buzatu, Dan, Ph.D.

Analysis of Proton MRS Data Using a Distributed Artificial Neural Network (E0719501)

Responsible Division: Systems Biology
Collaborating Division(s): Personalized Nutrition and Medicine
Objective(s):

Evaluate whether a self-optimizing, parallel-distributed neural network can use the data from *in vivo* proton magnetic resonance spectroscopy (MRS) exams to provide additional information about a brain lesion.

PI: Chelonis, John, Ph.D.

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

Responsible Division: Neurotoxicology
Collaborating Division(s): Office of Research
Objective(s):

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

PI: Chelonis, John, Ph.D.

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

Responsible Division: Neurotoxicology
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.

PI: Chen, James, Ph.D.

Benefit/Risk Classification Models for Regulatory Decision Making in Personalized Medicine (E0722001)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Division(s): Genetic and Molecular Toxicology, Microbiology, Systems Biology
Collaborating FDA Center(s): CDER
Objective(s):

Develop prediction models and computational methods for quantitative assessment of benefit/risk models for regulatory decisions in personalized medicine.

PI: Chen, James, Ph.D.

Sex Differences in Molecular Biomarkers for Individualized Treatment of Non-Gender-Specific Disease: A Novel Classification Algorithm for the Development of Genomic Signatures from High-Dimensional Data (E0727901)

Responsible Division: Personalized Nutrition and Medicine

Objective(s):

1) Find sex-specific high-dimensional biomarkers.

2) Develop classifiers for each sex using our CERP algorithm,, as well as several alternative algorithms.

3) Investigate the improvement in these high-dimensional biomarkers by using the variable importance derivative.

PI: Desai, Varsha, Ph.D.

Molecular Mechanisms Underlying Gender-Associated Differences in the Adverse Reactions to the Antiretroviral Agent, Zidovudine (AZT): Role of Mitochondrial Toxicity (E0725601)

Responsible Division: Systems Biology

Collaborating Division(s): Genetic and Molecular Toxicology

Objective(s):

Elucidate molecular mechanisms of mitochondrial dysfunction that will address gender-based differences in adverse effects of antiretroviral drugs, such as AZT.

PI: Dobrovolsky, Vasily, Ph.D.

Phosphatidylinositol Glycan—Complementation Group A (PIG-A) Mutagenesis: Development of Methods for the Identification and Molecular Characterization of Mutations in the PIG-A Gene in Human Lymphoblastoid Cells and C57Bl/6 Mice (E0720901)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Personalized Nutrition and Medicine

Objective(s):

Develop flow-cytometric methods for the detection of cells with mutations in the PIG-A gene using wild-type and mutant human-lymphoblastoid cells, TK6, and WTK1, as a model.

PI: Ferguson, Sherry, Ph.D.

Assessment of Specific Cognitive Domains in Girls with a History of Sexual Abuse (E0724701)

Responsible Division: Neurotoxicology

Objective(s):

Determine if childhood sexual abuse in 8-14 year-old girls has significant effects on cognitive tasks, which measure short-term memory, time perception, learning color/position discrimination, and motivation, as well as achievement and IQ scores.

PI: Ferguson, Sherry, Ph.D.

Methylphenidate (Ritalin) Exposure during Pregnancy: Assessment of Neurotoxicity in Offspring (E0731801)

Responsible Division: Neurotoxicology

Collaborating Division(s): Biochemical Toxicology, Genetic and Molecular Toxicology

Collaborating FDA Center(s): CDER

Objective(s):

Quantify the neurobehavioral toxicity associated with pre- and early-postnatal treatment with methylphenidate in rats.

PI: Fuscoe, James, Ph.D.

Assessment of the Global Gene Expression Changes During the Life Cycle of Rats (E0712201)

Responsible Division: Systems Biology

Collaborating Division(s): Genetic and Molecular Toxicology

Objective(s):

1) Use the NCTR rat-microarray chip

to quantitate the relative expression of approximately 4000 genes in the liver of rats at the following ages: 2 wks, 5 wks, 6 wks, 8 wks, 15 wks, 21 wks, 52 wks, 78 wks, and 104 wks. These data will serve as a baseline measurement of gene expression that will be available for future studies on drug metabolism, toxicity, and susceptibility.

2) Verify the relative expression levels by quantitative PCR or Northern analysis.

PI: Fuscoe, James, Ph.D.

Characterization of Whole Genome Amplified (WGA) DNA for Use in Genotyping Assay Development (E0735201)

Responsible Division: Systems Biology
Collaborating Division(s): Genetic and Molecular Toxicology, Personalized Nutrition and Medicine
Objective(s):

1) Determine WGA's impact on DNA copy-number variation throughout the genome using the CFTR (cystic fibrosis) gene as a model.

2) Determine the extent of mutations introduced into DNA by the WGA process. Extensive copy-number variation or mutations introduced by the WGA process would make this material unsuitable for use as a reference standard in supporting the performance of the genotyping assays. The CFTR gene will be used as a model system because it is a large gene (~200,000 bp) and is the target of genotyping assays in which it has been difficult to obtain rare allele samples.

PI: Fuscoe, James, Ph.D.

Improved Prediction and Monitoring of Drug Safety Through Assessment and Simulation of Injury/Reserve/Repair Pathways (E0741101)

Responsible Division: Systems Biology
Collaborating FDA Center(s): CDER
Objective(s):

1) Define the pathways associated with drug-induced injury, focusing on those reflecting the initial injury/repair process and the concept of "reserve."

2) Develop analytical tools and computer-based predictive models capable of identifying new classes of safety biomarkers that can serve as early signals of drug-induced injury and the amount of tissue-specific reserve available. These tools would be of particular utility in monitoring "hard to diagnose" toxicities that currently lack mechanistic understanding or a clear regulatory science path.

PI: Guo, Lei, Ph.D.

Differential Gene Expression in Rodent and Human Primary Hepatocytes Exposed to the Peroxisome Proliferators-Activated Receptor (PPAR) Alpha Agonists (E0721301)

Responsible Division: Biochemical Toxicology
Collaborating Division(s): Systems Biology
Objective(s):

1) Obtain the global gene-expression patterns response to PPAR agonists in rodent and human hepatocytes in both transcriptional and translational levels.

2) Compare mutual versus species-specific gene-expression response to PPAR-a agonists.

3) Investigate specific genes regulated by PPAR-a agonists in susceptible species, such as rat and mouse compared to human.

4) Identify novel target genes whose expression has not been previously reported to be affected by PPAR-a agonists.

5) Determine whether the expression of candidate target genes is PPAR-a dependent.

PI: Guo, Lei, Ph.D.

Study of Drug-Induced Liver Toxicity using State-of-the-Art In Vitro Liver Models Including Primary Rat and Mouse Hepatocytes and Stem Cells (E0732101)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Genetic and Molecular Toxicology

Objective(s):

Both rats and mice will be used in these studies. In addition, rodents of different ages and both sexes will be needed. The isolation of liver cells is performed to permit the analysis of drug metabolism and to determine whether the administration of agents cause direct toxicity to the cells or result in their death. The long-term goals of this endeavor will be to:

1) Obtain signature-gene and protein-expression patterns of each cell type for comparison to toxin-induced changes.

2) Determine the contribution of each cell type to overall liver toxicity from agent exposure once these isolated cell types are available reliably. To achieve these long-term goals, training must be provided to give confidence in the integrity of liver cells following perfusion, separation, and culture of the liver cells.

PI: Hart, Mark, Ph.D.

Application of Co-Culture and Simulated-Vaginal Models to Elucidate the Inhibitory Properties of Naturally Occurring and Bioengineered Strains of *Lactobacillus* Toward Toxic-Shock Syndrome Toxin-1 Producing Strains of *Staphylococcus* aureus (E0728601)

External Funding: Office of Women's Health (OWH)

Responsible Division: Microbiology

Collaborating FDA Center(s): CFSAN

Objective(s):

1) Determine the inhibitory effects of a select group of *Lactobacilli* with probiotic potential on a wide variety of *S. aureus* TSST-1-producing strains isolated from patients with TSS using previously developed co-culture system and the vaginal tract (toroid) model with recently developed genital tract secretion medium.

2) Generate transcriptional and proteomic profiles of *Lactobacillus* sp., and *S. aureus* strains using previously developed co-culture system and identify gene systems and proteins critical for inhibition of *S. aureus* growth and/or TSST-1 production.

3) Isolate and clone the lysostaphin gene, an endopeptidase that specifically cleaves the cell wall cross-

linking pentaglycine bridges of *S. aureus*, into a select group of *Lactobacilli* and determine expression levels of lysostaphin, as well as inhibitory capacity of engineered *Lactobacilli* against *S. aureus* in the co-culture system and the vaginal tract (toroid) model.

PI: Hart, Mark, Ph.D.

Co-Display of Hemagglutinin and CD154 on the Surface of Yeast Cells as a Vaccine Against Avian Influenza (E0733301)

Responsible Division: Microbiology
Collaborating Division(s): Veterinary Services
Objective(s):

1) Generate HA surface presented yeast recombinant avian-influenza vaccines.

2) Characterize humoral and cellular-mediated immune responses of yeast vaccines in mice.

3) Demonstrate protection of mice from lethal avian-influenza virus through yeast-based immunization.

PI: Hong, Huixiao, Ph.D.

Baseline Practices for Analyzing Genome-Wide Association Study (GWAS) Data (E0729701)

Responsible Division: Systems Biology
Collaborating Division(s): Personalized Nutrition and Medicine
Objective(s):

A comparative study of the latest methods for analyzing GWAS data will be conducted with a focus on developing baseline practices. Publicly available data sets will be used, as well as data sets received through collaborations between FDA and drug sponsors.

PI: Inselman, Amy, Ph.D.

In Vitro Differentiation of Embryonic Stem (ES) Cells and Induced Pluripotent Stem (IPS) Cells (P00727)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Division(s): Biochemical Toxicology
Objective(s):

1) Maintain and passage several mouse ES and human IPS cell lines in a pluripotent, undifferentiated state in the absence of feeder cells and serum.

2) Recapitulate early embryonic development by terminal differentiation of mouse ES and human IPS cells into a variety of cell types. Primary focus will be on the differentiation into osteoblasts.

3) Monitor differentiation process by examining gene expression in undifferentiated ES cells and in cells that have undergone differentiation.

PI: Kaput, James, Ph.D.

Delta Vitamin Obesity Prevention Summer Camp (E0733001)

Responsible Division: Personalized Nutrition and Medicine
Objective(s):

1) Analyze levels of 13 vitamins in 100 children in grades 4-6 to confirm food-frequency questionnaire data showing low intakes of certain nutrients and vitamins.

2) Provide fresh fruits, vegetables, and fortified snacks to supplement low-vitamin intake for a one-month

period to improve serum concentration levels of vitamins.

3) Analyze ancestry through whole-genome scans and candidate genes responsive to vitamin intake to associate individual responses with genetic polymorphisms.

4) Improve the nutrition and genetic education of the participants through lessons taught by local teachers with materials provided by NCTR, USDA, and local University of Arkansas for Medical Sciences/Area Health Education Centers diabetes educator.

5) Develop health-economic analyses of the intervention.

6) Begin developing a sustainable program for improving the foods of the children in the Marvell School District by analyzing economic impact of vitamin intervention.

PI: Leakey, Julian, Ph.D.

Subchronic Toxicity Studies of Chondroitin Sulfate and Glucosamine in Fischer 344 Rats and Diabetic Goto-Kakizaki Rats (E0215701)

External Funding: National Toxicology Program (IAG)

Responsible Division: Associate Director of Scientific Coordination

Objective(s):

1) Investigate the potential toxicity of chondroitin sulfate and glucosamine, administered by oral gavage in male rats.

2) Determine whether subchronic exposure of glucosamine or chondroitin sulfate potentiate the pathological effects of noninsulin-dependent diabetes in obese diabetic rats.

PI: Lyn-Cook, Beverly, Ph.D.

Sex Differences in Chemotherapeutic Toxicity: Profiling of Transporter Genes in Human Liver (E0725401)

Responsible Division: Associate Director for Regulatory Activities

Collaborating Division(s): Personalized Nutrition and Medicine, Biochemical Toxicology

Objective(s):

1) Identify sex differences in the gene expression of drug transporters known to be involved in the transport of chemotherapeutic drugs and in hepatic expression in human-liver tissues. This is a prerequisite to elucidating the mechanisms of interindividual variability in hepatic drug-transport systems.

2) Evaluate sex-related hepatic drug-transport functions, including both of the basolateral-transport systems that are responsible for translocating drugs across the sinusoidal membrane and the active canalicular transport systems that are responsible for the biliary excretion of drugs using sandwich-cultured human hepatocytes. Characterize the relationships between transporter gene expression and uptake or excretion of chemotherapeutic drugs defined with the sandwich model and transporter-transfected cell lines.

3) Evaluate the effects of sex hormones on hepatic-transporter gene expression in human cancer-cell lines and sandwich-cultured hepatocytes.

4) Identify and validate novel transporter-drug correlations using a chemogenomic approach followed by cytotoxicity and drug-uptake studies in cell lines over-expressing specific transporter genes.

5) Develop an *in silico* pharmacokinetic-modeling program based on the data from sandwich-cultured hepatocytes to predict potential *in vivo* drug pharmacokinetics and toxicity in men and women.

6) Develop guidelines and recommendations for clinical-trial design and analysis of sex differences in new drug applications.

PI: Lyn-Cook, Beverly, Ph.D.

Sex Differences in Systemic Lupus Erythematosus (SLE): Effects of a Single Nucleotide Polymorphism (SNP) in the Prolactin (PRL) Gene on Individual Response to Prasterone Therapy (E0727401)

Responsible Division: Associate Director for Regulatory Activities

Collaborating Division(s): Personalized Nutrition and Medicine, Regulatory Compliance & Risk Management, Biochemical Toxicology, Veterinary Services

Objective(s):

Elucidate whether the PRL-1149G SNP increases SLE susceptibility by modulating signal-transduction pathways in a manner reversible by prasterone.

PI: Lyn-Cook, Beverly, Ph.D.

Genotyping of Transporter Genes Associated with Gender Differences and Promoter Methylation of UGT1A1 in Human Liver: A Means of Assessing Safety and Toxicity of Chemotherapeutic Drugs (E0729801)

Responsible Division: Associate Director for Regulatory Activities

Collaborating Division(s): Office of Research/Immediate Office, Biochemical Toxicology, Personalized Nutrition and Medicine

Collaborating FDA Center(s): CDER

Objective(s):

1) Identify polymorphisms in drug-transporter genes identified to be differentially expressed according to gender in human-liver samples. This will further elucidate the mechanisms of inter-individual variability in hepatic-drug transporters.

2) Correlate polymorphism frequencies in male and female to gene expression. This will determine if differences in expression of transporter genes may be affected by genetic variation.

3) Evaluate the methylation profile of UGT1A1 promoter in human-liver samples from male and female and correlate it to expression of UGT and its activity. This will determine the role of methylation in silencing of UGT1A1.

4) Evaluate effects of polymorphisms in transporter genes on uptake and clearance of chemotherapeutic drugs in a functional assay using the B-CLEAR human *in vitro* model.

PI: Mei, Nan, Ph.D.

Development of a New T-cell Receptor (TCR) Gene Rat Model for Safety Screening of Pharmaceuticals and Other Chemicals for Potential Mutagenicity (E0719601)

Responsible Division: Genetic and Molecular Toxicology

Objective(s):

1) Develop an *in vivo* model using the TCR genes of the Fisher 344 rat for the rapid, cost-effective, and predictable identification of pharmaceuticals and other chemicals that can induce mutations.

2) Model mutagens, N-ethylnitrosourea (ENU) and cyclosphosphamide (CP) to investigate the potential utility of the TCR gene-mutation assay using isolated spleen lymphocytes derived from treated Fisher 344 rats.

3) Compare the mutant frequencies in the TCR genes and the Hprt gene in spleen lymphocytes of rats after mutagen exposure to validate the TCR assay.

PI: Moore, Martha, Ph.D.

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)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Systems Biology

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.

3) Determine the fundamental genetic mechanism(s) causing the small and large colony thymidine-kinase mutant phenotypes.

PI: Morris, Suzanne, Ph.D.

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

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Systems Biology, Personalized Nutrition and Medicine

Objective(s):

1) Determine the effect of mutation in the p53 tumor suppressor gene on gene-expression profiles in young and aged mice.

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

PI: Ning, Baitang, Ph.D.

Mechanisms of Gender Differences in Aspirin Effects: Metabolizing Enzymes and Therapeutic Targets (E0727101)

Responsible Division: Personalized Nutrition and Medicine

Objective(s):

1) Profile gender differences in the mRNA expression and protein production of drug-metabolizing enzymes known to be involved in aspirin metabolisms, using human liver samples from 50 males and 50 females.

2) Characterize molecular mechanisms of sex hormones (estrogens, progestogens, and androgens) in regulation of the expression of aspirin-metabolizing genes in human ER-positive hepatic-cell line HepG2-ER(+) using biochemical procedures, including DNA-protein binding assay and reporter-construct assay.

3) Measure sex-hormone modulation of aspirin effect on platelet aggregation and its related biomarkers (COX-1, COX-2, PGE2, TXA2, and LTB4) using human-platelet precursor cells.

4) Identify sex-hormone modulation of aspirin actions in human endothelial and epithelial cell lines, by measuring prostacyclin dynamics (PGE2, TXA2 and LTB4) and aspirin-targeting enzymes (COXs, NOSs, and LOX) expression.

5) Evaluate sex-hormone modulation of response to aspirin in apoliprotein E-deficient mice.

PI: Ning, Baitang, Ph.D.

Micronutrient Involvement in Differentiation of Multipotent Mesenchymal Stem Cells into Adipocytes through MicroRNA Regulation (P00720)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Systems Biology ; Biochemical Toxicology

Objective(s):

1) Identify mircroRNA biomarkers of adipogenesis process.

2) Investigate the effect of micronutrients on microRNA expression during the differentiation process of mesenchymal stem cells into adipocytes.

PI: Ning, Baitang, Ph.D.

Genetic Variants in Cardiovascular Disease Risks and Drug Responses: Exome Sequencing in the Amish Phenotypic Extremes (P00738)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Systems Biology

Collaborating FDA Center(s): CDER

Objective(s):

1) Identify genetic variants that are associated with cardiovascular diseases through the massively parallel-sequencing approach using a NextGen sequencer.

2) As a part of the SEQC project, to evaluate the technical performance and quality of NextGen instrument and results when applied to genomic DNA sequencing.

PI: Parsons, Barbara, Ph.D.

Analysis of p53 Codon 270 CGT to TGT Mutation in Simulated Solar Light-Induced Skin Tumors and Exposed Mouse Skin (E0715201)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Associate Director of Scientific Coordination

Objective(s):

1) Develop the ACB-PCR detection of mouse p53 codon 270 CGT->TGT mutation.

2) Measure the frequency of detection and levels of this mutation in mouse-skin tumors.

3) Measure the frequency of this mutation in skin tissue from tumor-bearing animals.

4) Measure the frequency of this mutation in skin exposed to decreasing levels of SSL.

PI: Parsons, Barbara, Ph.D.

Cancer Mutations as Biomarkers of Cancer Risk: Human Studies with Implications for Personalized Medicine (E0726501)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Personalized Nutrition and Medicine

Objective(s):

1) Develop the information necessary for the rational use of oncogene mutations as quantitative biomarkers of cancer risk; specifically allele-specific competitive blocker PCR (ACP-PCR) will be used to determine normal and pathological levels of relevant oncogene mutations in multiple human tissues and tumors.

2) Compare the information derived from human tissues with data generated in a parallel rodent protocol as an approach for incorporating carcinogenesis-relevant data into the rodent to human extrapolation necessary in cancer- risk assessment.

3) Validate a streamlined ACP-PCR methodology and develop the methodology necessary to measure oncogene MF in cell-free DNA isolated from plasma.

4) Through a series of publications, convey to the regulatory risk-assessment community the regulatory significance of the data regarding tumor-associated mutations, which have and will be generated.

PI: Paule, Merle, Ph.D.

Effects of Prenatal Cocaine on Behavioral Plasticity (E0663307)

Responsible Division: Neurotoxicology

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.

PI: Paule, Merle, Ph.D.

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

Responsible Division: Neurotoxicology

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.

PI: Pence, Lisa, Ph.D.

Development and Optimization of Quantitative LC/MS Metabolic Profiles for Amino Acids, Vitamins, and Other Important Metabolites (P00725)

Responsible Division: Systems Biology

Objective(s):

Metabolomics has the potential to provide noninvasive translational biomarkers. Most mass spectrometry (MS)-based metabolomics are based on semiquantitative analysis of MS data, which makes it difficult to translate directly into the clinical setting. We will develop and optimize quantitative LC/MS metabolic profiles for markers that are often mentioned in Personalized Nutrition and Medicine and have been identified as biomarkers in drug-safety studies such as amino acids, water soluble and fat soluble vitamins, bile acids (biomarkers of liver function), and other metabolites, like gluthathione and SAMe in biofluids. Once the method(s) have been tested and optimal procedure determined, an SOP will be written for that method. Sensitivity and reproducibility for all metabolites will be determined for each SOP. These methods will be used in quantitative-targeted metabolomics analyses in future protocols including the NCTR Healthy Challenge study, Delta Vitamin, biomarkers of hepatotoxicity, and other protocols, including melamine projects.

PI: Pogribny, Igor, Ph.D.

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

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Genetic and Molecular Toxicology, Systems Biology, Office of the Director

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 models.

2) Identify genes that are consistently up-regulated or down-regulated in target tissue during the promotion stage of carcinogenesis.

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.

PI: Pogribny, Igor, Ph.D.

Relationship between Liver Epigenetic Phenotype and Susceptibility to Nonalcoholic Steatohepatitis-Induced Hepatocarcinogenesis (NASH) in Mice (E0735301)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Personalized

Nutrition and Medicine, Associate Director for Regulatory Activities, Systems Biology

Objective(s):

1) Determine the role of epigenetic dysregulation in the etiology and pathogenesis of dietary nonalcoholic steatohepatitis (NASH)-induced hepatocarcinogenesis in mice.

2) Determine whether or not interstrain-specific susceptibility of mice to NASH-induced hepatocarcinogenesis is associated with differences in individual hepatic-epigenetic phenotypes.

3) Determine the role of epigenetic dysregulation in the etiology and pathogenesis of NASH-induced hepatocarcinogenesis in mice induced by tamoxifen administration.

4) Determine whether or not aberrant epigenetic markers can be used as targets for prevention of NASH-induced hepatocarcinogenesis in mice.

PI: Salminen, William, Ph.D.

Assessing Acetaminophen-Induced Liver Injury and the Influence of Dietary Supplements—Potential Synergistic Interactions (E0737901)

Responsible Division: Systems Biology

Collaborating Division(s): Personalized Nutrition and Medicine

Objective(s):

Most drug-dietary supplement interactions are unknown or, at best, poorly characterized. Women are at increased risk for any interactions, since they consume a greater quantity of dietary supplements compared to men. In addition, dietary supplement consumption continues to rise in the U.S., increasing the potential for any interactions. The situation is particularly important for APAP (acetaminophen)—women consume a larger quantity of APAP compared to men leading to a magnified exposure of women to APAP and dietary supplements. Identification of APAP-dietary supplement interactions will allow warnings to be provided to women and better assessment of the safety of the current therapeutic uses of APAP and the protection afforded by the U.S. dietary supplement regulations. Based on previously reported interactions between some drugs and dietary supplements, it is expected that the majority of tested dietary supplements will influence the toxicity of APAP. By testing each dietary supplement in females and males, differential sex-sensitivity to APAP in combination with the dietary supplements will be clearly identified. Subsequent analysis of the biological pathways that are affected by the dietary supplements and APAP, individually and when combined, will provide insight into the mechanism(s) of the interaction and why females are more sensitive to APAP DILI .

PI: Salminen, William, Ph.D.

Biomarkers of Liver Toxicity (E0732201)

Responsible Division: Systems Biology

Collaborating Division(s): Personalized Nutrition and Medicine

Objective(s):

1) Discover biomarkers of hepatotoxicity in preclinical studies that are more predictive of adverse

effects in humans. These biomarkers may or may not be directly applicable to the clinic, but they must be predictive of human responses so that they can be used to extrapolate preclinical data to humans in safety assessments. These results will be published and publicly available in order to fulfill FDA's goal of developing science-based best-practice standards, guidance, and tools to improve the regulatory process.

2) Farther-reaching goals include the qualification of such biomarkers (e.g., via the FDA/EMEA qualification process) and potential translation for clinical use.

PI: Schmued, Laurence, Ph.D.

Histochemical Test Battery for Evaluating the Efficacy and Toxicity of Putative Alzheimer's Disease Therapeutics of FDA Relevance (E0727301)

Responsible Division: Neurotoxicology

Objective(s):

The primary objective of this protocol is to test the hypothesis that Alzheimer's Disease (AD), which is characterized by the deposition of insoluble-amyloid plaques in the brain, is the result of a cascade of pathological processes, and that pharmacological intervention at various points within this sequence of events could attenuate the resulting pathology. To do this, the protocol will look at 13 different potential AD therapeutics, each with known but different mechanisms of action. If our hypothesis is correct, we would expect the attenuation of amyloid plaques to be observed following treatment with a variety of therapeutic agents, regardless of their specific mode of action. If, however, only one mechanistic class of compounds proves to be effective in reducing plaque deposition, then one would conclude that one identifiable event is primarily responsible for the generation of amyloid plaques. In addition to this primary goal, a secondary goal of the study will be to obtain efficacy and toxicity data on 12 of the more promising AD therapeutic agents, including many presently undergoing phase 2 or 3 FDA clinical trials.

PI: Schmued, Laurence, Ph.D.

Development of Novel Histochemical Markers of Brain-Vascular Elements and Their Application for Localizing Neurotoxicant-Induced Pathologies (E0731201)

Responsible Division: Neurotoxicology

Objective(s):

Numerous studies have developed histochemical tracers for the detection of brain cell-specific pathologies, such as Fluoro-Jade dyes, which has been used exclusively to stain degenerating neurons and Black-Gold, which detects myelinopathies; however, few specific markers are available to detect brain-vascular elements. Therefore, the goals of this project are:

1) Develop and characterize novel markers for brain-vascular elements and investigate the effects of three different classes of neurotoxicants viz., kainic acid, an NMDA agonist, 3-nitropropionic acid (3-NPA) , an

inhibitor of metabolic respiration and methamphetamine, a dopamine agonist on each of the above-mentioned vascular elements, such as perivascular pericytes, vascular lumen, and perivascular sheath.

2) Characterize the response of certain vascular elements to neurotoxic insults.

3) Provide fluorescent and bright field labeling at the vascular lumen.

PI: Shi, Leming, Ph.D.

SEQC (MACQ-III)—The Sequencing Quality Control Project (E0731901)

Responsible Division: Systems Biology

Collaborating Division(s): Genetic and Molecular Toxicology, Biochemical Toxicology, Veterinary Services, Associate Director of Scientific Coordination, Personalized Nutrition and Medicine, Office of Director

Collaborating FDA Center(s): CDER, CFSAN, CBER, CDRH

Objective(s):

Next-generation sequencing is expected to revolutionize transcriptome studies because of its claimed higher sensitivity and specificity, the capability of simultaneously detecting individual exons and alternative splicing, and the possibilities of genome-wide quantification (through single-molecule sequencing). In this collaborative SEQC (Sequencing Quality Control) project is a natural extension of the FDA-led MicroArray Quality Control (MAQC) project. The technical performance of different next-generation sequencing technologies and various bioinformatics strategies for handling and analyzing the massive sequence datasets will be objectively assessed by using the reference RNA samples previously established by the MAQC project. In addition, next-generation sequencing technologies will be used to profile RNA samples isolated from cells with or without treatment by nanoparticles and toxicants of known mechanisms-of-toxicity to further evaluate their performance in assessing the safety and toxicity of FDA-regulated products. The SEQC project may be extended from RNA-Seq to genomic DNA analysis. It is anticipated that next-generation sequencing will play an important role in Personalized Nutrition and Medicine and in ensuring the safety of FDA-regulated products. The proposed SEQC project will help prepare FDA for the next wave of submission of genomic data generated from the next-generation sequencing technologies.

PI: Shi, Leming, Ph.D.

Phase II of the MicroArray Quality Control Project (MAQC-II) Toward Personalized Medicine (S00705)

Responsible Division: Systems Biology

Collaborating Division(s): Personalized Nutrition and Medicine, Biochemical Toxicology

Objective(s):

1) Assess the reliability of microarray-based predictive models (classifiers) for clinical (diagnosis, prognosis, and treatment outcome) and preclinical (toxicogenomics) applications.

2) Issue consensus recommendations to the microarray community—a

critical component of personalized medicine. The MAQC effort will facilitate the appropriate application of microarray data in the discovery, development, and review of FDA-regulated products.

3) Draft guidance document on the development and validation of predictive models based on microarrays.

PI: Sonko, Bakary, Ph.D.

Evaluation of Glycolysis and TCA Fluxes in MPTP-Treated C57BL Mouse Model of Parkinson's Disease (E073260)

Responsible Division: Systems Biology
Collaborating Division(s):
Neurotoxicology
Objective(s):

1) Use the data to estimate the contributions of glycolysis and TCA-cycle pathways to energy metabolism in the model.

2) Identify potential energy metabolic biomarkers of PD in this setting.

PI: Starlard-Davenport, Athena, Ph.D.

Inactivation of UDP-Glucuronosyltransferases in Human Breast Tissues: Accessing Cancer Risk, Tamoxifen Safety and Toxicity (E0734001)

External Funding: Office of Women's Health (OWH)

Responsible Division: Associate Director for Regulatory Activities
Collaborating Division(s): Biochemical Toxicology
Collaborating FDA Center(s): CDER
Objective(s):

1) Characterize UGT mRNA expression in normal and malignant human-breast tissues isolated from the same donor and from different donors. This objective will be used to screen for inter-individual differences in UGT expression and to determine the association of UGT expression and breast-cancer risk.

2) Identify polymorphisms in those UGT genes that show significant inter-individual differences in UGT mRNA expression in all breast tissues. This objective will be used to determine if differences in UGT gene expression are affected by UGT genetic variations.

3) Determine the methylation profile of those UGTs identified in Objective 2 above and correlate it to UGT expression.

4) Determine the effects of polymorphisms in UGT genes on glucuronidation of E2, 4-OH-E1, and 4-hydroxy-Tamoxifen (4-OH-TAM) using glucuronidation-activity assay and MTT-cytotoxicity assays.

PI: Sun, Jinchun, Ph.D.

Preclinical Metabolomic investigation of Drug Pharmacokinetics in Multiple Drug Toxicity Studies (E0732401)

Responsible Division: Systems Biology
Objective(s):

1) Apply metabolomic methods to investigate a drug-metabolite profile in urine samples from preclinical studies using LC/MS and NMR with the combination of principal component analysis (PCA) and heterocorrelation analyses of NMR and MS data.

2) Determine the excretion kinetics of the drug-N-acetyl-cysteine conjugates

and S-adenosylmethionine, which is the primary source of the sulfur atom in the biosynthesis of glutathione using LC/MS/MS technique.

3) Investigate mercapturic acids profile using a highly sensitive and selective constant neural-loss technique developed on a triple quadrupole mass spectrometer.

PI: Teitel, Candee

Epigenetics, DNA Methylation, and Obesity (E0733101)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Biochemical Toxicology

Objective(s):

We propose to evaluate the effect of differences in DNA methylation and agouti-signaling protein (ASIP) in the offspring of Avy/a dams x a/a sires as a result of nutrient x gene interactions. These preliminary data will be used to select the appropriate diets for further studies on obesity and type 2 diabetes.

PI: Tong, Weida, Ph.D.

Development of Liver Toxicity Knowledge Base (LTKB) to Empower the FDA Review Process (E0721501)

External Funding: Critical Path Funding

Responsible Division: Systems Biology

Collaborating Division(s): Biochemical Toxicology

Objective(s):

1) Liver Ontology (LO)—Develop an LO that characterizes liver pathology and toxicity. LO will be used as guidance for data collection/curation, classification, and analysis described in the following specific aims.

2) Gene Expression Data—The primary data collected for this project will be existing gene-expression data. Other types of data, such as data from proteomics, metabonomics, and genotyping studies (including GWAS) will be considered as the project progresses.

3) Text Mining—We will conduct text mining on >13 million abstracts in PubMed and other public resources with an emphasis on liver-related data. The association between the liver-specific entities (i.e., genes/proteins, pathways, drugs, tissues, and toxicity) will be established.

4) Known Data—There is substantial knowledge available in public domains on liver toxicity , including genes/proteins (e.g., signatures and biomarkers), pathways/networks, and chemicals/drugs.

5) Experiment—We will conduct gene-expression studies on well-understood and characterized hepatic and nonhepatic compounds. The dataset will be used to validate the LTKB. Data from 50 compounds will be generated in the first year of this project and will serve as a proof of principle. Data for 150 additional compounds will be collected in the following two years, assuming the first set of data supports the LTKB approach.

6) LTKB—The data/information generated from Objectives 1-5 will be analyzed (combined and correlated) to establish liver toxicity- related regulatory networks and

genes/proteins-pathways-chemicals-disease associations. A set of training rules will be identified to minimize the false positives in LTKB.

PI: Tong, Weida, Ph.D.

Development of an FDA Resource and Knowledge Base for Sex Difference in Drug-Induced Liver Injury (DILI) (E0733801)

Responsible Division: Systems Biology
Collaborating Division(s): Biochemical Toxicology
Objective(s):

The project is to develop a knowledge base for the sex differences in drug-induced liver injury (DILI) through analyzing and modeling the molecular data in public domain. Specifically:

1) Further augment the collection of the genomic data from public resources and through collaborations.

2) Develop a standard data curation model for the sex-biased DILI in ArrayTrack™ to manage the collected data. The data curation model will be developed in accordance with the data standard developed in FDA for electronic-data submission.

3) We will conduct the meta-analysis, text mining, and network analysis to develop a relationship between drugs, molecular signatures, liver-specific biomarkers, genes/proteins functions, pathways, and sex-biased liver toxicity. Sex differences in DILI have been well described. Females develop liver injury more rapidly and have a lower-exposure threshold than men when exposed to many drugs. However, the mechanism for this difference is poorly characterized. The completion of the project will gain understanding, at molecular level, of sex-biased differences in DILI. We anticipate that the knowledge and resources derived from this project are useful for FDA to utilize and reference when sex-related DILI issues arise during the various stages of the regulatory-review process, thus impacting women's health.

PI: Tong, Weida, Ph.D.

Development and Refinement of the FDA Genomic Tool, ArrayTrack™ for Advancing Pharmacogenomics and Personalized Medicine in the Context of the FDA's Critical Path Initiative (S00671)

External Funding: Critical Path Funding

Responsible Division: Systems Biology
Objective(s):

Initial Objective: Data to be received from CDER drug-review offices and ArrayTrack™ used to analyze data and send results back to CDER collaborators.

Subsequent Objective: The agency has witnessed a shift of PGx technologies in the VGDS submission over the past three years. What began as predominately DNA microarray data has expanded to proteomics, metabolomics and, most recently, Genome Wide Association Study data. Recently, the name of VGDS was changed to Voluntary eXploratory Data Submission (VXDS) to reflect the diverse data being reviewed in this program. ArrayTrack™ as a key bioinformatics tool used in the VGDS/VXDS program that currently contains functionality only to address the need for reviewing DNA

microarray data. Thus, the specific goals of this proposal are to:

1) Develop the functionality in ArrayTrack™ to facilitate the review of nonmicroarray PGx data as relevant to the Critical Path Initiative. Specifically, the project will develop new modules in ArrayTrack™ to review proteomics and metabolomics data and data from genome-wide association studies.

2) Develop modules to allow electronic data submission in the VGDS/VXDS program.

a) Modules in ArrayTrack™ to store and analyze data from Genome-Wide Association Study.

b) Modules in ArrayTrack™ to store and analyze data from metabolomics.

c) Modules in ArrayTrack™ to store and analyze data from proteomics.

PI: Tong, Weida, Ph.D.

Interagency Collaboration on Identification of *In Vitro* and Omics Biomarkers for Liver Toxicity (E0734401)

Responsible Division: Systems Biology

Collaborating Division(s): Office of Director

Objective(s):

Hepatotoxicity is usually investigated using a set of standardized animal-based studies which, unfortunately, fail to detect all compounds that induce human adverse events and do not provide detailed mechanistic information of observed toxicity. An alternative approach, which is being widely evaluated, particularly in various government agencies, is to prioritize and/or supplement animal testing with a battery of mechanistically informative *in vitro* and omics assays. These can be used to develop novel biomarker identification models and methods to improve safety and risk assessment. Thus, the proposed study aims to identify novel biomarker- identification methods tailored to specific mechanisms of liver toxicity based on the experiment platforms adopted by the agencies participating in this project. A recursive process will be carried out in two phases of the project to enhance the validity of the biomarker models:

Phase I—The common sets of compounds from the FDA LTKB, the EPA ToxCast, the NCGC-NTP - EPA Tox21, and the CDC HazDat and HSEES databases will be identified as a test case to assess the current gaps of the existing data and further assay needs for development and validation/verification of biomarker identification models and methods.

Phase II—A new set of compounds (most are drugs) will be further identified for study in LTKB, ToxCast and Tox21 to improve the robustness and reliability of models for regulatory application. The completion of the project will result in a wealth of mechanistic

and molecular information, as well as associated biomarker models that are useful for FDA to utilize and reference when liver toxicity issues arise during the various stages of the regulatory review process. The project will also serve as a proof-of-concept approach for biomarker identification related to other drug-safety issues (e.g., cardiac disease) essential to the FDA regulatory mission.

PI: Tong, Weida, Ph.D.

JANUS (BIB Project) (S00699)

Responsible Division: Systems Biology

Objective(s):

Janus will integrate submitted review data from pre-clinical, clinical, and omics domains with external scientific data. NCTR's ArrayTrack™ software will be integrated with Janus to provide omics-data capability. Janus will enable electronic-data submission and review.

PI: Varma, Vijayalakshmi, Ph.D.

An Omics Approach To Investigate the Metabolic and Endocrine Effects of Fructose on Adipocytes Compared to Glucose (E0740401)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Systems Biology

Collaborating FDA Center(s): CFSAN

Objective(s):

Identify cellular mechanisms involved in fructose-induced metabolic and endocrine regulation of human adipocytes in culture using omic technologies.

PI: Wagner, Robert, Ph.D.

Gene-Expression Responses of Estrogen-Primed Vaginal-Epithelial Cells After Contact with *Lactobacillus* rhamnosus GR-1, *Lactobacillus reuteri* RC-14, and the Pathogenic Fungus, *Candida albicans* (E0729401)

Responsible Division: Microbiology

Objective(s):

1) Ascertain how VEC respond at the molecular level to contact with *C. albican* to give insight into what intracellular-molecular systems for signal transduction and intercellular signaling are activated; thereby, detecting any effect probiotic *Lactobacilli* have on these processes.

2) Establish whether probiotic *Lactobacilli* have an effect on VEC resistance to *C. albicans*, and how that effect is mediated.

3) Establish whether estrogen has an influence on these processes or not, as this is still a controversial issue.

PI: Wagner, Robert, Ph.D.

Maintenance of Germfree BALB/c Mice in Isolators for Use in Future Protocols (E0736701)

Responsible Division: Microbiology

Objective(s):

Maintain a small colony of germ-free BALB/c mice in gnotobiotic isolators between approved research protocols.

PI: Wagner, Robert, Ph.D.

Effects of Phytoestrogens on Gene Expression Responses of Vaginal Epithelial Cells After Contact With *Candida albicans* (E0740601)

External Funding: Office of Women's Health (OWH)

Responsible Division: Microbiology
Collaborating FDA Center(s): CFSAN
Objective(s):

The proposed study would examine whether phytoestrogens affect the ability of vaginal-epithelial cells to mobilize host defenses against *C. albicans* through changes in receptor-mediated signal transduction. This study will increase our limited understanding of whether phytoestrogens found in dietary supplements have a negative or positive effect on host responses to *C. albicans* at the point of contact with vaginal-epithelial cells. Since estrogens also stimulate pathogenicity in *C. albicans*, it will also be beneficial to know whether these components of dietary supplements also stimulate fungal pathogenicity. Estrogen receptor signal-transduction pathways are complicated and involve interactions with several transcriptional regulatory pathways that are also involved in the activation of apoptosis and in extracellular signaling to immune cells.

PI: Wiley, Kenneth, Ph.D.

The Role of Sex in Expression of DNA Cytosine 5-Metyltransferases, Histone Deacetylases, Acetylases, Metyltransferases, and Demethylases Among Patients with Systemic Lupus Erythematosus (SLE) : Elucidating Potential New Drug Targets (E0738601)

Responsible Division: Associate Director for Regulatory Activities
Objective(s):

Elucidate whether there is a sex and/or ethnic bias in expression levels of epigenetic markers in SLE patients.

PI: Wilkes, Jon, Ph.D.

Quantum Mechanical and NMR Spectral Approaches for the Rapid Prediction of Estrogen Activity of FDA-Regulated Chemicals (E0740701)

External Funding: Office of Women's Health (OWH)

Responsible Division: Systems Biology
Collaborating FDA Center(s): CFSAN
Objective(s):

The availability of artificial-neural networks trained on objective data and applicable for evaluation of a broad-structural range should provide a tool of use to new drug reviewers or for retrospective evaluation of grandfathered drugs with respect to estrogenic activity. The models could be added to NCTR's Endocrine Disrupor Knowledge Base (EDKB) or provided directly to CDER and other FDA Centers.

PI: Word, Beverly, Ph.D.

DNA Methylation is Modulated by Lifestyle Factors and Environmental Agents (P00713)

Responsible Division: Associate Director for Regulatory Activities

Objective(s):

1) Determine the effect of cigarette smoke condensate on DNA methylation of several genes in lung cells.

2) Assess the ability of other agents to modulate the effect of CSC (class-specific correlations) on gene DNA methylation, either singularly or in various combinations.

Strengthen Surveillance and Risk Analysis

PI: Ahn, Young, Ph.D.

Impact of Antimicrobial Residues on the Human Gastrointestinal Tract Microbiota (E0732701)

Responsible Division: Microbiology

Collaborating FDA Center(s): CVM

Objective(s):

1) Development of methodology to determine if antimicrobial-agent residues bound to fecal contents are microbiologically active.

2) Evaluate the use of current molecular biology, genomic, and proteomic technologies to determine the impact of antimicrobial-agent residues on the human-intestinal microbiota.

3) Determine the potential of the intestinal microbiota to metabolize antimicrobial residues.

PI: Beland, Frederick, Ph.D.

Carcinogenicity of Acrylamide and its Metabolite, Glycidamide, in Rodents: Neonatal Mouse Bioassay (E0718501)

Responsible Division: Biochemical Toxicology

Objective(s):

Compare the carcinogenicity of acrylamide and its metabolite glycidamide in B6C3F1 mice treated neonatally.

PI: Beland, Frederick, Ph.D.

Effect of Urinary pH upon the Nephrotoxicity of a Combined Exposure to Melamine and Cyanuric Acid (E0731501)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

Determine the effect of urinary pH on the renal toxicities elicited by a combined exposure of melamine and cyanuric acid.

PI: Beland, Frederick, Ph.D.

Genotoxicity and Carcinogenicity of Acrylamide and its Metabolite, Glycidamide, in Rodents—Rangefinding/Subchronic/Two-Year Chronic Carcinogenicity Studies (E0215001)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Genetic and Molecular Toxicology

Objective(s):

Compare the carcinogenicity of acrylamide and its metabolite glycidamide in B6C3F1 mice and Fischer 344 rats treated chronically for two years.

PI: Beland, Frederick, Ph.D.

Two-Year Carcinogenicity Bioassay of Furan in F344 Rats (E0216801)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Objective(s):

Determine the dose-response relationship for the carcinogenicity of furan in F344 rats.

PI: Binienda, Zbigniew, Ph.D.

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

Responsible Division: Neurotoxicology

Collaborating Division(s): Regulatory Compliance & Risk Management, Office of Director

Collaborating FDA Center(s): CFSAN

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 deficits associated with L-carnitine using enzymatic and neurochemical biomarkers of neurotoxicity in the rat model of 3-NPA-induced histotoxic hypoxia.

4) Establish the relationship between 3-NPA-induced physiological and neurochemical phenotypes and transcriptome profiles in the rat brain model.

5) Investigate the underlying control mechanisms of dopaminergic activation in mitochondrial dysfunction using 3-NPA and methamphetamine.

PI: Boudreau, Mary, Ph.D.

Bioassays in the Fischer 344 Rat and the B6C3F1 Mouse Administered *Aloe Vera* Plant Constituents in the Drinking Water (E0214201)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

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.

PI: Bowyer, John, Ph.D.

Implementation and Development of *In Vivo* Confocal Microscopy and Real-Time Body Temperature and Blood-Pressure Monitoring Methods (P00739)

Responsible Division: Neurotoxicology

Collaborating FDA Center(s): CDER

Objective(s):

This protocol allows the principal investigator and co-principal investigator to become proficient enough in the use of:

1) *In vivo* confocal microscopy to visualize the changes in the vasculature within the MAV and on the surface of the cerebral cortex.

2) Implant rats with telemetry devices to remotely and continuously monitor the temperature and blood pressure. Refinement of these two methods is necessary for gathering meaningful data that will be submitted when the methods necessary are fully developed and applicability understood.

PI: Buzatu, Dan, Ph.D.

FERN Level-One Validation Study of a Mobile, Field-Rugged Rapid Detection and Enumeration System for *Salmonella* in Foods (E0731601)

Responsible Division: Systems Biology

Collaborating Division(s): Microbiology

Collaborating FDA Center(s): ORA

Objective(s):

Conduct a Food Emergency Response Network (FERN) level-one validation for LITMUS Rapid Identification of Bacterial Pathogens (RAPID-B™) screening of viable pathogens in food. This technology originated in the NCTR Division of Systems Biology. The proposed work involves collaborative validation of the LITMUS RAPID-B™ system as a mobile, field-rugged instrumental and chemical assay for detection and enumeration of viable pathogens in food. This validation is designed to qualify the system for rapid screening of bacteria-contaminated food in FDA's Office of Regulatory Affairs (ORA) laboratories and mobile field response units, especially the FDA FERN network. The first validation exercise will be conducted by ORA's Arkansas Regional Laboratory. Pending successful completion of the Level-1 validation, the study will be expanded to include the Center for Food Safety and Applied Nutrition, OARSA.

PI: Cerniglia, Carl, Ph.D.

Proteomic Approaches to Elucidate Biodegradative Pathways (E0711801)

Responsible Division: Microbiology

Objective(s):

1) Use a proteomic approach to isolate putative catabolic proteins that are over-expressed when microorganisms are grown in the presence of polycyclic aromatic hydrocarbons.

2) Develop software to analyze 2-D gels.

PI: Cerniglia, Carl, Ph.D.

Impact of Melamine on Human Intestinal Microbiota: Does the Human Intestinal Microbiota Have the Enzymatic Capacity to Metabolize Melamine to Cyanuric Acid? (P00730)

Responsible Division: Microbiology

Collaborating Division(s): Biochemical Toxicology

Collaborating FDA Center(s): CVM

Objective(s):

1) Determine if melamine impacts the population dynamics of the human intestinal microbiota.

2) Determine if the human intestinal microbiota metabolizes melamine to cyanuric acid.

PI: Chen, James, Ph.D.

Integrated Genomics Knowledge Base for Rapid Threat-Assessment of Enteric Pathogens: *Salmonella* (E0733701)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Systems Biology, Microbiology

Objective(s):

Develop an integrated phenotypic and genotypic knowledge base for detection and characterization of *Salmonella*, and potentially other foodborne pathogens.

PI: Delclos, Kenneth, Ph.D.

Evaluation of the Toxicity of Bisphenol A (BPA) in Male and Female Sprague-Dawley Rats Exposed Orally from Gestation Day 6 through Postnatal Day 90—Subchronic II (E0217601)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Associate Director of Scientific Coordination

Collaborating FDA Center(s): CFSAN

Objective(s):

The goal of this subchronic study is to characterize the dose-response for orally administered BPA in the NCTR Sprague-Dawley (CD) rat to address the question of adverse effects in rodents near levels of exposure potentially attainable in humans. A broad dose-range will be covered, but the focus will be on doses less than 5 mg/kg body weight/day. Animals will be exposed throughout development. Pups will be directly dosed through the lactation period rather than relying on exposure through the dam's milk, and environmental conditions (background phytoestrogens, background BPA) will be strictly controlled and monitored. Since the effects of BPA that have been described in the literature largely involve perturbation of estrogen signaling, the potent orally active estrogen ethinyl estradiol (EE2) will be included as a reference to demonstrate the estrogen responsiveness of the animal model under these exposure conditions. Although there will be a focus on reproductive endpoints, endpoints related to the reported effects of BPA on other organ systems, including the development of obesity and cardiovascular disease, will be evaluated. The results will also be used to set doses for a chronic toxicity study.

PI: Doerge, Daniel, Ph.D.

Determination of Carcinogenic Mechanisms for Furan in Fischer 344 Rats (E0216401)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Genetic and Molecular Toxicology

Collaborating FDA Center(s):

Objective(s):

1) Develop and validate LC-ES/MS/MS assays to quantify the major furan-derived DNAadducts in liver, the major furan-derived hemoglobin adduct(s), and the major furan-derived urinary glutathione-derived metabolite.

2) Determine dose-response relationships for liver furan-derived DNA and hemoglobin adduct formation and repair/turnover and the major furan-derived urinary glutathione-derived metabolite in male and female Fischer 344 rats following single and multiple dose exposures of rodents to furan.

3) Determine concentration of furan in irradiated NIH-31 diet using headspace-GC/MS.

4) Determine toxicokinetics of furan in male and female Fischer 344 rats following exposure by single gavage administration.

5) Combine all data from single and repeated dose toxicokinetics of furan in rat blood and liver with the corresponding levels of liver DNA adducts, hemoglobin adducts, and urinary mercapturates to construct a PBPK model for future use in determining carcinogenic risks from human exposure to furan through the diet.

6) Determine mutagenicity of furan in liver *in vivo* using male Big Blue rats.

7) Determine the dose-response relationships for furan-mediated hepatotoxicity and cell proliferation in liver of male and female Fischer 344 rats.

8) Determine effects of furan on methylation status in rat liver and kidney DNA and histones as epigenetic changes related to carcinogenic process.

PI: Doerge, Daniel, Ph.D.

The Role of Perinatal Development on Toxicokinetics of Bisphenol A (E0216701)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CDRH

Objective(s):

1) Determine Bisphenol A (BPA) pharmacokinetics at low-dose (100 ug/kg bw single dose; 100 ug/kg bw/d repeated).

2) Measure free and conjugated forms of BPA separately.

3) Use deuterium-labeled BPA to avoid issues of background contamination. Use LC/MS/MS for sensitivity and selectivity of measurement.

4) Determine complete rat-data set for blood, tissue, and excreta across stages of development (pregnant females, fetuses, neonates).

5) Determine BPA pharmacokinetics from oral and intravenous administration in pregnant, lactating, and nonpregnant female rats; neonatal rats.

6) Determine plasma and urinary-pharmacokinetic data in neonatal and adult nonhuman primates.

7) Use the new pharmacokinetic data in conjunction with literature data from experimental animals and humans to build a physiologically based pharmacokinetic model for BPA with the ultimate goal of predicting target-tissue concentrations of active BPA in humans, including fetuses and children, from food and medical device exposures.

PI: Doerge, Daniel, Ph.D.

Effect of Soy-Containing Diets on Ammonium Perchlorate-Induced Thyroid Toxicity in Sprague-Dawley Rats—II (E0742201)

Responsible Division: Biochemical Toxicology

Objective(s):

The goal of this protocol is to determine the effect of dietary whole soy and purified genistein, the principal soy isoflavone, on the dose-response characteristics for perchlorate-induced thyroid toxicity in male Sprague-Dawley rats. A critical processing error in the original project makes it necessary to repeat the study. The results from the previous study, while incomplete and therefore insufficient to inform regulatory policy, are very provocative and largely substantiate the original hypothesis that soy diets can adversely affect thyroid function in the presence of additional risk factors.

PI: Ferguson, Sherry, Ph.D.

Training for Bisphenol A Studies (P00706)

Responsible Division: Neurotoxicology

Collaborating FDA Center(s):

Objective(s):

This study will develop the appropriate skills and techniques necessary to conduct subsequent studies of developmental treatment with Bisphenol A. Key personnel to be trained include principal investigators, technicians, and animal-care personnel. Techniques to be developed include complex behavioral assessments and quantitative volumetric analysis of sexually dimorphic brain regions.

PI: Fisher, Jeffrey, Ph.D.

PBPK Models for Bisphenol A (E0742601)

Responsible Division: Biochemical Toxicology

Objective(s):

1) Create physiologically based pharmacokinetic (PBPK) models for BPA in three species of adult, neonatal, and pregnant (mother and fetus) and lactating (mother and neonate) laboratory animals (mouse, rat, and rhesus monkey). These PBPK models will be used to calculate internal measures of dose for both aglycone (i.e., active) and conjugated (i.e., inactive) forms of BPA.

2) Human PBPK models for BPA (adult, child, and pregnant mother and fetus, and lactating mom and infant) will be created using

information obtained from the monkey, mouse, and rat, and limited information from the human published in the literature. The human suite of PBPK models will be used to extrapolate the internal doses of BPA associated with toxicity in laboratory animals to humans and to extrapolate dosimetry from regions of observation to low levels of exposure to BPA for which no experimental data exist. This simulation protocol will help reduce the uncertainty in the assessment of health risks posed by BPA to human populations. The human BPA-PBPK model will also be used to help interpret biomonitoring data for BPA in urine and blood. The type of modeling is called reverse dosimetry. Depending on the emerging knowledge about mode of action (MOA) research for relevant adverse health effects, a biologically based dose response (BBDR) model for BPA will be considered and proposed for follow-on modeling beyond 3 years of this proposed work to describe explicily the interaction of BPA at target organs. This effort is considered beyond the scope of the current proposed effort.

PI: Foley, Steven, Ph.D.

Characterization of Plasmid-Associated Antimicrobial Resistance in *Salmonella enterica* Serovars Associated with Poultry and Human infections (E0733501)

Responsible Division: Microbiology

Collaborating FDA Center(s): CVM

Objective(s):

Begin to identify and understand the genetic mechanisms associated with plasmids that facilitate the spread and persistence of virulence and multidrug resistance in *Salmonella* from poultry and egg-associated serovars; three objectives are proposed:

1) Sequence the plasmids from multidrug-resistant *Salmonella enterica* serovar, Enteritidis, Heidelberg and Typhimurium strains to identify genes likely associated with virulence and antimicrobial resistance.

2) Determine the relative selective potential of antimicrobial agents to trigger the dissemination of antimicrobial resistance and virulence factors to susceptible *Salmonella*.

3) Determine the contribution of plasmids transferred via conjugation to virulence in *Salmonella* strains.

PI: Fu, Peter, Ph.D.

Method Development for Study of Antioxidant Properties in Dietary Supplement (E0730501)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

1) Microsomal Metabolism-Mediated Studies

a) Determine whether or not the studied herbal dietary supplements can enhance or inhibit free-radical formation, mediated by microsomal metabolism, in a dose-

dependent manner.

b) Determine whether or not the studied herbal dietary supplements can enhance or inhibit microsomal metabolism mediated lipid peroxidation in a dose-dependent manner.

2) Cell Culture Studies

a) Determine the toxic effects, including mitochondrial dehydrogenase activity, intracellular ROS (reactive oxygen species) concentration, and mitochondrial membrane potential, of the studied herbal dietary supplements in cells, including A549 human-lung carcinoma cells and rabbit brain rBCECs cells (a normal cell line to assay the toxic effect on CNS) .

b) Use of ESR oximetry technique to determine the inhibition/induction of lipid peroxidation by the studied herbal dietary supplements in A549 human-lung carcinoma cells and rabbit brain rBCECs cells.

PI: Gamboa Da Costa, Gonçalo, Ph.D.

Assessment of the Nephrotoxic Effect of a Combined Exposure to Melamine and Cyanuric Acid (E0216901)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Systems Biology, Microbiology

Collaborating FDA Center(s): CDRH, CFSAN, CVM

Objective(s):

1) Conduct a pharmacokynetic study on F344/N rats on the absorption and disposition of melamine and cyanuric acid when administered individually by gavage, simultaneously as a separate base and acid, and simultaneously as a preformed salt (melamine cyanurate).

2) Determine the NOAEL of a combined exposure to melamine and cyanuric acid in F344/N rats for 28 and 90 days.

3) Investigate the occurrence of early metabonomic and proteomic biomarkers of melamine + cyanuric acid-induced nephrotoxicity obtainable by noninvasive methods.

4) Investigate the pharmacokinetics and determine the NOAEL of a combined exposure to melamine and cyanuric acid in a mini-pig model deemed to be representative of the human-kidney anatomy and physiology.

PI: Gamboa Da Costa, Goncalo, Ph.D.

Assessment of the Nephrotoxicity of a Seven-Day Combined-Exposure to Melamine and Cyanuric Acid (E0731701)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Microbiology

Collaborating FDA Center(s): CFSAN, CVM

Objective(s):

Investigate the nephrotoxic effect of a 7-day co-exposure to melamine and cyanuric acid in Fischer 344 rats.

PI: Guo, Lei, Ph.D.

Toxicokinetic Studies of Berberine in SKH-1 Hairless Mice and *In Vitro* Phototoxicity Testing for Berberine and Goldenseal: Phase-I Study for Phototoxicity and Photocarcinogenicity Studies of Goldenseal and Berberine (E0217701)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Objective(s):

Study the tissue distribution of berberine administered orally to SKH-1 hairless mice. The principal objective of this project is to study the photocarcinogenic effects of orally administered berberine and goldenseal in SKH-1 mice exposed to UVA light. The study will consist of a tiered approach with two phases, Phase I and Phase II. This current protocol outlines the Phase I study. The specific aim of Phase I study is to determine tissue distribution of berberine in SKH-1 mice following oral administration.

PI: Hansen, Deborah, Ph.D.

Developmental Toxicity of Bitter Orange in Rats (E0214701)

External Funding: National Toxicology Program (IAG)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Genetic and Molecular Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

Determine potential developmental toxicity of synthetic synephrine and *citrus aurantium* extract in rats.

PI: Hansen, Deborah, Ph.D.

Physiological Effects of Bitter Orange in Rats (E0214901)

External Funding: National Toxicology Program (IAG)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Genetic and Molecular Toxicology, Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

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.

PI: Khan, Saeed, Ph.D.

Gene Expression Responses by Avirulent *Bacillus anthracis* and Human Epithelial Cells During Initial Host-Pathogen Contact (E0733401)

Responsible Division: Microbiology

Collaborating Division(s): Systems Biology

Collaborating FDA Center(s): ORA, CDER

Objective(s):

1) Compare the gene-expression profiles of the bacteria and cell lines with and without co-culture. Analyze the data for tissue-related differences related to pathogenesis.

2) Validate gene expression data by RT-qPCR.

3) Identify key signal-transduction pathways and immune-system interaction genes involved in EC stimulation by *B. anthracis*.

PI: Leakey, Julian, Ph.D.

RANGE-FINDING ONLY—Studies of Usnic Acid and Usnea Barbata Herb in Fischer 344 Rats and B6C3F1 Mice (E0215911)

External Funding: National Toxicology Program (IAG)

Responsible Division: Associate Director of Scientific Coordination

Collaborating Division(s): Systems Biology

Collaborating FDA Center(s): CFSAN

Objective(s):

Establish appropriate doses of usnic acid and Usnea barbata administered in feed, in male and female Fischer 344 rats and B6C3F1 mice, for use in subsequent subchronic and chronic studies.

PI: Leakey, Julian, Ph.D.

Subchronic Studies of Usnic Acid in Fischer 344 Rats and B6C3F1 Mice (E0216501)

External Funding: National Toxicology Program (IAG)

Responsible Division: Associate Director of Scientific Coordination

Collaborating Division(s): Systems Biology

Objective(s):

Evaluate the subchronic toxicity of usnic acid in male and female Fischer 344 rats and B6C3F1 mice.

PI: Leakey, Julian, Ph.D.

Subchronic Toxicology Studies of Usnea Lichen in Fischer 344 Rats and B6C3F Mice (E0216601)

External Funding: National Toxicology Program (IAG)

Responsible Division: Associate Director of Scientific Coordination

Collaborating Division(s): Systems Biology

Objective(s):

Evaluate the sub-chronic hepatotoxicity of Usnea Lichen in male and female Fischer 344 rats and B6C3F1 mice.

PI: Lyn-Cook, Beverly, Ph.D.

Sex Differences in FDA-Regulated Products: Research for the Future—Seminar Series (S00722)

External Funding: Office of Women's Health (OWH)

Responsible Division: Associate Director for Regulatory Activities

Objective(s):

1) Identify critical gaps in FDA-regulated research addressing disparities in women's health as it relates to drug response, devices, incidence in diseases, and biological tests in various FDA Regulatory Centers.

2) Invite scientists from regulatory centers to NCTR for a seminar series and interaction with NCTR scientists to discuss research needed to address identified critical areas impacting regulatory products or regulatory guidelines and facilitate collaborative projects.

3) Develop and host a one-day Workshop titled, "Sex Differences in FDA-Regulated Products: Research for the Future."

4) Define a Women's Health Research Agenda.

PI: Melchior, William, Ph.D

Real-Time PCR Assays for Ricin and Related Potential Bioterrorism Agents in Foods (P00684)

Responsible Division: Biochemical Toxicology

Objective(s):

1) Develop the precise materials and methods needed to perform the proposed assays.

2) Prove that the assays work simply, rapidly, and reliably.

3) Prove that the assays function as desired in real-world situations, such as with contaminated food stuffs.

PI: Nawaz, Mohamed, Ph.D.

Isolation and Characterization of Fluoroquinolone-Resistant Bacteria from Shrimp (E0730701)

Responsible Division: Microbiology

Collaborating FDA Center(s): CVM; ORA

Objective(s):

1) Isolate and identify fluoroquinolone-resistant Gram-negative bacteria from shrimp imported from different countries.

2) Molecular characterization of fluoroquinolone-resistant determinants.

3) Molecular typing of fluoroquinolone-resistant bacteria.

PI: Nayak, Rajesh, Ph.D.

Antimicrobial-Resistance Genetics of "Emerging" *Salmonella Enterica* Serovar Javiana Phenotypes Involved in Clinical and Food-Related Outbreaks (E0726701)

Responsible Division: Microbiology

Objective(s):

1) Determine the intrinsic resistance of *Salmonella* Javiana isolates to multiple antimicrobials by the SensiTitre antimicrobial-susceptibility testing protocol using the Clinical and Laboratory Standards Institute (CLSI) guidelines.

2) Determine the variation in genetic clonality among the drug-resistance genotypes by fingerprinting the bacteria using the CDC's PulseNet pulsed-field gel electrophoresis (PFGE) protocol.

3) Identify the genes in the multiple antibiotic region (MAR) of the *Salmonella* Genomic Island (SGI)-class 1 integron gene cassettes in the resistant phenotypes.

4) Detect antimicrobial-resistance genes in select multidrug resistant Javiana isolates by PCR-based and microarray-biochip methodologies.

PI: Paule, Merle, Ph.D.

Developmental Neurotoxicity Assessment of Acrylamide in Rats: Long-Term Studies (E0215101)

External Funding: National Toxicology Program (IAG)

Responsible Division: Neurotoxicology

Collaborating Division(s): Biochemical Toxicology, Office of Director

Objective(s):

Determine the consequences of long-term exposure to acrylamide on a variety of developmental milestones and measures of nervous-system integrity throughout life.

PI: Rafii, Fatemeh, Ph.D.

Microarray Analysis for the Detection of Targeted Gene Expression Changes Resulting from Exposure of *Clostridium perfringens* to Fluoroquinolones (E0731101)

Responsible Division: Microbiology

Collaborating Division(s): Systems Biology

Collaborating FDA Center(s): CBER

Objective(s):

Determine the effect of fluoroquinolone exposure on gene expression, regulation of transcription and metabolic activities of *Clostridium perfringens*.

PI: Sung, Kidon, Ph.D.

Quantification Proteomic, Transcriptomic, and Phenotypic Microarray Analysis of *C*. jejuni for the Identification of Colonization Factors in Poultry (E0735601)

Responsible Division: Microbiology

Collaborating Division(s): Systems Biology

Collaborating FDA Center(s):

Objective(s):

1) Evaluate genomic and phenotyphic microarrays, and whole proteomic analyses to compare genes, phenotypes, and proteins from both good and poor C. jejuni chicken colonizers.

2) Investigate functional role of identified colonizing factors by mutant construction, *in vitro* assays, and *in vivo* assays.

3) Identify potential targets for vaccine that will enable us to eliminate the threat of Campylobacter infection in chickens.

PI: Sutherland, John, Ph.D.

Microbial Degradation of Fluoroquinolone Antimicrobial Agents (E0722701)

Responsible Division: Microbiology

Collaborating Division(s): Biochemical Toxicology, Personalized Nutrition and Medicine

Objective(s):

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

PI: Tolleson, William, Ph.D.

Laboratory Studies in Melamine and Cyanuric Acid Biochemical Toxicology (E0729101)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Systems Biology

Objective(s):

Determine chemical and biochemical properties of melamine and cyanuric acid that may influence their toxicity and retention as tissue residues.

PI: Tolleson, William, Ph.D.

Chemical Inactivation of Protein Toxins on Food Contact Surfaces (E0730301)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

1) Identify cleaning/sanitizing treatments that result in elimination and/or inactivation of protein toxins (abrin and ricin) on food-contact surfaces.

2) Identify surrogate(s) that can be used to study chemical inactivation of abrin or ricin.

3) Measure the loss of ricin and abrin biological and biochemical activities in the presence of cleaning/sanitizing solutions using RAW264.7 macrophage cytotoxicity assays and 28S rRNA adenosine N-glycosidase RTqPCR-based enzyme assays.

PI: Tolleson, William, Ph.D.

Rapid Detection of Ribosome-Inactivating Protein Toxins in Foods (E0736101)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Systems Biology, Microbiology

Collaborating FDA Center(s): CFSAN

Objective(s):

Provide robust methods for detecting the biological activity of the potential bioterrorism agents ricin, abrin, and shiga-like toxins, each of which is characterized as a ribosome-inactivating protein (RIP) toxin, in three selected foods (spinach, apple juice, and milk).

PI: Tolleson, William, Ph.D.

Thermodynamic Measurements for Inactivation of Bioterrorism Agents Ricin and Abrin (P00708)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

1) Measure forward-rate constants for thermal denaturation of ricin and abrin at seven temperatures (60, 65, 70, 75, 80, 85, 90, and 95 C) and three buffer combinations (0.10 M NaCl buffered with 20 mM lactate, pH 3.0; 20 mM acetate, pH 5.0; and 20 mM phosphate, pH 7.0) by monitoring the quenching of intrinsic protein (tryptophan) fluorescence (EX295, EM340) in a thermostatted spectrofluorimeter.

2) Exploit results (Tm, Delta H) gathered using differential-scanning calorimetry at NCFST to select Tm for toxin proteins and measure reverse-rate constants (protein renaturation) at one temperature and one buffer combination. Calculate Keq and Delta G from ratio of rates. Determine T Delta S from Delta G and Delta H.

3) Determine the influence of solvent pH on isothermal toxin folding/unfolding equilibria.

4) Identify time-, pH-, and temperature-dependent reversible and irreversible transitions in ricin conformation and correlate these with changes in toxin-dependent enzyme activity and cytotoxicity.

PI: Wagner, Robert, Ph.D.

Mechanistic Evaluation of the Induction of Lymphoproliferation and Apoptosis Inhibition by Probiotic Bacteria in Mice Infected with *Salmonella Enterica* (E0727601)

Responsible Division: Microbiology

Objective(s):

1) Orally challenge defined human microbiota-associated (HMA) BALB/c mice and probiotic-bacteria-treated HMA BALB/c mice with *Salmonella enterica* and isolate intestinal mucosal-associated lymphoid tissues (MALT) , including Peyer's patches, lamina propria, and mesenteric lymph nodes.

2) Use pathway-focused gene-expression profiles generated from real-time RT-PCR expression arrays to compare signal transduction in MALT from HMA mice treated with or without probiotic bacteria and orally challenged with S. *enterica*.

3) Develop immunohistochemical (IHC) and *in situ* hybridization (ISH) conditions to detect the expression of the signal pathway molecules implicated in activation and apoptosis inhibition in mucosal T cells and accessory cells in tissue sections of Peyer's patches, lamina propria, and mesenteric-lymph nodes.

4) Conduct IHC and ISH studies on tissue sections for detection of molecules involved in the regulation of lymphocyte activation and programmed cell-death pathways induced by bacterial-surface antigens.

5) Compare the probiotic-treated and untreated mice for expression of dendritic cell, macrophage, and IEC-derived cytokines.

PI: Wilkes, Jon, Ph.D.

Rapid Screening of Food or Drugs for Chemical or Microbiological Contamination (E0734701)

Responsible Division: Systems Biology

Collaborating Division(s): Office of Management

Objective(s):

A pyrolysis mass spectrometry instrumental method combined with pattern recognition can detect bacterial or chemical contamination in foods or drug ingredients at a concentration of 0.01 to 0.1% by weight in 15 seconds/sample. Real-time characterization is not precise but can be used to recognize variant batches, to indicate the probable contamination as biological or chemical, and to flag suspicious products for further analysis.

PI: Aidoo, Anane, Ph.D.

Development of Methods for Evaluating DNA Damage Using Single-Cell Gel Electrophoresis (Comet Assay) in Rodents (E0729001)

Responsible Division: Genetic and Molecular Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

Evaluate and establish methods and conditions that enhance the sensitivity and reproducibility of the *in vivo* alkaline-comet assay for use in preclinical-hazard identification and genotoxicity testing of food ingredients and chemicals for regulatory purposes.

PI: Ali, Syed, Ph.D.

Neurotoxicity Assessment of Cell Phone Radio Frequency Radiation using Rat and Bovine Brain Microvascular Endothelial Cells as Model Blood-Brain Barrier Systems, PC-12 Cultured Cells, and Whole-Animal Models (Mice and Rats). (E0217301)

External Funding: National Toxicology Program (IAG)

Responsible Division: Neurotoxicology

Collaborating Division(s): Regulatory Compliance & Risk Management

Collaborating FDA Center(s): CDRH

Objective(s):

Determine whether power levels of radiofrequency radiation (RFR) that are emitted from mobile phones produce any changes in the central nervous system (CNS) of mice and rats.

PI: Ali, Syed, Ph.D.

Neurotoxicity Assessment of Manganese (Mn) Nanoparticles in PC-12 Cells and in Mice (E0725701)

Responsible Division: Neurotoxicology

Collaborating Division(s): Regulatory Compliance & Risk Management

Objective(s):

1) Evaluate the neurotoxicity of differently sized manganese nanoparticles using PC-12 cultured cells.

2) Determine if *in vitro* exposure to manganese nanoparticles selectively induces specific genomic changes in PC-12 cultured cells using oligonucleotide microarrays.

3) Determine if multiple doses of Mn-nanoparticles produce reactive oxygen species, alterations in lipid peroxidation and/or changes in antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase), and levels of glutathione in various regions of the mouse brain.

4) Determine if single or multiple doses of manganese nanoparticles induce specific genomic changes in various regions of the mouse brain using oligonucleotide microarrays.

5) Determine if single or multiple doses of Mn-nanoparticles produce significant changes in neurotransmitter concentrations in

various regions of the mouse brain.

6) Determine if single or multiple doses of Mn-nanoparticles produce significant changes in the formation of 3-nitrotyrosine, an *in vivo* biomarker for oxidative stress, in various regions of the mouse brain.

7) Determine if multiple doses of Mn-nanoparticles produce morphological alterations in the brain or visceral organs of the mouse.

PI: Ali, Syed, Ph.D.

Neurotoxicity Assessment of Silver (Ag) Nanoparticles in PC-12 Cells and in Rats (E0728201)

External Funding: Office of Women's Health (OWH)

Responsible Division: Neurotoxicology

Collaborating Division(s): Regulatory Compliance & Risk Management

Objective(s):

1) Evaluate the neurotoxicity of different sizes of silver (Ag)-nanoparticles using cultured PC-12 cells.

2) Determine if *in vitro* exposure to Ag-nanoparticles selectively induces specific genomic changes in cultured PC-12 cells using microarrays.

3) Determine if single or multiple doses of Ag-nanoparticles produce reactive-oxygen species, alterations in lipid peroxidation and/or changes in antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) and glutathione levels in the rat brain.

4) Determine if single or multiple doses of Ag-nanoparticles induce specific genomic changes in the rat brain as indicated with microarrays.

5) Determine if single or multiple doses of Ag-nanoparticles produce significant changes in neurotransmitter concentrations in the brain in rat.

6) Determine if single or multiple doses of Ag-nanoparticles produce significant changes in the formation of 3-nitrotyrosine (3-NT), an *in vivo* biomarker for oxidative stress, in the rat brain.

7) Determine if multiple doses of Ag-nanoparticles produce morphological alterations in blood-brain barrier, brain, or other visceral organs of the rat.

PI: Beger, Richard, Ph.D.

3D- and 4D-QSDAR Modeling Applied to Various Toxicological Endpoints (E0734801)

Responsible Division: Systems Biology

Collaborating Division(s): Office of Management, Neurotoxicology

Collaborating FDA Center(s): CDER

Objective(s):

1) Develop 3D- and 4D- QSDAR models for endocrine disruptors, lowest-observed-adverse-effects level (LOEAL) and no observed-adverse-effects level (NOEALs), and other relevant toxicological endpoints.

2) The training models tested with external test sets and the training and testing results will be compared to previous QSDAR, quantitative structure-activity relationship (QSAR), and SAR models.

3) Determine how the technique used to predict 13C or 15N NMR spectra affects 3D-QSDAR modeling. One technique will use ACD/Labs Predictor software, which references 2D descriptors for compound fragments and approximately 20 million experimental NMR chemical shifts. The other determines 13C and 15N NMR spectra from ab initio calculations of structural conformations for each compound. The latter should show chemical shifts changing as the conformation changes in time. Spectral-structural conformation changes may improve 4D-QSDAR models when a specific 3D conformation determines a compound's biological effect.

PI: Beland, Frederick, Ph.D.

Perinatal Carcinogenicity of Drug Combinations Used to Prevent Mother-to-Child Transmission of HIV (E0214111)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Genetic and Molecular Toxicology

Objective(s):

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

PI: Beland, Frederick, Ph.D.

Mechanisms of Nevirapine Carcinogenicity (E0217101)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Objective(s):

Determine the mechanisms by which nevirapine induces liver tumors in rats.

PI: Beland, Frederick, Ph.D.

Benzocaine-Induced Methemoglobinemia in an Acute Rat Model (E0730201)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CVM

Objective(s):

The potential for benzocaine-induced methemoglobinemia in humans consuming meat from benzocaine-treated fish is of particular concern. The current study is intended to produce data that will put this particular concern in perspective.

PI: Bhalli, Javed, Ph.D.

Development of a High-Throughput Assay for Measuring In Vivo Mutation in an Autosomal Gene (E0741301)

Responsible Division: Genetic and Molecular Toxicology

Objective(s):

The major objective of this study is to develop a high-throughput *in vivo* mutation model that detects mutations induced by a range of mechanisms, including gene

mutation, large deletions, and LOH. The basic properties and sensitivity of the model will be evaluated in experiments employing well-characterized mutagens.

PI: Bhattacharyya, Sudeepa, Ph.D.

Quality Control for Focused and Unfocused LC-MS Based Metabolomic Profiling of Blood Samples (E0738401)

Responsible Division: Systems Biology

Objective(s):

For the ongoing, as well as future projects in the lab involving LC-MS-based metabolomic profiling studies on blood, we will develop and test a quality control (QC) protocol on existing preclinical hepatotoxicity protocols that can potentially be translated to clinical metabolomics experiments. Our working hypothesis is that adding quality control will improve both intra-lab and inter-lab reproducibility of LC-MS-based metabolomics of blood samples.

PI: Boudreau, Mary, Ph.D.

Effect of Topically Applied Skin Creams Containing Retinyl Palmitate on the Photocarcinogenicity of Simulated Solar Light in SKH-1 Mice (E0214301)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Regulatory Compliance & Risk Management, Associate Director of Scientific Coordination

Collaborating FDA Center(s): CFSAN

Objective(s):

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

PI: Bowyer, John, Ph.D.

Characterizing the Amphetamine-Induced Changes in Vascular Tone, Vasotrauma, and Alterations in Angiogenesis in Rodent Brain (E0729501)

Responsible Division: Neurotoxicology

Collaborating FDA Center(s): CDER

Objective(s):

1) Evaluate the effects of both acute and chronic amphetamine (AMP) exposure on the vasculature of the rat brain. In particular, we propose to examine vasculature within the parenchyma of three brain regions; the striatum, parietal cortex, and the combined piriform and amygdaloid nuclear cortices where AMP-induced neurodegeneration can occur.

2) Look at the effects of AMP on the vasculature associated with pial and arachnoid membranes (part of the meninges) and vasculature of the choroid plexus. The vasculature of the meninges is particularly interesting, because it may be associated with the subarachnoid hemorrhage that is often reported in humans abusing amphetamine and methamphetamine (METH). We have already conducted one preliminary study looking at the initial effects of a one-day exposure to high doses of AMP that produce hyperthermia and significant

neurotoxicity but do not normally lead to stroke or hemorrhage in rat (Bowyer *et al.*, submitted for publication). The results of this study clearly show that AMP can produce profound changes in genes associated with vascular tone, damage, and angiogenesis.

3) Conduct experiments involving AMP exposures to determine the alterations in vascular gene expression after:

a) One-day 4-dose exposure that produces hyperthermia (full time-course of effects, not just 1st day).

b) Single exposure to a very high dose.

c) Nine-day exposure that does not produce significant hyperthermia.

d) One-month exposure to AMP included in the drinking water.

PI: Cao, Xuefei, Ph.D.

Dose-Response Genotoxicity of Ethylmethane Sulfonate (EMS) in Mice using the PIG-A and Transgenic gpt Delta Assays (E0739001)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Personalized Nutrition and Medicine

Collaborating FDA Center(s): CDER

Objective(s):

This project will conduct a thorough examination on the dose response of EMS genotoxicity in mice, both to challenge the conclusions reached by the Roche study described in this protocol and to gain experience with how best to examine the induction of no-effect thresholds by genotoxic agents. In order to address some of the weakness of the Roche study that were cited in the FDA review, the study will:

1) Use sensitive genotoxicity endpoints with low-background frequencies to increase the sensitivity of the assays for detecting low-dose effects.

2) Measure genotoxicity using a design to detect the maximum responses.

3) Measure the effects of EMS exposure in neonatal, as well as adult animals.

4) Measure genotoxicity in the major target tissues for EMS carcinogenicity.

PI: Chelonis, John, Ph.D.

ASK CHILDREN Study—Assess Specific Kinds of Children Challenges for Neurologic Devices (E0734301)

Responsible Division: Neurotoxicology

Collaborating Division(s):

Neurotoxicology

Objective(s):

The purpose of this research plan is to develop a framework of science-based recommendations important to help expedite pediatric prostheses to market, including recommendations for the research and development of neurologic devices. The objectives are to:

1) Collect qualitative and quantitative self-report clinical data (through interviews) and identify scientific and medical issues

associated with pediatric devices when used in children undergoing treatment, to develop more efficient strategies for evaluating these types of products regulated by FDA. Data that are important to developing more efficient strategies in evaluating these types of products regulated by FDA will be organized by multiple categories, including (but not limited to) device type, pediatric subpopulations, disorder or condition, and intended use.

2) Establish a science-based framework of recommendations to help develop more efficient strategies in evaluating pediatric products regulated by FDA.

PI: Chelonis, John, Ph.D.

Long-Term Neurodevelopmental Follow-Up of Children Administered Ketamine Prior to Cardiac Surgery in Infancy (SAFEKIDS study) (E0738801)

Responsible Division: Neurotoxicology

Objective(s):

Immature neurons in the infant brain are more susceptible to excitotoxic cell death. Ketamine, an anesthetic agent used commonly for infants, is a potent, noncompetitive NMD-receptor antagonist, which also has anti-inflammatory effects and may decrease excitotoxic-neuronal injury. However, animal studies have also suggested that the use of high doses of ketamine may also cause neuronal death in the immature brain. We have conducted a pilot, randomized and controlled trial in human infants undergoing cardiac surgery using ketamine prior to cardiopulmonary bypass and showed possible neuroprotective and anti-inflammatory effects. This pilot study did not reveal any evidence of neurotoxicity in short term follow-up of these patients two to three weeks after surgery. Our current objective is to:

1) Assess the possibility of any long-term neuroprotective or neurotoxin effects in the previously studied population of patients who were enrolled as infants in the previous trial. Specifically we will administer neuropsychological tests after their 5th birthday to assess cognitive function, academia achievement, memory, operant behavioral endpoints, and executive functioning.

2) Determine the behavioral outcomes of the cases and controls.

PI: Chen, Huizhong, Ph.D.

Assessment of Effects and Metabolism of Synthetic Azo Colorants Used in Women's Cosmetics on Human-Skin Microbiota (E0729301)

Responsible Division: Microbiology

Collaborating Division(s): Personalized Nutrition and Medicine

Collaborating FDA Center(s):

Objective(s):

The overall objective is to evaluate the metabolism and effect of color additives used in cosmetics on the skin microbiota and potential to adversely affect women's health. Specific objectives are to:

1) Assess the degradability of the synthetic azo colorants in cosmetics by skin bacteria.

2) Identify and quantify the potential carcinogenic and toxic aromatic amines in the metabolites.

3) Elucidate the role of the microflora with potential genotoxic effects of cosmetic azo dyes on women's health.

4) Determine physicochemical properties of the azo dye-degrading enzymes from the skin bacteria.

5) Establish a standardized assay to determine the reductive capacity of the skin on the azo colorants.

PI: Chen, James, Ph.D

Data-Mining Strategy to Identify Hepatotoxic Drugs and Sensitive Patients (E0740301)

Responsible Division: Personalized Nutrition and Medicine

Collaborating FDA Center(s): CDER

Objective(s):

The main objective of this study is to build a prototype computerized-visualization system as an integration of FDA safety data-mining system for further analysis of the AERS data. The system will be able to explore the similarities and differences among drugs and among events and identify unusual drug-event combinations for further investigation.

PI: Chen, James, Ph.D.

Modification and Application of Quantitative Risk-Assessment Techniques to FDA-Regulated Products (S00174)

Responsible Division: Personalized Nutrition and Medicine

Collaborating FDA Center(s): CDER, CDRH, CFSAN

Objective(s):

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

PI: Chen, Tao, Ph.D.

Development of a New Safety Evaluation Method Using MicroRNA (miRNA) Expression Analysis as a Biomarker for Detecting Carcinogens (E0728101)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Systems Biology

Objective(s):

1) Determine miRNA expression profiles of the tumor target tissues of rats and mice treated with genotoxic carcinogens aristolochic acid (AA), riddelliine, and comfrey; and nongenotoxic carcinogens, propiconazole and triadimeforn, as well as noncarcinogen myclobutanil using microarray technologies.

2) Develop a PCR array containing

the primers that are specifically used to amplify carcinogensis-related miRNAs and use the PCR array to conduct time-course and dose-response studies for miRNA expression alterations in tissues of rats treated with carcinogens.

3) Define the miRNA biomarker genes that are associated with carcinogen exposure by prediction of their target genes and determination of their biological functions.

PI: Delclos, Kenneth, Ph.D.

Effects of Sedatives on the Metabolism of Di(2-ethylhexyl)phthalate (DEHP) Administered by Intravenous Injection and the Relationship of DEHP Metabolism to Biological Effects in Neonatal Rats (E0216201)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CBER; CDRH

Objective(s):

1) Determine if sedatives potentially useful for intravenous-injection studies of DEHP in neonatal rhesus monkeys and/or in common use in neonatal intensive care units (NICU) affect the metabolic profile of DEHP.

2) Examine DEHP metabolism in neonatal rodents dosed intravenously and orally and relate this metabolism to biological effects.

PI: Delclos, Kenneth, Ph.D.

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

Responsible Division: Biochemical Toxicology

Objective(s):

1) 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) Evaluate the renal toxicity of dietary DEHP in developing rats maintained on a soy-free diet.

3) Evaluate potential early markers of renal cystogenesis in p-nonylphenol- and DEHP-treated rats and their modulation by soy-containing diets.

4) Evaluate the roles of modulation of antioxidant defenses and cyclooxygenase activities in the protective effect of soy against p-nonylphenol and, if demonstrated, DEHP-induced renal toxicity.

5) As secondary objectives in the above studies, the effect of diet on hepatic, testicular, and lung toxicity of DEHP will be assessed.

PI: Desai, Varsha, Ph.D.

Development of a Translational Mouse Model of Drug-Induced Cardiac Tissue Injury. (P00744)

Responsible Division: Systems Biology

Collaborating Division(s): Associate Director of Scientific Coordination

Collaborating FDA Center(s): CDER

Objective(s):

1) Perform noninvasive measurements of heart rate, heart rate variability, and electrocardiogram using ECGenie system.

2) Measure cardiac troponin T and creatine kinase MB in plasma/serum as indicators of DOX-induced cardiac tissue damage.

3) Identify cardiac lesions by light microscopy and morphological changes in cardiac mitochondria by electron microscopy.

4) Measure levels of cardiolipin, amino acids, and Krebs cycle intermediates in serum using metabolomics.

PI: Dobrovolsky, Vasily, Ph.D.

Development of High-Throughput Methodology for Detection of *In Vivo* Mutation in the Endogenous PIG-A Gene of Human-Blood Cells Using Flow Cytometry (E0728301)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Systems Biology

Collaborating FDA Center(s): CDRH, CDER

Objective(s):

1) Design high-throughput methods for detecting PIG-A mutant human red- and white- blood cells by flow-cytometric detection of cells lacking cell-surface protein markers anchored by glycosyl phosphatidyl inositol (e.g., CD59, CD48).

2) Using the methods developed in Objective 1, establish a normal range of PIG-A mutant frequencies in red- and white- blood cells and compare these ranges with those of different groups of human subjects hypothesized to have increased mutational loads. The groups will include, but will not be limited to:

a) Patients with the disease paroxysmal nocturnal hemoglobinurea.

b) Patients undergoing radiation treatment or chemotherapy with DNA reactive drugs.

c) Patients predisposed to cancer due to inherited deficiencies in endogenous pathways.

3) When the volumes of blood samples permit, compare red-blood cell PIG-A mutant frequencies determined in Objective 2 with PIG-A mutant frequencies in white-blood cells from these samples determined by limiting-dilution cloning, and determine the PIG-A DNA sequence changes responsible for the white-blood cell mutants.

PI: Doerge, Daniel, Ph.D.

Di(2-ethylexl)phthalate (DEHP) and Bisphenol A (BPA) Exposure in Pediatric Patients (E0742501)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CDRH

Objective(s):

1) Determine the pharmacokinetics of the production of urinary metabolites of DEHP and BPA after cardiopulmonary bypass in children.

2) In a pilot study, quantify the exposure of children to DEHP and BPA while undergoing cardiopulmonary bypass (CPB) as compared to critically ill children without cardiac surgery and healthy controls.

3) Evaluate the ability of a battery of urinary biomarkers to detect acute kidney injury in patients following CPB.

PI: Fang, Jia-long, Ph.D.

Vehicle Selection for Triclosan Dermal Toxicity Studies in B6C3F1 Mice (E0217501)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Objective(s):

Determine the appropriate vehicle for use in triclosan dermal toxicity studies in B6C3F1 mice.

PI: Ferguson, Sherry, Ph.D.

Long-Term Effects of Morphine Treatment in Preterm Infants Exposed to Repetitive Neonatal Pain (E0724301)

Responsible Division: Neurotoxicology

Objective(s):

Determine if Neonatal Intensive Care Unit morphine treatment in preterm infants is associated with long-term alterations in short-term memory and/or motivation at approximately six years of age.

PI: Fu, Peter, Ph.D.

Use of Electron Spin Resonance Spectroscopy to Characterize the Interactions Between Nanoscale Materials and Model Biological Systems (E0730601)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

1) Chemical Reactions

a) Determine whether or not nanomaterials can catalyze Fenton reaction to initiate hydroxyl radical formation in a nanoparticle size-dependent manner.

b) Determine whether or not nanomaterials and/or their cations can be reduced by natural reducing agents, such as ascorbic acid and glutathione, leading to the formation of ROS.

2) Microsomal Metabolism-Mediated Studies

a) Determine whether or not nanomaterials enhance or inhibit

free-radical formation, mediated by microsomal metabolism, in a nanoparticle size-dependent manner.

b) Determine whether or not nanomaterials and/or their cations can enhance or inhibit microsomal metabolism-mediated lipid peroxidation in a nanoparticle size-dependent manner.

3) Cell Culture Studies

a) Determine the toxic effects, including mitochondrial-dehydrogenase activity, intracellular-ROS concentration, and mitochondrial- membrane potential, of nanomaterials of different particle size in cells, including A549 human-lung carcinoma cells and rabbit brain rBCECs cells (a normal cell line to assay the toxic effect on CNS).

b) Use of ESR oximetry technique to determine the inhibition/induction of lipid peroxidation by nanomaterials of different particle size in A549 human lung carcinoma cells and rabbit brain rBCECs cells.

PI: Hammons, George, Ph.D.

Assessment of Interindividual Variability in Expression of DNA Methyltransferases, DNMT3a, and DNMT3b, in Liver and Identification of Factors Influencing Expressions (E0716701)

Responsible Division: Associate Director for Regulatory Activities

Objective(s):

1) Determine levels of expression of DNMT3a and DNMT3b 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.

3) Assess the polymorphism frequency identified in DNMT3b in the sample pool included in the study and assess whether it is correlated with expression.

PI: He, Zhen, Ph.D.

Brain Sexual Dimorphic Structures and Sex Hormone-like Compounds: Animal Request for Methods Development and Training (P00710)

Responsible Division: Neurotoxicology

Objective(s):

Explore the utility of a more comprehensive evaluation of the effects of SHLCs (sex hormone-like compounds). We propose establishing a series of standardized procedures for evaluating SHLC-induced changes in brain morphology utilizing immmunohistochemical and other, more traditional, techniques.

PI: Heflich, Robert, Ph.D.

Phosphatidylinositol Glycan Complementation Group A (PIG-A) Mutagenesis; an International Validation Study Comparing PIG-A Mutation in Rats with Other Biomarkers of Genetic Toxicity (E0741201)

Responsible Division: Genetic and Molecular Toxicology

Collaborating FDA Center(s): CDER, CVM

Objective(s):

The major objective of this study is to generate data using a standardized protocol that, in combination with results from other investigators, will be used to determine the sensitivity, specificity, and portability of the rat RBC/RET PIG-A gene-mutation assay. By performing the *in vivo* Comet, MN, and the PIG-A and Hprt lymphocyte gene-mutation assays in conjunction with the RBC/RET PIG-A assay, we also will be able to determine how the RBC/RET PIG-A assay compares in terms of sensitivity and specificity with these other *in vivo* assays that have been used or considered for use as regulatory assays.

PI: Howard, Paul, Ph.D.

The Immunogenicity of Permanent Makeup Inks and Their Components (E0216101)

External Funding: National Toxicology Program (IAG)

Responsible Division: Associate Director of Scientific Coordination

Collaborating Division(s): Veterinary Services

Objective(s):

Determine the immunogenicity of permanent-makeup inks using a modified LNPA (lymph node proliferation assay) protocol.

PI: Howard, Paul, Ph.D.

Methodology for Safety Testing of Pigments Used for Tattooing, Including Permanent Makeup (E0710501)

Responsible Division: Associate Director of Scientific Coordination

Collaborating Division(s): Biochemical Toxicology

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-makeup inks in SKH-1 hairless mice in the presence and absence of simulated-solar light.

5) Determine if tattoo pigments are photococarcinogenic in the SKH-1 hairless mouse using simulated solar light.

PI: Howard, Paul, Ph.D.

Analytical Assay for Photochemical Generation of Hydroxyl Radical (S00728)

Responsible Division: Associate Director of Scientific Coordination

Collaborating Division(s): Biochemical Toxicology

Objective(s):

1) Provide support for analysis of the

photoactivation of nanomaterials using the OH/coumarin-3-carboxylic acid assay.

2) Provide particle-size analysis for all materials being analyzed by OH method and other nanomaterials used in studies at NCTR and ARL/ORA.

3) Improve the assay using ultraviolet-light diode laser (on hand) as a replacement to the existing broad-band ultraviolet-light A source.

PI: Kanungo, Jyotshnabala, Ph.D.

Methods Development for Toxicity Assays Using the Zebrafish Embryo as a Model System: Whole-Animal High-Throughput Assays for Chemical Testing (E0735901)

Responsible Division: Neurotoxicology

Objective(s):

Establish a high-throughput assay system using zebrafish embryos for toxicity assessments of FDA-relevant compounds. We propose to focus on establishing a high-throughput screening assay to monitor both traditional morphological and behavioral endpoints of toxicity and the newer, more subtle organ-specific toxicities.

PI: Kanungo, Jyotshnabala, Ph.D.

Effect of Pediatric Anesthetics on Zebrafish Embryos: Neurotoxicity vs. Gene Expression Changes and Neuronal Kinase (Cdk5) as a Mediator of Toxicity (E0736301)

Responsible Division: Neurotoxicology

Collaborating Division(s): Office of Director

Objective(s):

Determine whether ketamine will have neurotoxic effects (on neurogenesis and axonogenesis) in zebrafish and to determine if the window of such effects varies between early- and late-differentiating neurons (sensory and motor neurons, respectively).

PI: Kanungo, Jyotshnabala, Ph.D.

Zebrafish Breeding and Maintenance for Embryo Studies (E0738701)

Responsible Division: Neurotoxicology

Objective(s):

This protocol utilizes methods that will enable us to maintain the zebrafish for breeding purposes. In the NCTR zebrafish facility, the zebrafish will be maintained in recirculating water at 28 degrees Celcius and will be fed two times a day. The breeders will be fed three times a day. The food constitutes flake foods, brine shrimp, and/or Spirulina algae. All the feed will be procured from scientific suppliers.

PI: Leakey, Julian, Ph.D.

Toxicity Studies of Combination of AIDS Drugs in p53 (+/-) Transgenic Mice (E0215201)

External Funding: National Toxicology Program (IAG)

Responsible Division: Associate Director of Scientific Coordination

Collaborating Division(s): Biochemical Toxicology, Office of Research

Objective(s):

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.

PI: Levi, Mark, Ph.D.

Studies Comparing the Neurotoxicology of Amphetamine with Methamphetamine and Methylphenidate (E0740101)

Responsible Division: Neurotoxicology

Collaborating Division(s): Biochemical Toxicology

Collaborating FDA Center(s): CDER

Objective(s):

1) Determine the appropriate dose range and plasma levels of MP that produce a hyperthermic profile similar to that produced by neurotoxic doses of AMP and METH. Then, using that dose range, compare the neurotoxicity produced by MP with that of AMP and METH.

2) Evaluate the hyperthermic profiles resulting from the selected doses of MP, AMP, or METH and their relationship between the hyperthermic profile and neurotoxic outcome of these compounds.

3) Determine the effects of non-neurotoxic doses of MP, AMP, or METH on body temperature and gene expression, which is to be compared to neurotoxic doses.

4) Determine whether a single very high dose of either AMP or METH, which produces seizure activity, has substantially different effects on body temperature than the multiple "lower" dose neurotoxic exposures (four consecutive doses in the 3-10mg/kg range) that do not produce seizures.

PI: McKinzie, Page

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)

Responsible Division: Genetic and Molecular Toxicology

Objective(s):

1) 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.

2) 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.

PI: McKinzie, Page

Development of Cancer-Relevant Biomarkers for Identification of Potential Carcinogens: Research to Understand the Normal Background Frequencies in Rats (E0733601)

Responsible Division: Genetic and Molecular Toxicology

Objective(s):

Understand the distribution and range of spontaneous oncogene mutant frequencies in the major organs of rats and mice to provide important basic information for the

validation of these oncogene mutant frequencies as biomarkers of chemically induced carcinogenesis.

PI: Moore, Martha, Ph.D.

Development of a Method To Use *In Vivo* Mutagenicity Data to Address the Question as to Whether a Specific Chemical Induces Cancer Via a Mutagenic or a Non-mutagenic Mode-of-Action (MOA)

External Funding: Toxicology Excellence for Risk Assessment (CRADA)

Responsible Division: Genetic and Molecular Toxicology

Objective(s):

The primary goal of this project is to further develop, evaluate, and disseminate a new NCTR method that utilizes *in vivo* mutagenicity and other key event data to address the question of whether a specific chemical induces cancer via a mutagenic or a nonmutagenic mode-of-action (MOA). Specific objectives are to:

1) Work collaboratively (NCTR/TERA scientists) to select appropriate chemicals for this analysis.

2) Investigate design alternatives and various strategies for selecting doses, selecting the number of animals in each dose group and for conducting the *in vivo* mutation studies required for this analysis. The oral *in vivo* studies will be conducted by NCTR, as well as the mutation assays.

3) Investigate various modeling approaches for mutagenic and non-mutagenic MOAs with the goal of understanding the relationship between the dose response for the induction of mutations and the dose response for the induction of tumors.

4) Evaluate several dose-response modeling methods (e.g., benchmark dose, categorical regression) to determine the appropriate model(s) for this analysis.

5) Develop the optimal experimental design and the optimal modeling methods to address the question as to whether a particular chemical is inducing cancer via a mutagenic MOA.

6) Share the utility of this new approach with the larger risk assessment community by publishing and making presentations on the results.

PI: Moore, Martha, Ph.D.

Evaluation of the Ability of Both the Agar and Microwell Versions of the Mouse Lymphoma Assay (MLA) to Optimally Detect the Mutagenic Potential and Potency of Complex Chemical Mixtures (E0728401)

Responsible Division: Genetic and Molecular Toxicology

Objective(s):

Develop science-based best-practice standard and tools to incorporate translational and applied toxicological advancements into the regulatory-science process to create a seamless bench-to-bedside continuum.

PI: Morris, Suzanne, Ph.D.

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

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Personalized Nutrition and Medicine, Neurotoxicology, Biochemical Toxicology, Office of the Director

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.

4) Determine the plasma concentration of methylphenidate and its major metabolite, ritalinic acid, during the chronic exposure of juvenile rhesus monkeys to the drug.

PI: Parsons, Barbara, Ph.D.

Evaluating the Utility of ACB-PCR in Dose-Response Assessment and Mode-of-Action Evaluation (E0726901)

External Funding: The Hamner Institutes for Health Sciences (CRADA)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Neurotoxicology

Objective(s):

The primary goal of this Cooperative Research and Development Agreement (CRADA) and associated protocol is to further develop, evaluate, and disseminate a new NCTR method, Allele-specific competitive blocker-PCR (ACB-PCR) and to determine whether ACB-PCR measurements of specific oncogenic base substitutions can be used to inform and improve the dose-response and mode-of-action assessments required in cancer-risk assessment.

PI: Patterson, Tucker, Ph.D.

Pramipexole: Thirty-Week Toxicity Study in Juvenile Rhesus Monkeys Followed by a Twelve-Week Recovery Period: Use of a Nonhuman Primate Model for Studying the Consequences of Long-Term Dopaminergic Receptor Stimulation on Complex Brain Functions Using the (E0725201)

External Funding: Boehringer Ingelheim Pharmaceuticals Inc. (CRADA)

Responsible Division: Neurotoxicology

Collaborating Division(s): Neurotoxicology

Objective(s):

1) Establish acquisition curves for several operant behaviors in juvenile rhesus monkeys during chronic oral exposure to pramipexole and vehicle.

2) Determine whether such exposure results in any significant changes in the acquisition and performance of these operant and other observable behaviors.

3) Determine whether such exposure results in any significant changes in clinical chemistry or ophthalmic parameters.

4) Determine plasma distribution profiles and concentrations of pramipexole at various stages of chronic exposure.

5) Conduct standard postmortem toxicological investigations, including histopathology.

6) Conduct a focused neuropathological evaluation.

PI: Paule, Merle, Ph.D.

Complex Brain Function in Children as Measured by Performance in the NCTR Operant Test Battery (E0703301)

Responsible Division: Neurotoxicology

Collaborating Division(s):
Neurotoxicology

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.

PI: Paule, Merle, Ph.D.

Long-Term Consequences of Neonatal Ketamine Anesthesia in Rhesus Monkeys: Extended Cognitive Assessments (E0736401)

Responsible Division: Neurotoxicology

Collaborating Division(s): Office of the Director, Regulatory Compliance & Risk Management

Collaborating FDA Center(s): CDER

Objective(s):

1) Continue monitoring the cognitive capabilities of rhesus monkey subjects that were exposed to a single, 24-hour bout of ketamine-induced anesthesia during the first week of life. Data to date indicate that, compared to control animals, ketamine-exposed subjects exhibit significant deficits in several aspects of brain function including learning, the ability to perform simple visual discriminations, motivation and speed of psychomotor processing. Continuing these observations will provide valuable information on the ultimate time-course and severity of the observed deficits.

2) Extend the functional domains that are being assessed. Performance of a temporal discrimination task (timing task), a counting task and reversal learning tasks (cognitive flexibility) will be added to the current assessment battery.

PI: Paule, Merle, Ph.D.

Implementation of a New Computer Configuration for Administration of NCTR's Operant Test Battery (P00746)

Responsible Division: Neurotoxicology

Objective(s):

1) Develop a new USB-based system to replace and improve the droid system.

2) Compare the function of the newly developed system with that of the older system to ensure system continuity and compatibility.

3) Test, debug, and validate the new system prior to future use in the human and nonhuman primate OTB testing laboratories.

PI: Petibone, Dayton, Ph.D.

Development of a Molecular Cytogenetics Laboratory in the Division of Genetic and Molecular Toxicology (E0734201)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division(s): Personalized Nutrition and Medicine

Objective(s):

The overall objective of the molecular cytogenetics laboratory will be to establish methods for the culture and harvest of peripheral blood lymphocytes, application of whole chromosome FISH probes to metaphase cells, and data collection and analysis. Protocols will be established for the culture and harvest of primary peripheral blood lymphocytes from nonhuman primates and peripheral-blood lymphocytes, splenocytes, and bone marrow from rodents (mice and rats).

PI: Salminen, William, Ph.D.

Evaluation of Growth and Pubertal Development in Male Rhesus Monkeys (Macaca mulatta) Chronically Exposed to Methylphenidate Hydrochloride (MPH) (E0728701)

Responsible Division: Systems Biology

Collaborating Division(s): Regulatory Compliance & Risk Management, Neurotoxicology, Biochemical Toxicology, Veterinary Services, Personalized Nutrition and Medicine, Office of the Director

Objective(s):

The initial experiment was designed to examine the genetic toxicity associated with chronic MPH treatment. After approval of the two-year experiment described here, the initial year will begin after the completion of the genetic toxicity experiments. It is necessary to avoid procedures which could compromise those genetic toxicity data, specifically anesthesia and x-ray radiation. We are now proposing the continuation of dosing through the completion of puberty, to allow for evaluation of changes in pharmacokinetics and operant behavior testing.

PI: Salminen, William, Ph.D.

Magnetic Resonance Spectroscopy (MRS) of 13C-glucose and 13C-cysteine Fluxes in Rats after Treatment with Usnic Acid or Acetaminophen (P00740)

Responsible Division: Systems Biology

Collaborating Division(s):
Neurotoxicology

Objective(s):

In order to isolate rodent-liver cells, scientists and support staff in NCTR's Center for Hepatotoxicity , within the Division of Systems Biology require, training on the specialized isolation technique. Both rats and mice are required since both species are used in the whole-animal toxicology studies that are being modeled with the liver cells. In addition, some drugs exhibit greater toxicity in mice vs. rats and vice versa; therefore, it is important to be able to use both types of liver cells.

PI: Salminen, William, Ph.D.

Training and Maintaining Proficiency in Primary Rodent Hepatocyte Isolation (P00741)

Responsible Division: Systems Biology

Objective(s):

The isolation and culturing of rat and mouse liver cells is a critical technique for the Division of Systems Biology. These liver cells greatly reduce animal usage by allowing a single animal to supply enough cells for several experiments, as opposed to having to use multiple animals in a single experiment. Liver cells also allow detailed investigations into why and how certain drugs cause toxicity that are difficult to determine in the whole animal. These factors allow research into FDA-related issues to be conducted faster and with fewer animals.

PI: Tolleson, William, Ph.D.

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

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Associate Director of Scientific Coordination

Objective(s):

1) Characterize photochemical DNA damage in the skin of TP-ras/ink-4a mice exposed to UVA+UVB radiation.

2) Determine whether cutaneous malignant melanoma can be induced in neonatal TP-ras (+) ink4a (+/-) transgenic mice using UVA+UVB radiation.

3) Identify photochemically induced mutations within the ink4a/p16/CDKN2A and p53 loci in tumor tissues.

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

PI: Wang, Cheng, Ph.D.

Assessment of Ketamine in the Developing Nonhuman Primate (E0718901)

Responsible Division: Neurotoxicology

Collaborating Division(s): Office of Director, Biochemical Toxicology

Collaborating FDA Center(s): CDER

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.

4) Determine, at the most sensitive stage and dose, genomic and proteomic responses to ketamine treatment.

PI: Wang, Cheng, Ph.D.

Methods Development for High-Resolution Dedicated Positron Emission Tomography (microPET) to Rodent Neuroplasticity and Toxicity During Development (E0726401)

Responsible Division: Neurotoxicology

Collaborating Division(s): Office of the Director

Objective(s):

Advances in pediatric and obstetric surgery have resulted in an increase in complexity, duration, and number of anesthetic procedures. To minimize risks to children resulting from the use of anesthesia, it is necessary to understand the effects of anesthetic drugs on the developing nervous system. This study will utilize microPET to screen and evaluate *in vitro* and *in vivo* measurements from a broad range of pathophysiological or pharmacological parameters using specific tracers in the developing rat. Three different age groups of developing rats will be used: pregnant day 18 female rats, PND-7 rat pups, and PND-35 rats. This study will also attempt to elucidate the relationship between apoptosis identifying ligands (specific tracers) and subsequent behavioral deficits.

PI: Wang, Cheng, Ph.D.

Assessment of Gaseous Anesthetics in the Developing Nonhuman Primate (E0728501)

Responsible Division: Neurotoxicology

Collaborating Division(s): OD/Immediate Office

Collaborating FDA Center(s):

Objective(s):

1) Evaluate dose-response effects of gaseous anesthetics to determine if prolonged exposure to nitrous oxide or isoflurane alone affects the developing nonhuman primate.

2) Determine if a relative high dose or prolonged exposure of the developing nonhuman primates to nitrous oxide or isoflurane alone, or their combination will induce long-term behavioral deficits, as well as

long-lasting pathological changes.

3) Determine, using noninvasive imaging techniques, if a high dose or prolonged exposure of the developing nonhuman primates to nitrous oxide or isoflurane alone, or in combination will induce long-lasting pathological changes. MRI will be used to verify pathological evidence and look for volume changes. MicroPET will be used to examine the sensitivity for tracing low picomolar concentrations of radiolabeled molecules, which is useful for studying dynamic imaging in animal models of human diseases.

4) Identify potential underlying mechanisms that could link alteration of mitochondrial function and elevation of reactive oxygen species (ROS) to gaseous anesthetic-induced neuronal-cell death. L-carnitine will be used to attenuate neurological brain injury associated with mitochondria-related degenerative effects induced by gaseous anesthetics in the developing nonhuman primate.

PI: Wang, Cheng, Ph.D.

In Vitro Assay To Predict Developmental Neurotoxicity of Pediatric Anesthetics (E0740501)

Responsible Division: Neurotoxicology

Collaborating Division(s): Office of the Director

Collaborating FDA Center(s): CDER

Objective(s):

Expanding on findings from our previous protocol, we propose to use rodent *in vitro* organotypic and primary culture models to:

1) Examine additional anesthetics, including propofol (GABAA agonist), baclofen (GABAB agonist), diazepam (GABAA agonist), pentobarbital (GABAA agonist & AMPA antagonist), etomidate (GABAA agonist), sevoflurane (NMDA antagonist & GABA agonist), fentanyl (opiate agonist), and anesthetic combinations commonly used in pediatric surgical procedures.

2) Determine the utility of *in vitro* culture systems to predict *in vivo* outcomes in subsequent studies.

3) Determine the dose and time-course over which the potential neurotoxic effects of anesthetics are expressed in the developing brain.

4) Determine effective ways to protect against anesthetic-induced developmental neurotoxicity that have potential clinical utility.

5) Identify mechanisms that link altered NMDA receptor function and/or elevation of ROS to anesthetic-induced neuroapoptosis.

6) Identify biomarkers such as genomic pathway signatures and determine their validity for predicting *in vitro* outcomes of pediatric anesthetic exposure.

PI: Zhang, Xuan, Ph.D.

Assessment of the Pharmacokinetics, Pharmacodynamics, and Neurotoxic Effects of an Anesthetic in Juvenile Nonhuman Primates Undergoing Various Surgical Procedures (E0738101)

Responsible Division: Neurotoxicology

Collaborating Division(s): Office of the Director, Systems Biology, Biochemical Toxicology

Objective(s):

We propose a randomized controlled preclinical trial to assess the pharmacokinetics, pharmacodynamics, and neurologic effects of ketamine anesthesia in neonatal primates undergoing surgery. Neonatal rhesus monkeys will be randomly assigned to receive Ketamine 2 mg/kg prior to thoracotomy followed by a ketamine infusion of 0.5 mg/kg/hr during surgery (group A), OR an equivalent amount of placebo prior to initiation of surgery followed by a placebo infusion during surgery (group B.)

FY 2010 Publications

Publication is an essential component of research. All documents authored by NCTR investigators must undergo the NCTR Document Review and Approval Process, which consists of the review, clearance, and approval by the Center Director prior to submitting the publication to a journal. The list below identifies the NCTR-approved publications that were **accepted or published in journals in FY 2010, and book chapters that were accepted in FY 2010**.

1. Aidoo, A. and Manjanatha, M. (2011). Influence of Dietary Isoflavones Genistein and Daidzein on Genotoxicity and Mammary Carcinogenesis in Rats Exposed to the Model Carcinogen 7,12-dimethylbenz(a)anthracene (DMBA). *Vegetables, Whole Grains and Their Derivatives in Cancer Prevention*. 2: 142-173.
   Responsible Division: Genetic and Molecular Toxicology

2. Ali, S.F., Jiang, H., Rongzhu, L., Milatiovic, D., and Aschner, M. (2009). Methamphetamine dysregulates redox status in primary astrocyte and neuronal rat cultures. *American Journal of Neuroprotection and Neurodegeneration*. 1: 52-59.
   Responsible Division: Neurotoxicology

3. Alusta, P.S., Im, I., Pearce, B.A., Beger, R., Kretzer, R.M., Buzatu, D.A., and Wilkes, J.G. (2010). Improving proton MR spectroscopy of brain tissue for noninvasive diagnostics. *Journal of Magnetic Resonance Imaging.* 32: 818-829.
   Responsible Division: Systems Biology
   Co-Author Division: Office of Management

4. Andrade, J.E., Twaddle, N.C., Helferich, W.G., and Doerge, D.R. (2010). Absolute Bioavailability of Isoflavones from Soy Protein Isolate-Containing Food in Female BALB/C Mice. *Journal Agricultural Food Chemistry.* 58(7): 4529-36.
   Responsible Division: Biochemical Toxicology

5. Antunes, A.M., Godinho, A.L., Martins, I.L., Justino, G.C., Beland, F.A., and Marques, M.M. (2010). Amino acid adduct formation by the nevirapine metabolite, 12-hydroxynevirapine—a possible factor in nevirapine toxicity. *Chemical Research in Toxicology.* 23(5): 888-899.
   Responsible Division: Biochemical Toxicology

6. Antunes, A.M., Godinho, A.L., Martins, I.L., Oliveira, M., Gomes, R., Coelho, A., Beland, F.A., and Marques, M.M. (2010). Protein adducts as prospective biomarkers of nevirapine toxicity. *Chemical Research in Toxicology.* 23 (11): 1714-1725.
   Responsible Division: Biochemical Toxicology

7. Arasappan, D., Fang, H., Perkins, R.G., and Tong, W. (2010). Interpretation of toxicogenomics data. *Comprehensive Toxicology.* 2(34): 663-683.
Responsible Division: Systems Biology

8. Beger, R., Sun, J., and Schnackenberg, L. (2010). Metabolomics approaches for discovering biomarkers of drug-induced hepatotoxicity and nephrotoxicity *Toxicology and Applied Pharmacology.* 243: 154-166.
Responsible Division: Systems Biology

9. Binienda, Z.K., Beaudoin, M.A., Gough, B.J., Ali, S.F., and Virmani, A. (2010). Assessment of 3-nitropropionic acid-evoked peripheral neuropathy in rats: neuroprotective effects of acetyl-L-carnitine and resveratrol. *Neuroscience Letters.* 480: 117-121.
Responsible Division: Neurotoxicology

10. Boctor, S.Y. and Ferguson, S.A. (2010). Altered adult locomotor activity in rats from phencyclidine treatment on postnatal days 7, 9, and 11, but not repeated ketamine treatment on postnatal day 7. *Neurotoxicology and Teratology.* 31: 42-54.
Responsible Division: Neurotoxicology

11. Hong, H., Shi, L., Su, Z., Ge, W., Jones, W.D., Czika, W., Miclaus, K., Lambert, C.G., Vega, S.C., Zhang, J., Ning, B., Liu, J., Green, B., Xu, L., Fang, H., Perkins, R., Lin, S.M., Jafari, N., Park, K., Ahn, T., Chierici, M., Furlanello, C., Zhang, L., Wolfinger, R.D., Goodsaid, F., and Tong, W. (2010). Assessing sources of inconsistencies in genotypes and their effect on genome-wide association studies with HapMap samples. *The Pharmacogenomics Journal*. 10(4): 364-374.
Responsible Division: Systems Biology
Co-Author Division: Personalized Nutrition and Medicine

12. Castillo-Lluva, S., Tatham, M.H., Jones, R.C., Jaffray, E.G., Edmondson, R.D., Hay, R.T., and Malliri, A. (2010). SUMOylation of the GTPase Rac1 is required for optimal cell migration. *Nature Cell Biology.* 12(11):1078-85.
Responsible Division: Systems Biology

13. Chan, Y., Patterson, T.A., Bouaynaya, N., Chowdhury, P., Lesing, S., and Tarasenko, O. (2010). Predictive models of cognitive outcomes of developmental insults. *AIP Conference Proceedings.* 1229: 87-93.
Responsible Division: Neurotoxicology
Co-Author Division: Regulatory Compliance and Risk Management

14. Chen, H., Feng, J., Kweon, O., Xu, H., and Cerniglia, C.E. (2010). Identification and molecular characterization of a novel flavin-free NADPH preferred azoreductase encoded by azoB in Pigmentiphaga kulllae K24. *BMC Biochemistry.* 11: 13.
Responsible Division: Microbiology

15. Chen, J.J. and Tsai, C. (2010). Testing Significance of a Class of Genes. *Computational and Statistical Methods to Analyze Complex Diseases.* Book Chapter: 167-180.
Responsible Division: Personalized Nutrition and Medicine

16. Chen, T. (2010). The role of microRNA in chemical carcinogenesis. *Journal of Environment Science and Health, Part C Environmental Carcinogenesis and Ecotoxicology Reviews.* 28: 89-124.
Responsible Division: Genetic and Molecular Toxicology

17. Chen, T., Mei, N. and Fu, P.P. (2010). Genotoxicity of Pyrrolizidine Alkaloids. *Journal of Applied Toxicology.* 30(3): 183-96.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Division: Biochemical Toxicology

18. Chiang, H.H., Yin, J., Xia, Q., Zhao, Y., Fu, P.P., Wen, K., and Yu, H. (2010). Photoirradiation of azulene and guaiazulene—Formation of reactive oxygen species and induction of lipid peroxidation. *Journal of Photochemistry and Photobiology.* 211: 123-128.
Responsible Division: Biochemical Toxicology

19. Cimafranca, M.A., Davila, J., Ekman, G.C., Neese, S.L., Woodling, K.A., Flaws, J.A., Schantz, S., Doerge, D.R., and Cooke, P.S. (2010). A physiological mouse model for testing neonatal effects of the soy phytoestrogen genistein. *Biology of Reproduction.* 83: 114-121.
Responsible Division: Biochemical Toxicology
Co-Author Divisions: Neurotoxicology , Genetic and Molecular Toxicology

20. Cuevas, E., Lantz, S., Newport, G.D., Divine, B.L., Wu, Q., Paule, M.G., Tobon-Velasco, J.C., Ali, S.F., and Santamaria, A. (2010). On the early toxic effect of quinolinic acid: involvement of RAGE. *Neuroscience Letters.* 474: 74-78.
Responsible Division: Neurotoxicology
Co-Author Division: Biochemical Toxicology

21. David, D.E., Lynne, A.M., Han, J., and Foley, S.L. (2010). Evaluation of virulence factor profiling in the characterization of veterinary *Escherichia coli*. *Applied and Environmental Microbiology.* 76 (22):7509-13.
Responsible Division: Microbiology

22. Desai, V.G. (2011). Mitochondria-specific mouse gene array and its application in toxicogenomics. *Systems Toxicology: A Handbook.* 1 (3): 125-146.
Responsible Division: Systems Biology

23. Ding, D., Xu, L., Fang, H., Hong, H., Perkins, R.G., Harris, S.C., Bearden, E.D., Shi, L., and Tong, W. (2010). The EDKB, an established knowledge base for endocrine disrupting chemicals. *BMC Bioinformatics*. 11 Suppl 6:S5.
Responsible Division: Systems Biology

24. Dobrovolsky, V.N., Miura, D., Heflich, R.H., and Dertinger, S.D. (2010). The *in vivo* PIG-A gene mutation assay, a potential tool for regulatory safety assessment. *Environmental Molecular Mutagenesis.* 51: 825-835.
Responsible Division: Genetic and Molecular Toxicology

25. Doerge, D.R., Twaddle, N.C., Vanlandingham, M., and Fisher, J.W. (2010). Pharmacokinetics of Bisphenol A in neonatal and adult Sprague-Dawley rats. *Toxicology and Applied Pharmacology.* 247(2): 158-165.
Responsible Division: Biochemical Toxicology

26. Doerge, D.R., Twaddle, N.C., Woodling, K.A., and Fisher, J.W. (2010). Pharmacokinetics of Bisphenol A in neonatal and adult rhesus monkeys. *Toxicology Applied Pharmacology.* 248(1): 1-11.
Responsible Division: Biochemical Toxicology

27. Fan, X., Shi, L., Fang, H., Cheng, Y., Perkins, R.G., and Tong, W. (2010). DNA microarrays are predictive of cancer prognosis: a re-evaluation. *Clinical Cancer Research.* 16(2): 629-636.
Responsible Division: Systems Biology
Co-Author Division: Microbiology

28. Fang, H., Bushel, P.R., Woods, M., and Zhang, J. (2010). A comparison of batch effect removal methods for enhancement of prediction performance using MAQC-II microarray gene expression data. *The Pharmacogenomics Journal.* 10: 278-291.
Responsible Division: Systems Biology

29. Fang, H., Shi, L., and Tong, W. (2010). Consistency of predictive signature genes and classifiers generated using different microarray platforms. *The Pharmacogenomics Journal.* 10: 247-257.
Responsible Division: Systems Biology

30. Fang, H., Xu, Z., Ding, D., Jackson, S.A., Patel, I., Frye, J.G., Zou, W., Nayak, R.R., Foley, S.L., Chen, J.J., Su, Z., Ye, Y., Turner, S.A., Harris, S.C., Zhou, G., Cerniglia, C.E., and Tong, W. (2010). An FDA bioinformatics tool for microbial genomics research on molecular characterization of bacterial foodborne pathogens using microarrays. *BMC Bioinformatics.* 11(Supp 6): S4.
Responsible Division: Systems Biology
Co-Author Divisions: Personalized Nutrition and Medicine, Microbiology

31. Fang, J., Stingley, R.L., Beland, F.A., Harrouk, W., Lumpkins, D.L., and Howard, P. (2010). Occurrence, efficacy, metabolism, and toxicity of triclosan. *Journal of Environmental Carcinogenesis and Ecotoxicology Reviews/Journal of Environmental Science and Health-Part C.* 28(3): 147-71.
Responsible Division: Biochemical Toxicology
Co-Author Division: Associate Director of Scientific Coordination

32. Ferguson, S.A. and Berry, K.J. (2010). Chronic oral treatment with isotretinoin alters measures of activity, but not anxiety in male and female rats. *Neurotoxicology and Teratology.* 32: 573-578.
Responsible Division: Neurotoxicology

33. Ferguson, S.A. and Boctor, S.Y. (2010). Cocaine responsiveness or anhedonia in rats treated with methylphenidate during adolescence. *Neurotoxicology and Teratology.* 32: 432-442.
Responsible Division: Neurotoxicology

34. Formeister, E.J., Threadgill, D., Shpyleva, S.I., Pogribny, I.P., and Rusyn, I. (2010). Comparative analysis of promoter methylation and gene expression endpoints between tumorous and non-tumorous tissues from HCV-positive patients with hepatocellular carcinoma. *Mutation Research.* 692(1-2): 26-33.
Responsible Division: Biochemical Toxicology

35. Fu, P.P., Chou, M.W., Churchwell, M.I., Wang, Y., Zhao, Y., Xia, Q., Gamboa Da Costa, G., Marques, M.M., Beland, F.A., and Doerge, D.R. (2010). High-performance liquid chromatography electrospray ionization tandem mass spectrometry for the detection and quantitation of pyrrolizidine alkaloid-derived DNA adducts *in vitro* and *in vivo*. *Chemical Research in Toxicology.* 23(3): 637-652.
Responsible Division: Biochemical Toxicology

36. Gamboa Da Costa, G., Singh, R., Volker, A.M., Mirza, A., Takamura-Enya, T., Farmer, P.B., and Phillips, D.H. (2009). Quantification of 3-nitrobenzanthrone-DNA adducts using online column-switching HPLC-electrospray tandem mass spectrometry. *Chemical Research in Toxicology.* 22(11): 1860-8.
Responsible Division: Biochemical Toxicology

37. Garey, J.D. and Paule, M.G. (2010). Effects of Chronic Oral Acrylamide Exposure on Incremental Repeated Acquisition (Learning) Task Performance in Fischer 344 Rats. *Neurotoxicology and Teratology.* 32: 220-225.
Responsible Division: Neurotoxicology

38. Goodsaid, F.M. and Mendrick, D. (2010). Translational medicine and the value of biomarker qualification. *Science Translational Medicine.* 2(47): 47-44.
Responsible Division: Systems Biology

39. Guo, L., Fu, P.P., Fuscoe, J., and Mei, N. (2010). Application of DNA microarray in studies of herbal dietary supplements. *Handbook of Systems Toxicology.*
Responsible Division: Biochemical Toxicology
Co-Author Divisions: Genetic and Molecular Toxicology, Systems Biology

40. Guo, L., Mei, N., Liao, W., Chan, P.C., and Fu, P.P. (2010). Ginkgo biloba extract-induced gene expression changes in xenobiotics metabolism and Myc-centered network. *OMICS.* 14(1): 75-90.
Responsible Division: Systems Biology
Co-Author Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology

41. Guo, L., Mei, N., Xia, Q., Chen, T., Chan, P., and Fu, P.P. (2010). Gene expression profiling as an initial approach for mechanistic study of toxicity and tumorigenicity of herbal plants and herbal dietary supplements. *Journal of Environmental Health Sciences, Part C.* 28(1): 60-87.
Responsible Division: Biochemical Toxicology
Co-Author Divisions: Genetic and Molecular Toxicology, Systems Biology

42. Guo, L., Shi, Q., Dial, S.L., Xia, Q., Mei, N., Li, Q., Chan, P.C., and Fu, P.P. (2010). Gene expression profiling in male B6C3F1 mouse livers exposed to kava identifies changes in drug metabolizing genes and potential mechanisms linked to kava toxicity. *Food and Chemical Toxicology.* 48: 686-696.
Responsible Division: Systems Biology
Co-Author Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology

43. Hansen, D.K. (2010). Developmental Toxicity of Antiepileptic Drugs. *Comprehensive Toxicology, 2nd edition.* 12: 177-187.
Responsible Division: Personalized Nutrition and Medicine

44. Hart, M.E., Hart, M.J., and Roop, A.J. (2009). Genotypic and phenotypic assessment of hyaluronidase among type strains of a select group of *Staphylococcus* species. *International Journal of Microbiology.* 2009: 1-8.
Responsible Division: Microbiology

45. Holthoff, J.H., Woodling, K.A., Doerge, D.R., Burns, S.T., Hinson, J.A., and Mayeux, P.R. (2010). Resveratrol, a dietary polyphenolic phytoalexin, is a functional scavenger of peroxynitrite. *Biochemical Pharmacology.* 80(8): 1260-1265.
Responsible Division: Biochemical Toxicology
Co-Author Divisions: Neurotoxicology, Systems Biology

46. Hong, H., Goodsaid, F.M., Shi, L., and Tong, W. (2010). Molecular biomarker: a U.S. FDA effort. *Biomarkers in Medicine.* 4(2): 215-225.
Responsible Division: Systems Biology

47. Hong, H., Su, Z., Ge, W., Shi, L., Perkins, R.G., Fang, H., Mendrick, D., and Tong, W. (2010). Evaluating variations of genotype calling: a potential source of spurious associations in genome-wide association studies. *Journal of Genetics.* 89(1): 55-64.
Responsible Division: Systems Biology

48. Hong, H., Xu, L., and Tong, W. (2010). Assessing consistency between versions of genotype-calling algorithm Birdseed for the genome-wide human SNP Array 6.0 using HapMap samples. *Advances in Computational Biology.* 680(4): 355-360.
Responsible Division: Systems Biology

49. Hsu, A., Bray, T., Helferich, W., Doerge, D.R., and Ho, E. (2010). Differential effects of whole soy extract and soy isoflavones on apoptosis in prostate cancer cells. *Exerimental Biology and Medicine.* 235(1): 90-7.
Responsible Division: Biochemical Toxicology

50. Iverson, A.R., Boyd, K.L., McAuley, J.L., Plano, L.R., Hart, M.E., and Mccullers, J.A. (2011). Influenza virus primes mice for pneumonia from *Staphylococcus aureus*. *Journal of Infectious Diseases*. 203(6): 880-8.
Responsible Division: Microbiology

51. Jackson, L.S., Zhang, Z., and Tolleson, W.H. (2010). Thermal stability of ricin in orange and apple juices. *Journal of Food Science.* 75: T65-T71.
Responsible Division: Biochemical Toxicology

52. Kaput, J. (2010). Challenges of molecular nutrition research 6: The Nutritional Phenotype database to store, share, and evaluate nutritional systems biology studies. *Genes and Nutrition.* 5(3):189-203.
Responsible Division: Personalized Nutrition and Medicine

53. Kaput, J. (2010). Using genetics to tackle malnutrition. *Science and Development Network.* January 20, 2010: Epub.
Responsible Division: Personalized Nutrition and Medicine

54. Kaput, J., Van Ommen, B., Perozzi, G., Evelo, C., and Cotton, R. (2010). Connecting the Human Variome Project to Nutrigenomics. *Genes and Nutrition.* 5(4): 275–283.
Responsible Division: Personalized Nutrition and Medicine

55. Kodell, R.L. and Chen, J.J. (2010). Is pre-market identification of hepatotoxic drugs and sensitive patients possible based on hi-dimensional 'omic' data? *Future Medicine—Personalized Medicine.* 7: 8.
Responsible Division: Personalized Nutrition and Medicine

56. Kulkarni, R.M., Petibone, D., Chang, C., Chen, J.J., Tolleson, W.H., Melchior, W.B., Churchwell, M.I., Beland, F.A., and Morris, S.M. (2011). Effect of p53 Genotype on Gene Expression and DNA Adducts in ENU-Exposed Mice. *Handbook of Systems Toxicology.* Volume 1: 53-66. ISBN: 978-0-470-68401-6.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Divisions: Biochemical Toxicology, Personalized Nutrition and Medicine

57. Kutanzi, K.R., Koturbash, I., Bronson, R.T., Pogribny, I.P., and Kovalchuk, O. (2010). Imbalance between apoptosis and cell proliferation during early stages of mammary gland carcinogenesis in ACI rats. *Mutation Research.* 694(1-2): 1-6.
Responsible Division: Biochemical Toxicology

58. Kwekel, J.C., Desai, V.G., Moland, C.L., Branham, W.S., and Fuscoe, J. (2010). Age- and sex- dependent changes in liver gene expression during the life cycle of the rat. *BMC Genomics*. 11:675.
Responsible Division: Systems Biology

59. Kweon, O., Kim, S., Freeman, J.P., Song, J., Baek, S., and Cerniglia, C.E. (2010). Substrate specificity and structural characteristics of the novel rieske nonheme iron aromatic ring-hydroxylating oxygenases NidAB and NidA3B3 from Mycobacterium vanbaalenii PYR-1. *Molecular BIology.* 1(2): 1-11.
Responsible Division: Microbiology
Co-Author Divisions: Biochemical Toxicology, Personalized Nutrition and Medicine

60. Goodsaid, F.M., Amur, S., Aubrecht, J., Burczynski, M.E., Carl, K., Catalano, J., Charlab, R., Close, S., Cornu-Artis, C., Essioux, L., Fornace, A.J., Jr, Hinman, L., Hong, H., Hunt, I., Jacobson-Kram, D., Jawaid, A., Laurie, D., Lesko, L., Li, H.H., Lindpaintner, K., Mayne, J., Morrow, P., Papaluca-Amati, M., Robison, T.W., Roth, J., Schuppe-Koistinen, I., Shi, L., Spleiss, O., Tong, W., Truter, S.L., Vonderscher, J., Westelinck, A., Zhang, L., and Zineh, I. (2010). Voluntary exploratory data submissions to the U.S. FDA and the EMA: experience and impact. *Nature Reviews Drug Discovery*. 9: 435-445.
Responsible Division: Systems Biology

61. Levis, S., Strickman-Stein, N., Doerge, D.R., and Krischer, J. (2010). Design and baseline characteristics of the soy phytoestrogens as replacement estrogen (SPARE) study—a clinical trial of the effects of soy isoflavones in menopausal women. *Contemporary Clinical Trials.* 31(4): 293-302.
Responsible Division: Biochemical Toxicology

62. Li, Z., Branham, W.S., Dial, S.L., Wang, Y., Guo, L., Shi, L., and Chen, T. (2010). Genomic analysis of microRNA time-course expression in liver of mice treated with genotoxic carcinogen N-ethyl-N-nitrosourea. *BMC Genomics.* 11: 609.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Division: Systems Biology

63. Liao, G., Wen, Z., Irizarry, K., Huang, Y., Mitsouras, K., Darmani, M., Leon, T., Shi, L., and Bi, X. (2010). Abnormal gene expression in cerebellum of Npc1-/- mice during postnatal development. *Brain Research.* 1325: 128-140.
Responsible Division: Systems Biology

64. Lin, G., Xia, Q., Li, N., and Fu, P.P. (2011). Hepatotoxicity and tumorigenicity induced by metabolic activation of pyrrolizidine alkaloids in herbs. *Current Drug Metabolism.* Publication pending.
Responsible Division: Biochemical Toxicology

65. Lin, W. and Chen, J.J. (2010). Power and sample-size estimation in microarray studies. *BMC Bioinformatics.* 11:48.
Responsible Division: Personalized Nutrition and Medicine

66. Lin, Y., Barker, E., Kislow, J., Kaldhone, P., Stemper, M.E., Pantrangi, M., Moore, F.M., Hall, M., Fritsche, T.R., Novicki, T., Foley, S.L., and Shukla, S.K. (2010). Evidence of multiple virulence subtypes in nosocomial and community-associated MRSA genotypes in companion animals from the upper midwestern and northeastern United States. *Clinical Medicine and Research*. September 2, 2010: Epub ahead of print.
Responsible Division: Microbiology

67. Liu, F., Paule, M.G., Ali, S.F., and Wang, C. (2011). Ketamine-induced neurotoxicity and changes in gene expression in the developing rat brain. *Current Neuropharmacology.* 9: 256-261.
Responsible Division: Neurotoxicology

68. Liu, F., Zou, X., Sadovova, N.V., Zhang, X., Shi, L., Guo, L., Qian, F., Wen, Z., Patterson, T.A., Hanig, J.P., Paule, M.G., Slikker, W., and Wang, C. (2010) Changes in gene expression after phencyclidine administration in developing rats: a potential animal model for schizophrenia. *International Journal of Developmental Neuroscience.* August 5, 2010: Epub ahead of print.
Responsible Division: Neurotoxicology
Co-Author Divisions: Office of the Director, Systems Biology

69. Lucas, P.W., Schmit, J.M., Peterson, Q.P., West, D.C., Hsu, D.C., Novotny, C.J., Dirikolu, L., Churchwell, M.I., Doerge, D.R., Garrett, L.D., Hergenrother, P.J., and Fan, T.M. (2010). Pharmacokinetics and derivation of an anticancer dosing regimen for PAC-1 in healthy dogs*. Investigational New Drugs*. May 25, 2010: Epub ahead of print.
Responsible Division: Biochemical Toxicology

70. Lyn-Cook, Jr., L.E., Tareke, E., Word, B.R., Starlard-Davenport, A., Lyn-Cook, B.A., and Hammons, G.J. (2011). Food contaminant acrylamide increases expression of cox-2 and nitric oxide synthase in breast epithelial cells. *Toxicology and Industrial Health.* 27(1): 11-8.
Responsible Division: Associate Director for Regulatory Activities
Co-Author Divisions: Biochemical Toxicology, Neurotoxicology

71. MAQC Consortium, T. (2010). The MicroArray Quality Control (MAQC)-II study of common practices for the development and validation of microarray-based predictive models. *Nature Biotechnology.* 28: 827-838.
Responsible Division: Systems Biology

72. Mei, N., Guo, L., Luan, Y., Fu, P.P., Fuscoe, J., and Chen, T. (2010). Metabolism, genotoxicity, and carcinogenicity of comfrey. *Journal of Toxicology and Environmental Health, Part B: Critical Reviews.* 13(7-8): 509-26.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Divisions: Biochemical Toxicology, Systems Biology

73. Mei, N., Guo, X., Verkler, T., Chen, Y., Polzin, G.M., Richter, P., Moore, M., and Mei, N. (2010). Mutagenicity of 11 cigarette smoke condensates in two versions of the mouse lymphoma assay. *Mutagenesis*. Oct 27, 2010: Epub ahead of print.
Responsible Division: Genetic and Molecular Toxicology

74. Mei, N., McDaniel, L.P., Dobrovolsky, V.N., Guo, X., Shaddock, J.G., Mittelstaedt, R.A., Azuma, M., McGarrity, L.J., Doerge, D.R., and Heflich, R.H. (2010). The genotoxicity of acrylamide and glycidamide in Big Blue Rats. *Toxicological Sciences.* 115(2): 412-421.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Division: Biochemical Toxicology

75. Mei, N., McDaniel, L.P., Wang, R., Manjanatha, M., Shelton, S.D., and Doerge, D.R. (2010). Mutagenicity of Acrylamide and glycidamide in the testes of Big Blue Mice. *Toxicological Sciences*. 117(1): 72-80.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Division: Biochemical Toxicology

76. Mei, N., Xia, Q., Fu, P.P., Moore, M., and Chen, T. (2010). Cytotoxicity and mutagenicity of retinol with ultraviolet A irradiation in mouse lymphoma cells. *Toxicology In Vitro.* 24(2): 439-44.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Division: Biochemical Toxicology

77. Melendez, S., Hanning, I., Han, J., Nayak, R.R., Clement, A., Wooming, A., Hererra, P., Jones, F., Foley, S.L., and Ricke, S. (2010). *Salmonella enterica* isolates from pasture-raised poultry exhibit antimicrobial resistance and class I integrons. *Journal of Applied Microbiology*. 109: 1957-1966.
Responsible Division: Microbiology

78. Mendoza, M. (2010). The path to personalized medicine—future challenges and opportunities for health information management professionals in the post-genomic era. *Journal of the American Health Information Management Association.* 11: 38-42.
Responsible Division: Personalized Nutrition and Medicine

79. Meng, F., Parsons, B.L., Andersen, M.E., Mckinzie, P.B., Bermudez, E., and Clewell, H. (2010). Measurement of tumor-associated mutations in the nasal mucosa of rats exposed to varying doses of formaldehyde. *Regulatory Pharmacology and Toxicology.* 57: 274-283.
Responsible Division: Genetic and Molecular Toxicology

80. Mercer, K.E., Apostolov, E.O., Gamboa Da Costa, G., Yu, X., Lang, P.M., Roberts, D.W., Davis, W., Basnakian, A.G., Kadlubar, F.F., and Kadlubar, S. (2010). 4-sulfoxy-tamoxifen produced in SULT1A1 expressing breast-cancer cells induces apoptosis. *International Journal of Cancer.* 1: 92-103.
Responsible Division: Biochemical Toxicology

81. Miclaus, K., Chierici, M., Lambert, C.G., Zhang, L., Vega, S.C., Hong, H., Yin, S., Furlanello, C., Wolfinger, R.D., and Goodsaid, F.M. (2010). Variability in GWAS analysis: the impact of genotype calling algorithm inconsistencies. *The Pharmacogenomics Journal.* 10: 324-335.
Responsible Division: Systems Biology

82. Miclaus, K., Wolfinger, R.D., Vega, S.C., Chierici, M., Furlanello, C., Lambert, C.G., Hong, H., Zhang, L., Yin, S., and Goodsaid, F.M. (2010). Batch effects in the BRLMM genotype calling algorithm influence GWAS results for the Affymetrix 500K array. *The Pharmacogenomics Journal.* 10: 336-346.
Responsible Division: Systems Biology

83. Moore, M., Thybaud, V., MacGregor, J.T., Dearfield, K.L., and Douglas, G. (2010). Strategies in case of positive *in vivo* results in genotoxicity testing. *Mutation Research*. Sep 17, 2010: Epub ahead of print.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Division: Associate Director for Regulatory Activities

84. Nawaz, M.S., Khan, S.A., Khan, A.A., Sung, K., Tran, Q., Kerdahi, K.F., and Steele, R.S. (2010). Detection and characterization of virulence genes and integrons in *Aeromonas* veronii isolated from catfish. *Food Microbiology.* 27: 327-331.
Responsible Division: Microbiology

85. Nayak, R.R., Foley, S.L. (2011). *Campylobacter jejuni*. *FDA.gov.* Chapter 2.
Responsible Division: Microbiology

86. Neese, S.L., Wang, V.C., Doerge, D.R., Woodling, K.A., Helferich, W.G., Korol, D.L., and Schantz, S. (2010). Impact of dietary genistein and aging on executive function in rats. *Neurotoxicology and Teratology.* 32(2): 200-11.
Responsible Division: Biochemical Toxicology
Co-Author Division: Neurotoxicology

87. Oberthuer, A., Juraeva, D., Li, L., Kahlert, Y., Westermann, F., Eils, R., Berthold, F., Shi, L., Wolfinger, R.D., Fischer, M., and Brors, B. (2010). Comparison of performance of one-color versus two-color gene-expression analyses in predicting clinical endpoints of neuroblastoma patients. *Pharmacogenomics.* 10: 258-266.
Responsible Division: Systems Biology

88. Tong, W., Shi, L., and Nikolsky, Y. (2010). Functional analysis of multiple genomic signatures demonstrates that classification algorithms choose phenotype-related genes. *The Pharmacogenomics Journal.* 10: 310-323.
Responsible Division: Systems Biology

89. Park, M., Rooney, A.P., Hecht, D., Li, J., Mcclane, B.A., Nayak, R.R., Paine, D.D., and Rafii, F. (2010). Phenotypic and genotypic characterization of tetracycline and minocycline resistance in *Clostridium perfringens*. *Archives of Microbiology.* 192(10): 803-10.
Responsible Division: Microbiology

90. Parry, R., Jones, W.D., Stokes, T.H, Phan, J.H., Moffitt, R.A., Fang, H., Shi, L., Oberthuer, A., Fischer, M., Tong, W., and Wang, M.D. (2010). K-nearest neighbors models for microarray gene expression analysis and clinical outcome prediction. *The Pharmacogenomics Journal.* 10: 292-309.
Responsible Division: Systems Biology

91. Parsons, B.L. and Meng, F. (2009). K-RAS mutation in the screening, prognosis, and treatment of cancer. *Biomarkers in Medicine.* 3(6): 757-769.
Responsible Division: Genetic and Molecular Toxicology

92. Parsons, B.L., Marchant-Miros, K.E., Delongchamp, R.R., Verkler, T., Patterson, T.A., McKinzie, P.B., and Kim, L.T. (2010). Defining normal and pathological levels of tumor-associated mutations. *Regulatory Research Perspectives.* 9(1): 1-14.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Division: Neurotoxicology

93. Parsons, B.L., Myers, M.B., Meng, F., Wang, Y., and McKinzie, P.B. (2010). Oncomutations as biomarkers of cancer risk. *Environmental and Molecular Mutagenesis.* 51(8-9): 836-50.
Responsible Division: Genetic and Molecular Toxicology

94. Parsons, B.L., Verkler, T., Patterson, T.A., McKinzie, P.B., Marchant-Miros, K.E., Delongchamp, R.R., and Kim, L.T. (2010). ACB-PCR Quantification of K-RAS codon 12 GAT and GTT mutant fraction in colon tumor and non-tumor tissue: Insight into field cancerization. *Cancer Investigation.* 28: 364-365.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Division: Neurotoxicology

95. Patterson, T.A., Li, M., Hotchkiss, C.E., and Paule, M.G. (2010). Toxicity assessment of pramipexole in juvenile rhesus monkeys. *Toxicology.* 276(3): 164-171.
Responsible Division: Neurotoxicology

96. Patterson, T.A. and Paule, M.G. (2011). Cognitive assessments in nonhuman primates. *Fundamental Neuropathology for Pathologists and Toxicologists: Principles and Techniques.* Book chapter at publisher.
Responsible Division: Neurotoxicology

97. Patterson, T.A., Schnackenberg, B., Slikker, W., and Wang, C. (2011). Systems biology approaches to neurotoxicity studies during development. *Developmental Neurotoxicology Research: Principles, Models, Techniques, Strategies and Mechanisms.* Chapter 2: 25-38.
Responsible Division: Neurotoxicology
Co-Author Division: Office of the Director

98. Paule, M.G. (2010). The nonhuman primate as a translational model for pesticide research. *Hayes Handbook of Pesticide Toxicology 3rd Edition.* Chapter 34: 847-859.
Responsible Division: Neurotoxicology

99. Paule, M.G. (2010). Behavioral approaches for assessing nervous system function during development in animal models. *Developmental Neurotoxicology Research: Principles, Models, Techniques, Strategies and Mechanisms.* Chapter 3: 39-52.
Responsible Division: Neurotoxicology

100. Paule, M.G. and Slikker, W. (2010). Developmental Neurotoxicity. *Reproductive Toxicology 3rd Edition, Informational Healthcare.* 157-167.
Responsible Division: Neurotoxicology
Co-Author Division: Office of the Director

101. Piper, B.J., Ali, S.F., Daniels, L.G., and Meyer, J. (2010). Repeated intermittent methylenedioxymethamphetamine exposure protects against the behavioral and neurotoxic, but not hyperthermic, effects of an MDMA binge in adult rats. *Synapse.* 64(6): 421-431.
Responsible Division: Neurotoxicology

102. Pogribny, I.P., Filkowski, J., Tryndyak, V.P., Golubov, A., Shpyleva, S.I., and Kovalchuk, O. (2010). Alterations of microRNAs and their targets are associated with acquired resistance of MCF-7 breast cancer cells to cisplatin. *International Journal of Cancer.* 31: 1785-1794.
Responsible Division: Biochemical Toxicology

103. Pogribny, I.P., Muskhelishvili, L., Tryndyak, V.P., and Beland, F.A. (2010). The role of epigenetic events in genotoxic hepatocarcinogenesis induced by 2-acetylaminofluorene. *Mutation Research*. Feb 25, 2010: Epub ahead of print.
Responsible Division: Biochemical Toxicology

104. Pogribny, I.P., Starlard-Davenport, A., Tryndyak, V.P., Han, T., Ross, S., Rusyn, I., and Beland, F.A. (2010). Difference in expression of hepatic microRNAs miR-29c, miR-34a, miR-155, and miR-200b is associated with strain-specific susceptibility to dietary nonalcoholic steatohepatitis in mice. *Lab Investigation.* 90(10): 1437-46.
Responsible Division: Biochemical Toxicology
Co-Author Division: Systems Biology

105. Powell, C., Bradford, B., Craig, C., Tsuchiya, M., Uehara, T., O'Connell, T., Pogribny, I.P., Melnyk, S.B., Koop, D., Bleyle, L., Threadgill, D., and Rusyn, I. (2010). Mechanism for prevention of alcohol-induced liver injury by dietary methyl donors. *Toxicology Science.* 115(1): 131-139.
Responsible Division: Biochemical Toxicology

106. Prabhu, B.M., Ali, S.F., Srivatsan, M., and Hussain, S.M. (2010). Copper nanoparticles exert size and concentration-dependent toxicity on somatosensory neurons of rat. *Nanotoxicology.* 4(2): 150-160.
Responsible Division: Neurotoxicology
Co-Author Division: Systems Biology

107. Rafii, F., Park, M., Gamboa Da Costa, G., and Camacho, M. (2009). Comparison of the metabolic activities of four wild-type *Clostridium perfringens* strains with their gatifloxacin-selected resistant mutants. *Archieves of Microbiology.* 191: 895-902.
Responsible Division: Microbiology
Co-Author Division: Biochemical Toxicology

108. Robison, T.W., Roth, J., Schuppe-Koistinen, I., Shi, L., Tong, W., Truter, S.L., Vonderscher, J., Westelink, A., Zhang, L., and Zineh, I. (2010). Biomarkers in drug development: experience and impact of the U.S. FDA Voluntary Exploratory Data Submissions (VXDS). *Nature Reviews in Drug Discovery.* 9: 435-450.
Responsible Division: Systems Biology

109. Rocca-Serra, P., Brandizi, M., Maguire, E.J., Sklyar, N., Taylor, C.F., Begley, K., Field, D., Harris, S.C., Hide, W., Hofmann, O., Neumann, S., Sterk, P., Tong, W., and Sansone, S. (2010). ISA software suite: supporting standards-compliant experimental annotation and enabling curation at the community level. *Bioinformatics.* 26(18): 2354.
Responsible Division: Systems Biology

110. Rodriguez, J.., Morris, S.M., Hotchkiss, C.E., Doerge, D.R., Allen, R.R., Mattison, D., and Paule, M.G. (2010). The effects of chronic methylphenidate administration on cognitive function in juvenile rhesus monkeys. *Neurotoxicology and Teratology.* 32(2): 142-151.
Responsible Division: Neurotoxicology
Co-Author Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology

111. Ross, J., Blackman, C.F., Thai, S., Li, Z., Chen, T., Kohan, M., and Jones, C.P. (2010). A potential microRNA signature for tumorigenic conazoles in mouse liver. *Molecular Carcinogenesis.* 49(4): 320-3.
Responsible Division: Genetic and Molecular Toxicology

112. Sarkar, S. and Schmued, L.C. (2010). Kainic acid and 3-nitropropionic acid-induced expression of laminin in vascular elements of the rat brain. *Brain Research*. 1352: 239-47.
Responsible Division: Neurotoxicology

113. Sarkar, S. and Schmued, L.C. (2010). Neurotoxicity of ecstasy (MDMA): An overview. *Current Pharmaceutical Biotechnology.* 11(5): 460-469.
Responsible Division: Neurotoxicology

114. Schnackenberg, B., Saini, U.T., Robinson, B.L., Ali, S.F., and Patterson, T.A. (2010). An acute dose of gamma-hydroxbutyric acid (GHB) alters gene expression in multiple mouse brains. *Neuroscience*. 170(2): 523-41.
Responsible Division: Neurotoxicology

116. Schnackenberg, L., Sun, J., and Beger, R. (2011). Metabolomics in systems toxicology: towards personalized medicine. *Systems Toxicology: A Handbook.* 1(5): 267-290.
Responsible Division: Systems Biology

117. Schnackenberg, L., Sun, J., Pence, L.M., Bhattacharyya, S., Sonko, B.J., and Beger, R. (2010). Metabolomics for personalized nutrition research and clinical applications: a 2010 update. *Current Pharmacogenomics and Personalized Medicine.* 8(3): 202-217.
Responsible Division: Systems Biology

118. Shao, L., Wu, L., Fang, H., Tong, W., and Fan, X. (2010). Does applicability domain exist in microarray-based genomic research? *PLoS ONE.* 5(6): e11055.
Responsible Division: Systems Biology

119. Sharma, H.S., Ali, S.F., Tian, Z.R., Patnaik, S., Sharma, A., Boman, A., Lek, P., Seifert, E., and Lunsdstedt, T. (2010). Nanowired-drug delivery enhances neuroprotective efficacy of compounds and reduces spinal cord edema formation and improves functional outcome following spinal cord injury in the rat. *Acta Neurochirurgica Supplement.* 106: 343-350.
Responsible Division: Neurotoxicology

120. Shi, Q., Guo, L., Patterson, T.A., Dial, S.L., Li, Q., Sadovova, N.V., Zhang, X., Hanig, J.P., Paule, M.G., Slikker, W., and Wang, C. (2010). Gene expression profiling in the developing rat brain exposed to ketamine. *Neuroscience.* 166(3): 852-863.
Responsible Division: Systems Biology
Co-Author Divisions: Office of the Director, Neurotoxicology, Genetic and Molecular Toxicology

121. Shi, Q., Hong, H., and Tong, W. (2010). Biomarkers for drug-induced liver injury. *Expert Review of Gastroenterology and Hepatology.* 4(2): 225-234.
Responsible Division: Systems Biology

122. Shpyleva, S.I., Tryndyak, V.P., Kovalchuk, O., Starlard-Davenport, A., Chekhun, V.F., Beland, F.A., and Pogribny, I.P. (2011). Role of ferritin alterations in human breast cancer cells. *Breast Cancer Research.* 126(1): 63-71.
Responsible Division: Biochemical Toxicology

123. Singh, R., Arlt, V.M., Henderson, C.J., Wolf, R.C., Steward, W.P., Phillips, D.H., Farmer, P.B., and Gamboa Da Costa, G. (2010). Detection and quantitation of N-(deoxyguanosin-8-yl)-2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine adducts in DNA using online column-switching liquid chromatography tandem mass spectrometry. *Journal of Chromatography B.* 878(23): 2155-2162.
Responsible Division: Biochemical Toxicology
Co-Author Division: Genetic and Molecular Toxicology

124. Slikker, W., Liu, F., Zhang, X., Zou, X., Patterson, T.A., Paule, M.G., and Wang, C. (2011). Perinatal rhesus monkey models and anesthetic-induces neuronal cell death. *Developmental Neurotoxicology Research: Principles, Models, Techniques, Strategies and Mechanisms.* Chapter 6: 95-110.
Responsible Division: Neurotoxicology
Co-Author Division: Office of the Director, Regulatory Compliance and Risk Management

125. Starlard-Davenport, A., Tryndyak, V.P., James, S.R., Karpf, A.R., Latendresse, J.R., Beland, F.A., and Pogribny, I.P. (2010). Mechanisms of epigenetic silencing of the *Rassf1a* gene during estrogen-induced breast carcinogenesis in ACI rats. *Carcinogenesis.* 31(3): 376-81.
Responsible Division: Biochemical Toxicology

126. Starlard-Davenport, A., Tryndyak, V.P., Rusyn, I., Beland, F.A., and Pogribny, I.P. (2010). Dietary methyl deficiency, microRNA expression and susceptibility to liver carcinogenesis. *World Review of Nutrition and Dietetics.* 101: 123-130.
Responsible Division: Biochemical Toxicology

127. Stumbo, P.M., Weiss, R., Newman, J.W., Pennington, J.A., Tucker, K.L., Illner, A., Troiano, R.P., Klurfeld, D.M., and Kaput, J. (2010). Omics and environmental assessments: Survey, needs, and plans of the U.S. nutrition and nutrigenomic communities. *Journal of Nutrition.* 5: 275-283.
Responsible Division: Personalized Nutrition and Medicine

128. Su, Z., Fang, H., Tong, W., Hong, H., Perkins, R.G., and Shi, L. (Scheduled for publication in 2011). Approaches and practical considerations for the analysis of toxicogenomics data. *Application of Toxicogenomics in Safety Evaluation and Risk Assessment.*
Responsible Division: Systems Biology

129. Sullivan, S.Z., Ghosh, A., Biris, A.S., Pulla, S., Brezden, A.M., Collom, S.L., Woods, R.M., Munshi, P., Schnackenberg, L., Pierce, B.S., and Kannarpady, G.K. (2010). Fe-complex of a tetraamido macrocyclic ligand: spectroscopic characterization and catalytic oxidation studies. *Chemical Physics Letters.* 498: 359-365.
Responsible Division: Systems Biology

130. Sun, J., Hansen, D.K., Schnackenberg, L., and Beger, R. (2010). Study of valproic acid-induced endogenous and exogenous metabolite alterations using LC-MS-based metabolomics. *Bioanalysis.* 2: 207-216.
Responsible Division: Systems Biology
Co-Author Division: Personalized Nutrition and Medicine

131. Sun, J., Schnackenberg, L., Pence, L.M., Bhattacharyya, S., Doerge, D.R., Bowyer, J.F., and Beger, R. (2010). Metabolomic analysis of urine from rats chronically dosed with acrylamide using NMR and LC/MS. *Metabolomics.* 6: 550-563.
Responsible Division: Systems Biology
Co-Author Divisions: Biochemical Toxicology, Neurotoxicology

132. Thomas, M., George, N., Saini, U.T., Patterson, T.A., Hanig, J.P., and Bowyer, J.F. (2010). Endoplasmic reticulum stress responses differ in meninges and associated vasculature, striatum, and parietal cortex after a neurotic amphetamine exposure. *Synapse.* 64: 579-593.
Responsible Division: Neurotoxicology
Co-Author Division: Personalized Nutrition and Medicine

133. Tong, W. and Mendrick, D. (2010). Genomics. *Biomarkers in Medicine, Drug Discovery, and Environmental Health.* 1(2): 7-24.
Responsible Division: Systems Biology

134. Tong, W. and Mendrick, D. (Scheduled for publication Sept 2012). Biomarker discovery, knowledge base. *Encyclopedia of Systems Biology.*
Responsible Division: Systems Biology

135. Tong, W. and Mendrick, D. (Scheduled for publication Sept 2012). Biomarkers. *Encyclopedia of Systems Biology.*
Responsible Division: Systems Biology

136. Tong, W., Fang, H., and Bushel, P.R. (2010). Genomic indicators in the blood predict drug-induced liver injury. *Pharmacogenomics.* 10: 267-277.
Responsible Division: Systems Biology

137. Tong, W., Shi, Q., Salminen, W.F., Chen, M., Fang, H., Suzuki, A., and Mendrick, D. (2011). Unraveling sex differences in drug-induced liver injury. *Systems Toxicology: A Handbook.* 1(7): 419-432.
Responsible Division: Systems Biology

138. Trickler, W.J., Lantz, S., Robinson, B.L., Newport, G.D., Paule, M.G., Slikker, W., and Ali, S.F. (2010). Silver nanoparticles-induced blood-brain barrier inflammation and increased permeability in primary rat brain microvessel endothelial cells. *Journal of Toxicological Science.* 118(1): 160-170.
Responsible Division: Neurotoxicology
Co-Author Division: Office of the Director

139. Tryndyak, V.P., Beland, F.A., and Pogribny, I.P. (2010). E-cadherin transcriptional down-regulation by epigenetic and microRNA-200 family alterations is related to mesenchymal and drug-resistant phenotypes in human breast-cancer cells. *Journal of Cancer.* 126(11): 2575-83.
Responsible Division: Biochemical Toxicology

140. Tryndyak, V.P., Han, T., Muskhelishvili, L., Fuscoe, J., Ross, S., Beland, F.A., and Pogribny, I.P. (2010). Coupling global methylation and gene expression profiles reveals key pathophysiological events in liver injury induced by a methyl-deficient diet. *Molecular Nutrition and Food Research*. Oct 11, 2010: Epub ahead of print.
Responsible Division: Biochemical Toxicology
Co-Author Division: Systems Biology

141. Twaddle, N.C., Churchwell, M.I., Vanlandingham, M., and Doerge, D.R. (2010). Quantification of deuterated Bisphenol A in serum, tissues, and excreta from adult Sprague-Dawley rats using liquid chromatography with tandem mass spectrometry. *Rapid Communication Mass Spectrometry.* 24(20): 3011-20.
Responsible Division: Biochemical Toxicology

142. Valentine, C.R., Delongchamp, R.R., Pearce, M.G., Rainey, H.F., Dobrovolsky, V.N., and Malling, H. (2010). *In vivo* mutation analysis using the X174 Transgenic Mouse and comparison with other transgenes and endogenous genes. *Mutation Research*. 705(3): 205-16.
Responsible Division: Genetic and Molecular Toxicology
Co-Author Division: Personalized Nutrition and Medicine

143. Varma, V., Wise, C.K., and Kaput, J. (2010). Data mining carbohydrate metabolic pathway genes associated with Quantitative Trait Loci (QTLs) for obesity and type 2 diabetes. *Biotechnology Journal.* 5(9): 942-949.
Responsible Division: Personalized Nutrition and Medicine

144. Wang, C., Guo, L., Patterson, T.A., and Slikker, W. (2011) Application of Systems Biology in Neurotoxicology Studies during Development. *Handbook of Systems Biology.* Chapter 9: 115-123.
Responsible Division: Neurotoxicology
Co-Author Divisions: Office of the Director, Systems Biology

145. Wang, C., Zhang, X., Liu, F., Paule, M.G., and Slikker, W. (2010). Anesthetic-induced oxidative stress and potential protection. *The Scientific World Journal.* 10: 1472-1482.
Responsible Division: Neurotoxicology
Co-Author Division: Office of the Director

146. Wang, C., Zhang, X., Liu, F., Paule, M.G., and Slikker, W. (2011). Alterations in *N*-methyl-D-Aspartate (NMDA) Receptor Function and Potential Involvement in Anesthetic-Induced Neurodegeneration. *Neurodegeneration: Theory, Disorders, and Treatments.* Book Chapter: 155-172.
Responsible Division: Neurotoxicology
Co-Author Division: Office of the Director

147. Wang, J.C., Ali, S.F., and Sun, W. (2009). Nanoparticles: Is neurotoxicity a concern? *Nanotoxicology: From In Vivo and In Vitro Models to Health Risks.* 171-180.
Responsible Division: Neurotoxicology

148. Wang, J.C., Rahman, M.F., Duhart, H.M., Newport, G.D., Patterson, T.A., Schlatter, J., and Ali, S.F. (2009). Expression changes of dopaminergic system-related genes in PC12 cells induced by manganese, silver, or copper nanoparticles. *Journal of Neurotoxicology.* 30(6): 926-33.
Responsible Division: Neurotoxicology
Co-Author Division: Biochemical Toxicology

149. Wen, Z., Su, Z., Liu, J., Ning, B., Guo, L., Tong, W., and Shi, L. (2011). The MicroArray Quality Control (MAQC) Project and cross-platform analysis of microarray data. *Handbook of Computational Statistics: Statistical Bioinformatics.*
Responsible Division: Systems Biology
Co-Author Division: Personalized Nutrition and Medicine

150. Wen, Z., Wang, C., Shi, Q., Huang, Y., Su, Z., Hong, H., Tong, W., and Shi, L. (2010). Evaluation of gene expression data generated from expired Affymetrix GeneChip microarrays using MAQC reference RNA samples. *BMC Bioinformatics.* 11(6): 11 Supplement 6:S10.
Responsible Division: Systems Biology
Co-Author Division: Biochemical Toxicology

151. Williams, A.J. (2011). A Community-Based Participatory Research/Translational Biomedical Research Strategy for Personalizing Nutrition, Medicine, and Healthcare. *Handbook of Systems Toxicology.* Book Chapter accepted, not yet published.
Responsible Division: Personalized Nutrition and Medicine

152. Witt, K.L. (2011). Follow-up actions from positive results on *in vitro* genetic toxicity testing. *Environmental and Molecular Mutagenesis.* 52: 177-204.
Responsible Division: Genetic and Molecular Toxicology

153. Xu, Y., Karmakar, A., Wang, D.Y., Mahmood, M.W., Watanabe, F., Zhang, Y., Fejleh, A., Feileh, P., Li, Z.R., Kannarpady, G., Ali, S.F., Biris, A.R., and Biris, A.S. (2010). Multifunctional Fe3O4 cored magnetic-quantum dot fluorescent nanocomposites for RF nanohyperthermia of cancer cells. *Journal of Physical Chemistry.* 114(11): 5020-5026.
Responsible Division: Neurotoxicology
Co-Author Division: Associate Director of Scientific Coordination

154. Xu, Z., Kelly, R., Fang, H., and Tong, W. (2010). ArrayTrack™: a free FDA bioinformatics tool to support emerging biomedical research—an updated, human genomics. *Human Genomics.* 4(6): 428-434.
Responsible Division: Systems Biology

155. Xu, Z., Wise, C.K., Varma, V., Fang, H., Ning, B., Hong, H., Tong, W., and Kaput, J. (2010). Two new ArrayTrack™ libraries for personalized biomedical research. *BMC Bioinformatics.* 11(6): S5.
Responsible Division: Systems Biology
Co-Author Division: Personalized Nutrition and Medicine

156. Yin, J., Xia, Q., Lutterodt, H., Wamer, W., and Fu, P.P. (2011). Application of electron spin resonance spectroscopy to study dietary ingredients and supplements—dual antioxidant and prooxidant functions of retinyl palmitate. *Magnetic Resonance in Food Science*.
Responsible Division: Biochemical Toxicology

157. Yu, L., Gao, Y., and Mendrick, D. (2011). Mass Spectrometry-Based Proteomics in Systems Toxicology. *Handbook of Systems Toxicology.* 1(4): 177-196.
Responsible Division: Systems Biology

158. Zhang, L., Yin, S., Miclaus, K., Chierici, M., Vega, S.C., Lambert, C.G., Hong, H., Wolfinger, R.D., Furlanello, C., and Goodsaid, F.M. (2010). Assessment of variability in GWAS with CRLMM genotyping algorithm on WTCCC coronary artery disease. *The Pharmacogenomics Journal.* 10: 347-354.
Responsible Division: Systems Biology

159. Zhang, X., Patterson, T.A., Paule, M.G., Slikker, W., and Wang, C. (2011). Neurotoxic Effects of Anesthetics and Potential Protective Agents. *Developmental Neurotoxicology Research: Principles, Models, Techniques, Strategies and Mechanisms.* Chapter 5: 79-94.
Responsible Division: Neurotoxicology
Co-Author Divisions: Office of the Director, Regulatory Compliance and Risk Management

160. Zhang, X. and Paule, M.G. (2010). *In vivo* systems: Animal models of neurodegeneration. *Comprehensive Toxicology, Nervous System and Behavioral Toxicology.* 13: 399-413.
Responsible Division: Neurotoxicology

161. Zhang, Y., Ali, S.F., Xu, Y., Dervishi, E., Li, Z., Casciano, D.A., and Biris, A.S. (2010). Cytotoxicity of graphene and single-wall carbon nanotubes in neural phaeochromocytoma-derived PC 12 cells. *ACS Nano.* 4(6): 3181.
Responsible Division: Neurotoxicology
Co-Author Divisions: Genetic and Molecular Toxicology, Systems Biology

162. Zou, W., Al-Khaldi, S.F., Branham, W.S., Han, T., Fuscoe, J., Han, J., Foley, S.L., Xu, J., Fang, H., Cerniglia, C.E., and Nayak, R.R. (2011). Microarray analysis of virulence gene profiles in *Salmonella* serovars from food/food animal environment. *Letters in Applied Microbiology.* 5(2): 94-105.
Responsible Division: Microbiology
Co-Author Division: Systems Biology

163. Zou, W., Lin, W., Foley, S.L., Chen, C., Nayak, R.R., and Chen, J.J. (2010). Evaluation of pulsed-field gel electrophoresis profiles for the identification of *Salmonella* Serotypes. *Journal of Clinical Microbiology (JCM).* 48: 3122-3126.
Responsible Division: Personalized Nutrition and Medicine
Co-Author Division: Microbiology

Glossary of Acronyms and Abbreviations

This glossary is provided to assist you in interpreting acronyms, abbreviations, and phrases you encounter while reading this publication. This is not meant to take the place of standard language or scientific dictionaries, which should be referred to if any short form of a scientific term does not appear in this glossary. Also, you may refer to the Index of Key Terms, located at the end of this publication, as a quick reference to locate other occurrences of a specific term.

Acronym/ Abbreviation	Name
3-NPA	3-nitropropionic acid or methamphetamine
3-NT	3-nitrotyrosine
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care, International
AALAS	American Association for Laboratory Animal Science
ACB-PCR	allele competitive blocker-polymerase chain reaction
ACLAM	American College of Laboratory Animal Medicine
AD	Alzheimer's Disease
ADHD	Attention Deficit Hyperactivity Disorder
AFMID	arylformamidase
Ag	silver
AIDS	acquired immunodeficiency syndrome
AMPH	amphetamine
ASK	Access Specific Kinds of CHILDREN Challenges for Neurological Devices STUDY (ASK)
AZT	zidovudine or azidothymidine
BAM	bacterial analytical manual
BBB	blood-brain barrier
BGACDC	Boys, Girls, and Adults Community Development Center
BPA	bisphenol A
CA	*Citrus aurantium*
CBER	Center for Biologics Evaluation and Research, FDA
CBPR	community-based participatory research
CDC	Centers for Disease Control
CDER	Center for Drug Evaluation and Research, FDA
cDNA	complementary DNA
CDRH	Center for Devices and Radiological Health, FDA

Acronym/ Abbreviation	Name
CERP	Classification by Ensembles from Random Partitions
CFSAN	Center for Food Safety and Applied Nutrition, FDA
CMAR	certified managers of animal resources
CNS	central nervous system
COTR	contracting officer's technical representative
CoV	coronaviruses
CP	cyclosphosphamide
CRADA	Cooperative Research and Development Agreement
CSC	class-specific correlations
CVM	Center for Veterinary Medicine, FDA
DBS	deep-brain stimulation
DEHP	di-(2-ethylhexyl)phthalate
DHHS	Department of Health and Human Services
DILI	drug-induced liver injury
DNMT	DNA methyltransferase
ENU	*N*-ethyl-*N*-nitrosourea
EPA	Environmental Protection Agency
ESR	electron spin resonance
FDA	Food and Drug Administration
FERN	Food Emergency Response Network
GABA	gamma-aminobutyric acid
GC/MS	gas chromatography-mass spectrometry
GGT	guanine guanine thymidine
GTT	guanine thymidine thymidine
GWAS	Genome-Wide Association Study
HIV	human immunodeficiency virus
HMA	human microbiota-associated
HPLC	high-performance liquid chromatography
IACUC	Institutional Animal Care and Use Committee
IHC	immunohistochemical
in silico	modeled on a computer
in situ	in place; localized and confined to one area
in vitro	in animal models
in vivo	in cell cultures

Acronym/ Abbreviation	Name
IND	Investigational New Drug
ISH	*in situ* hybridization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC/MS	liquid chromatography-mass spectrometry
LTKB	Liver Toxicity Knowledge Base
MALT	mucosal-associated lymphoid tissues
MAQC	MicroArray Quality Control
miRNA	microRNA
MLA	mouse lymphoma assay
MPH	methylphenidate hydrochloride
MPTP	1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine
MRI	magnetic resonance imaging
mRNA	messenger RNA
MS	mass spectrometry
NASH	nonalcoholic steatohepatitis
NCFST	National Center for Food Safety and Technology
NCTR	National Center for Toxicological Research, FDA
NDA	New Drug Application
NICHD	National Institute of Child Health and Human Development
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NMDA	n-methyl-d-aspartate
NMR	nuclear magnetic resonance
NOAEL	no observable adverse effect level
NP	*p*-nonylphenol
NTP	National Toxicology Program
ORA	Office of Regulatory Affairs, FDA
OTB	Operant Test Battery, NCTR
OWH	Office of Women's Health, FDA
PBPK	physiologically based pharmacokinetic
PCR	polymerase chain reaction
PD	Parkinson's Disease
PET	positive emission tomography
PI	Principal Investigator

Acronym/ Abbreviation	Name
PIG-A	phosphatidylinositol glycan anchor biosynthesis, class A
PPAR	peroxisome proliferator-activated receptor
PPX	Pramipexole
PRL	prolactin
RAPID-B™	Rapid Identification of Bacterial Pathogens
RLS	restless leg syndrome
RNA	Ribonucleic Acid
ROS	reactive-oxygen species
RT-PCR	reverse transcriptase-polymerase chain reaction
SAB	Science Advisory Board
SHLC	sex hormone-like compound
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphism
SOP	standard operating procedure
SSL	simulated-solar light
TCA	tricarboxylic acid cycle
TCR	T-cell receptor
TK	thymidine kinase
TSST-1	toxic-shock syndrome toxin-1
UGT	UDP-glucuronosyltransferase
USDA	United States Department of Agriculture
UV, UVA, or UVB	ultraviolet (A or B indicates the region)
VEC	vaginal epithelial cells
VGDS	Voluntary Genomic Data Submission
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
VXDS	Voluntary eXploratory Data Submission
WPAFB	Wright-Patterson Air Force Base

Index of Authors and Co-Authors of Publications Accepted in FY 10

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Index of Principal Investigators/Division Directors

R

S

T

V

W

Z

Index of Key Terms

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

Y

Z