

**National
Center for
Toxicological
Research
Annual
Report**



FY 2009 – FY 2010

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Preface

The National Center for Toxicological Research (NCTR) is an important research component of the U.S. Food and Drug Administration (FDA) that plays a critical role in FDA's and the Department of Health and Human Services' (DHHS) mission to promote and protect public health. The vision of NCTR is to be an internationally recognized FDA research center that provides innovative, vital scientific technology, training, and technical expertise 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. The Center—located in Jefferson, Arkansas, approximately 30 miles south of Little Rock—is co-located with the Office of Regulatory Affairs' Arkansas Regional Laboratory.

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 1 (Strengthen FDA for Today and Tomorrow), FDA's Strategic Goal 2 (Improve Patient and Consumer Safety), FDA's Strategic Goal 3 (Increase Access to New Medical and Food Products), and FDA's Strategic Goal 4 (Improve the Quality and Safety of Manufactured Products and the Supply Chain).

Customized bioassessment 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 reproductive/developmental 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 the 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 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. This collaboration is one that identifies FDA as a catalyst in the development of new standards that will facilitate drug 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, NCTR

NCTR Overview

Vision

NCTR is an internationally recognized FDA research Center that provides innovative and vital scientific technology, training, and technical expertise 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.

Mission

NCTR conducts peer-reviewed scientific research in support of the FDA mission and provides expert technical advice and training that enables FDA to make sound science-based regulatory decisions and improve the health of the American people. The research at NCTR supports FDA's goals: 1) to understand critical biological events in the expression of toxicity, 2) to develop and characterize methods, and incorporate new technologies to improve the assessment of human exposure, susceptibility, and risk, and 3) to increase the understanding of the interaction between genetics, metabolism, and nutrition.

NCTR is dedicated to supporting the FDA 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 such as personalized nutrition and medicine, bioimaging, systems biology, bioinformatics, nanotechnology, food protection technologies, and biomarker development
- 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 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 methods in risk assessment and for personal and public health

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 of promoting and protecting the public health. This Strategic Plan charts NCTR's course for the future, focusing on four strategic goals: 1) strengthening FDA, 2) improving the safety of patients and consumers, 3) increasing access to new medical and food products, and 4) improving the safety and quality of manufactured products and the supply chain. Each of these goals represents a fundamental public-health task that is crucial to fulfilling our mission.

To accomplish its mission, NCTR has established five strategic goals:

- Goal 1: Advance scientific approaches and tools to promote personalized nutrition and medicine for the public
- Goal 2: Develop science-based best-practice standards, guidance, and tools to incorporate toxicological advancements that improve the regulatory process.
- Goal 3: Conduct research and develop strategic technologies to protect the food supply
- Goal 4: Conduct bioinformatics research and development in support of FDA's regulatory mission
- Goal 5: Strengthen and improve scientific and human capital management and expand training and outreach to retain and train scientific experts critical to address FDA's scientific needs

The NCTR Strategic Plan is on the FDA Web site at <http://www.fda.gov/AboutFDA/CentersOffices/NCTR/NCTRStrategicPlan/default.htm>.

Research Structure

Established by executive order in 1971, the National Center for Toxicological Research (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 divisions 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 to address agency concerns in Food Protection Plan, Modernizing Science, and Product Safety. NCTR research divisions include:

- Biochemical Toxicology
- Genetic and Reproductive Toxicology
- Microbiology
- Neurotoxicology
- Personalized Nutrition and Medicine
- Systems Toxicology
- 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 2009 Accomplishments

The NCTR SAB held three separate subcommittee site visits in 2009 to perform an in-depth site-visit review of the research programs within the Division of Systems Toxicology, the Division of Genetic and Reproductive Toxicology, and the Division of Personalized Nutrition and Medicine.

The first subcommittee review, which occurred in February 2009, reviewed the Division of Systems Toxicology. The Division of Systems Toxicology supports the development of new technologies and works to facilitate integration of data from multiple technology platforms for scientific application to questions that are in direct support of the FDA mission. Six Centers of Excellence comprise the Division of Systems Toxicology: Functional Genomics, Hepatotoxicity, Innovative Technologies, Metabolomics, Proteomics, and Toxicoinformatics. In a report provided to the chair of the NCTR SAB, the subcommittee noted the capable leadership, strong scientific expertise of the staff, and well-equipped laboratories within the division. The subcommittee confirmed that the research being conducted within the division is highly aligned with the FDA mission and has a major emphasis for impacting the areas of biomarker development, adverse event detection, and translation of both of these from preclinical to clinical models. The site-visit team members identified a number of general recommendations to help guide and advice the division as it plans a strategy for developing future research programs at NCTR and noted that the full potential of systems toxicology required working across the Centers of Excellence as a fully integrated multidisciplinary team.

The subcommittee reviewed of the Division of Genetic and Reproductive Toxicology in July 2009. The Division of Genetic and Reproductive Toxicology conducts fundamental 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 a report to the Chair of the NCTR SAB, the subcommittee was uniformly positive regarding the overall strategic directions and accomplishments of the Division of Genetic and

Reproductive Toxicology and concluded that the overall program is appropriately focused on research areas that are highly valued by its regulatory clients, and has positioned the division as a well recognized and respected leader in the global regulatory and research genetic toxicology communities.

The subcommittee reviewed of the Division of Personalized Nutrition and Medicine in August 2009. The Division of Personalized Nutrition and Medicine is charged with the developing strategies, methods, and resources needed to improved public health by focusing on the individual. The research in this division seeks to develop an evidence-based understanding of how the unique genetic identity of each individual contributes to the wide array of biochemical, physiological, and morphological phenotypes in human populations. In a report to the Chair of the NCTR SAB, the subcommittee noted that it was strongly supportive of the research activities of the division and noted that the research conducted within the division will provide FDA with information and validated protocols that are critical for the agency to understand the individual's health and safety implications of environmental factors, including nutrition, nutritional supplements, and drugs. Based on this understanding, FDA will be able to develop the guidance and best practices needed to regulate the application of personalized medicine to public health.

The full NCTR SAB meeting was held at NCTR on November 17 and 18, 2009. The NCTR Director provided an overview of center-wide scientific endeavors and discussed the alignment and strategic focus of NCTR. The overview included a discussion of the current and new research programs at NCTR and the strategies NCTR uses to prioritize its research programs. In addition to the presentation, discussion and acceptance of each of the subcommittee reports, the meeting included presentations on ongoing research activities from each of the other NCTR divisions. Each Division Director updated the SAB on the major research findings of the past year, the important implications of the findings for FDA, and major scientific issues with current research. The NCTR SAB was also updated on several NCTR research initiatives including the Interagency Agreement with the National Toxicology Program and the Nanotechnology activities with FDA's Office of Regulatory Affairs.

SAB Membership Roster

Chair:

James A. Popp, D.V.M., Ph.D.

Term: 07/31/06-06/30/10

Expertise: Toxicology

Co-founder and Co-owner Stratoxon LLC

E-mail: popp@stratoxon.com

Designated Federal Officer:

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Members:**Cynthia A. Afshari, Ph.D., DABT**

Term: 08/01/08-06/30/12

Expertise: Molecular Toxicology and
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Ronald Hines, Ph.D.

Expertise: Pediatric Clinical Pharmacology

Term: 11/03/09 – 11/03/13

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Janice W. Yager, Ph.D., MPH

Expertise: Genetic Toxicology, epidemiology

Term: 11/03/09 – 11/03/13

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James S. Bus, Ph.D., DABT

Term: 07/31/06-06/30/10

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Jose M. Ordovas, Ph.D.

Term: 08/01/08-06/30/12

Expertise: Nutrition

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Stephen M. Roberts, Ph.D.

Term: 07/31/06-06/30/10

Expertise: Nanotoxicology

Professor and Director

University of Florida

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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 guidances, mechanistic understanding, and advanced methodology.

Interagency Agreements

Interagency Agreements (IAGs) are formal financial partnerships with other government agencies. 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 Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP)

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In 1992, FDA entered into an IAG with NIEHS. The NIEHS National Toxicology Program (NTP) conducts toxicology studies at the request of FDA and other federal agencies. 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 to study design input 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 byproducts, 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 B1 (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 include listed below.

- Furan—Determination of carcinogenic mechanisms and low-dose carcinogenesis in rats
- Acrylamide—Developmental neurotoxicity assessment in rats
- Bitter Orange (*Citrus aurantium*)—Developmental and physiological toxicity in rats
- Bisphenol A—Determination of the pharmacokinetics in rats and nonhuman primates, physiologically based pharmacokinetics (PBPK) modeling, and subchronic toxicity in rodents
- Di(2-ethylhexyl)phthalate (DEHP)—Toxicokinetics in neonatal male rhesus monkeys following intravenous and oral dosing
- Melamine and cyanuric acid—Toxicity studies and biomarker identification in rodents and rabbits
- Retinyl palmitate—Effect of topically applied skin creams containing retinyl palmitate on the photocarcinogenicity of simulated-solar light (SSL) in SKH-1 mice

- Acrylamide—Genotoxicity and carcinogenicity of acrylamide and its metabolite, glycidamide, in rodents (range-finding, subchronic, two-year chronic carcinogenicity studies)
- Ketamine—NMDA antagonist/GABA agonist-induced cell death in the developing rat brain
- AIDS therapeutics—Perinatal carcinogenicity of drug combinations used to prevent mother-to-child transmission of HIV
- 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
- Usnic acid, *Usnea* lichen—Toxicity studies in Fischer 344 rats and B6C3F1 mice
- Glucosamine and chondroitin sulfate—Subchronic toxicity in Fischer 344 rats and diabetic Zucker rats
- Permanent makeup inks—Determination of the immunogenicity of inks and their components
- AIDS therapeutics—Toxicity studies of combinations in p53 (+/-) haploinsufficient transgenic mice

In addition to the IAG with NIEHS/NTP, In FY 2009 NCTR received support from other government agencies. The FY 2009 or FY 2010 IAGs supporting NCTR research projects include those listed below.

Centers for Disease Control—Mouse Lymphoma Assay

Evaluation of the Ability of the Agar and Microwell Versions of the Mouse Lymphoma Assay to Optimally Detect the Mutagenic Potential and Potency of Complex Chemical Mixtures () (page 52)

National Institute for Child Health and Human Development

Evaluation of Growth and Pubertal Development in Male Rhesus Monkeys (*Macaca mulatta*) Exposed to Methylphenidate Hydrochloride (MPH) (E0728701) (page 81)

National Cancer Institute/DNA Hypomethylation

Global and Locus-Specific DNA Hypomethylation: A Common Mechanism Involved in Genotoxic and Non-Genotoxic Rat Hepatocarcinogenesis (E0718101) (page 67)

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 2009 or FY 2010 CRADAs supporting NCTR research projects include those listed below.

BG Medicine, Inc.

Liver Toxicity Biomarkers Study: Phase 1, Entacapone and Tolcapone (E0726601) (page 53)

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) (page 82)

Pfizer, Inc.

Evaluation of the Mechanisms of Inactivation and Degradation of Third-Generation Cephalosporins by the Bovine Intestinal Microflora (E0721901) (page 58)

The Hamner Institutes for Health Sciences

Evaluating the Utility of ACB-PCR in Dose-Response Assessment and Mode-of-Action Evaluation (E0726901) (page 66)

University of Arkansas for Medical Sciences)

Ketamine Pharmacokinetics in Children (E0726201) (page 76)

University of Arkansas for Medical Sciences

Novel Studies on Sites-of-Action and Mechanisms in Chronic Balance Dysfunction (E0722301) (page 66)

University of Illinois

Phytoestrogens and Aging: Dose, Timing, and Tissue (E0721001) (page 57)

Office of Women's Health

The 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 the health of women. This program has provided support for approximately 30 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, 2) comparative effectiveness of chemotherapeutic medicines, and 3) understanding the biological changes that cause lupus. The OWH Science Program also provided funding for NCTR's Endocrine Disruptor Knowledge Base, which has been extensively utilized to determine a compound's estrogenic activity and has served as the prototype for newer bioinformatics tools, such as ArrayTrack™. 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: 1) understanding the molecular basis of drug efficacy and safety and 2) how genetics, sex, diet, and other environmental factors influence drug efficacy and safety.

The NCTR projects listed below were supported by OWH in FY 2009 or have been approved for support in FY 2010.

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) (page 60)

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) (page 62)

Assessment of Effects and Metabolism of Synthetic Azo Colorants Used in Women's Cosmetics on Human Skin Microbiota (E0729301) (page 74)

Inactivation of UDP-Glucuronosyltransferases in Human-Breast Tissues: Accessing Cancer Risk, Tamoxifen Safety and Toxicity (E0734001) (page 68)

Development of a FDA Resource and Knowledge Base for Sex Difference in Drug-Induced Liver Injury (DILI) (E0733801) (page 69)

Division of Biochemical Toxicology ***Summary of Activities***

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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 the introduction of new techniques to assess toxicities and carcinogenic risks. The risk-assessment research is firmly rooted in mechanistic studies focused on the understanding of toxicological endpoints, an approach that allows greater confidence in the subsequent 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 2009 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 the 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 2009, final pathology reports were 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 are presently being used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to establish the risk of dietary exposures to acrylamide. This risk estimate will include consideration of data from two-year chronic rodent bioassays of glycidamide, a genotoxic metabolite of acrylamide. In further work, results from an *in vivo* mutagenicity study of acrylamide and glycidamide in newborn mice were published. These data will be used to help interpret a recently completed newborn mouse bioassay and to determine if infants are particularly susceptible to the carcinogenic properties of acrylamide and glycidamide.

In a continuation of investigations of foodborne carcinogens, experiments were initiated 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. Preliminary results indicate a preferential accumulation of furan in liver, the target organ for furan's carcinogenicity.

During FY 2009, final pathology reports were completed for rodent bioassays on *Aloe vera*, a widely used dietary supplement. The results indicate the presence of colonic tumors in rats, the mechanisms for which are currently under investigation.

A major emphasis within the division is elucidating potential toxicities associated with endocrine-disrupting chemicals. During FY 2009, a final report was completed on *p*-nonylphenol, an industrial chemical used in the production of non-ionic surfactants. In addition, manuscripts were published comparing the effects of genistein and ethinyl estradiol in multigenerational reproductive and chronic toxicity studies.

In response to a nomination by the Center for Biologics Evaluation and Research (CBER), division investigators conducted pharmacokinetic studies with male neonatal male rats and nonhuman primates on di(2-ethylhexyl)phthalate (DEHP), a 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 there is significant formation of the putative toxic metabolite mono(2-ethylhexyl)phthalate (MEHP), with a high percentage being unconjugated. Additional experiments have addressed the metabolism and effects of DEHP in neonatal male rats, with particular emphasis upon repeated anesthetic use—a problem relevant to clinical settings where DEHP exposure occurs.

During FY 2009, studies on endocrine-disrupting chemicals were expanded to include bisphenol A, a chemical to which there is ubiquitous environmental exposure. There has been an increasing concern over bisphenol A due to a large and growing body of research that has reported effects of this chemical at doses approaching human exposure levels. To address these concerns, the experiments include subchronic studies in rodents that encompass a wide range of exposures, including those to which humans are exposed, and endpoints that are not typically measured in reproductive and developmental toxicity experiments. The subchronic studies are being supported by extensive pharmacokinetic measurements in rodents and nonhuman primates.

An area of concern to FDA, in particular CFSAN, is the potential toxicity of cosmetic ingredients due to their interaction with light. During FY 2009, the final report on photocarcinogenesis studies of retinyl palmitate was completed. In addition, a manuscript was published describing the dermal penetration of nanoscale titanium dioxide, a component of certain sunscreens and other cosmetics, in mice.

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 2009, a final report was completed on rodent bioassays in which antiretroviral drugs were administered transplacentally to mice. In addition, division investigators published data describing the effects of antiretroviral drugs on cell-cycle kinetics, and in further experiments, the DNA and protein adducts of the antiretroviral drug nevirapine were characterized.

In response to reports that pet food and infant formula were adulterated with melamine, division investigators designed studies to characterize the toxicities of melamine in the presence of its oxidation product cyanuric acid. The results to date indicate that as the length of exposure is increased, toxicity occurs at even lower doses and that this toxicity is due to the formation of melamine cyanurate crystals in the kidney.

During FY 2009, division investigators collaborated with scientists at the National Center for Food Safety and Technology (NCFST) to investigate the stability and activity of the toxins of ricin and abrin in various foods as a function of time, temperature, and pH. As part of this effort, a manuscript was published describing a sensitive enzyme-activity assay method for detecting ricin, abrin, and other agents that act by a similar mechanism.

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 studies to assess the relationship between alterations in microRNAs (miRNAs) and the development of nonalcoholic steatohepatitis (NASH), a condition that has been shown to progress to cirrhosis and liver cancer. The results demonstrate that alterations in expression of miRNAs are a prominent event during early stages of NASH and strongly suggest that the severity of NASH and differences in the susceptibility to NASH-induced liver carcinogenesis may be determined by the variations in miRNA expression response.

FY 2010 Plans

In FY 2010, division investigators will complete final reports on the perinatal carcinogenicity of antiretrovirals drugs. Final reports will also be prepared on chronic two-year rodent bioassays of glycidamide, and on the carcinogenicity of acrylamide and glycidamide in the newborn mouse bioassay. Chronic and mechanistic studies will continue on the food contaminant furan. Experiments will be initiated to determine the toxicities associated with exposure to nanoscale silver particles. These investigations will initially focus on pharmacokinetic measurements, which will then be expanded to include subchronic studies.

Studies will be 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. These studies will include pharmacokinetic measurements and also assess the ability of triclosan to undergo photodecomposition to more toxic derivatives.

Investigations will continue to determine the pharmacokinetics and testicular toxicity of intravenously administered DEHP in neonatal rhesus monkeys and rats. These experiments will indicate if this plasticizer poses an undue risk to infants—especially those in neonatal intensive care units. Experiments will also be conducted with bisphenol A in rodent models, especially with regard to developmental exposures.

Division investigators will continue to evaluate the toxicities of melamine in combination with cyanuric acid with an emphasis on increasing the length of exposure. Division personnel will also continue collaborations with investigators at the NCFST to 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 Goals

The research conducted by the Division of Biochemical Toxicology contributes primarily to FDA Strategic Goals 2 and 4.

FDA Strategic Goal 2 (Improve Patient and Consumer Safety)

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. Additional experiments are assessing the toxicity of DEHP, a chemical present in a variety of medical devices.

Division investigators have developed new techniques that have improved the scientific capabilities of the agency. These include high-performance liquid chromatography (HPLC), coupled with tandem mass-spectrometric methods, to assess pharmacokinetic and toxicokinetic parameters of chemicals and drugs of interest to FDA and the introduction of new techniques for assessing the phototoxicity of chemicals.

FDA Strategic Goal 4 (Improve the Quality and Safety of Manufactured Products and the Supply Chain)

A major emphasis of the division's research is to ensure the safety of food products. For example, ongoing assessments include 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 bisphenol A, a chemical to which there is ubiquitous 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. The division also emphasizes toxicological assessments of chemicals found in cosmetic products. These chemicals include *Aloe vera*, retinyl palmitate, triclosan, and nanoparticles.

Division of Genetic and Reproductive Toxicology Summary of Activities

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Introduction

The Division of Genetic and Reproductive Toxicology (DGRT) 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, DGRT 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 DGRT are actively involved in national and international efforts to harmonize the conduct of genetic-toxicology tests and to improve their interpretation and use for regulatory decision making. DGRT scientists frequently provide expert advice to 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, and 4) research to improve risk assessment. DGRT 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 2009 Accomplishments

In FY 2009, DGRT 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. DGRT scientists participated in the Genetic Toxicology Working Group of the ICCVAM (Interagency Coordination Committee on the Validation of Alternative Methods) and provided advice on new international guidelines for the conduct of the *in vitro* micronucleus assay. DGRT scientists were, and will continue to be, involved in discussions concerning the appropriate follow-up strategies for chemicals (primarily pharmaceuticals) found to be positive in genetic-toxicology tests conducted as part of the drug-safety evaluation.

Specific 2009 research accomplishments involving the current regulatory assays include:

- In direct response to an FDA need, a project was initiated to evaluate various measures of cytotoxicity and other assay parameters for the *in vitro* micronucleus assay
- Initiated 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)
- Initiated a study to evaluate whether the current genetic toxicology assays are appropriate for evaluating the potential toxicity of nanomaterials.

DGRT was actively involved in research addressing specific chemicals and generating data that can be used by the regulatory Centers.

- Continued 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)
- Continued a study in collaboration with CDC (Centers for Disease Control) 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
- Initiated a study to evaluate the cancer mode-of-action for furan
- In collaboration with scientists in the Division of Biochemical Toxicology, continued the AIDS studies to evaluate the potential for various antiretroviral-drug combinations to induce genetic damage. These drugs are given to prevent the transmission of the virus from HIV-pregnant women to their children.

Substantial progress was made in 2009 in the development of new methods and in bringing new methodologies to NCTR.

- Initiated a project to evaluate whether microRNA expression analysis can be used to detect carcinogens from noncarcinogens
- Initiated a project to establish and evaluate the gpt/delta transgenic mouse model for use at NCTR
- Initiated 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. This project received special funding as a Critical Path Project to develop this approach for use in humans.

DGRT expanded its research program during 2009 addressing research to improve risk assessment.

- Continued two studies evaluating the presence of *p53* mutations in colon cancer from both mice and humans
- Initiated a project to use *in vivo* mutation analysis to inform cancer mode-of-action
- Initiated a project to investigate whether mutagens can have thresholds
- Continued research using an ACB-PCR (allele-specific competitive blocker-polymerase chain reaction) 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.

FY 2010 Plans

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

- Continue project to evaluate various measures of cytotoxicity and other assay parameters for the *in vitro* micronucleus assay
- Continue 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
- Continue research to understand the mechanistic basis of the *in vitro* regulatory assay—the mouse lymphoma assay (MLA)
- Continue research to evaluate whether the current genetic toxicology assays are appropriate for evaluating the potential toxicity of nanomaterials
- Complete study to assess any potential methylphenidate-induced adverse effects
- Complete study in collaboration with CDC to evaluate the relative mutagenic potential for a series of cigarette condensates.
- Continue a study to evaluate the cancer mode-of-action for furan
- In collaboration with scientists in the Division of Biochemical Toxicology, continue the AIDS studies to evaluate the potential for various antiretroviral-drug combinations to induce genetic damage. These drugs are given to prevent the transmission of the virus from HIV-pregnant women to their children
- Continue 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
- Continue a project to evaluate whether microRNA expression analysis can be used to detect carcinogens from noncarcinogens

- Continue a project to establish and evaluate the gpt delta transgenic mouse model for use at NCTR
- Continue a project to establish chromosome painting technology at NCTR
- Continue 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
- Continue two studies evaluating the presence of *p53* mutations in colon cancer from both mice and humans
- 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
- Continue a project to use *in vivo* mutation analysis to inform cancer mode-of-action
- Continue a project to investigate whether mutagens can have thresholds.

Contribution to FDA’s Strategic Goals

The research conducted by the Division of Genetic and Reproductive Toxicology contributes primarily to FDA Strategic Goals 2 and 3.

FDA’s Strategic Goal 2 (Improve Patient and Consumer Safety)

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.

FDA’s Strategic Goal 3 (Increase Access to New Medical and Food Products)

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

The division received funding for a special Critical Path project to develop the *PIG-A* assay for use in humans.

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.

Division of Microbiology Summary of Activities

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Introduction

The Division of Microbiology serves a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology as well as respond to microbial surveillance and diagnostic needs for research projects. The Division of Microbiology research projects are based on FDA strategic goals and programmatic expertise. The research program is divided into five areas: 1) Food Safety, Food Biosecurity, and Methods Development, 2) Antimicrobial Resistance, 3) Microbes and Host Interactions, 4) Environmental Biotechnology, and 5) Microbiological Surveillance and Diagnostic Support of Research. During FY 2009, the Division of Microbiology scientists engaged in research addressing a variety of FDA issues with special emphasis on food safety, antimicrobial resistance, women's health, environmental risk assessments, and human-intestinal microbiota and host interactions.

FY 2009 Accomplishments

Food Safety, Food Biosecurity, and Methods Development

- Studied the microbial genetics of *Salmonella* Javiana in human populations
- Conducted preliminary studies on cytotoxicity and pathogenicity in non-O157 Shiga toxin producing *Escherichia coli* (STEC)
- Data-mined bioinformatics databases on bacterial enteric pathogens and integrated the omics data into ArrayTrack™
- Determined the effect of egg white on two *Bacillus* species and found that they were inactivated in the order *B. anthracis* > *B. pumilus*. A slight growth of *B. cereus* was observed in egg white
- Screened *Aeromonas veronii* isolates from catfish for virulence genes
- Isolated virulent strains of *Pseudomonas* spp. harboring toxin encoding genes from imported shrimp
- Performed a survey on the metabolism of Sudan dyes found as contaminants in the food supply by prevalent intestinal microorganisms and elucidated the mechanism of azo dye reduction
- Reported on the inhibition of animal-associated bacteria by dietary resveratrol, a beneficial compound found in red wine and grape juice, and the identification of resveratrol metabolites produced by some of these bacteria.

Antimicrobial Resistance

- Studied, using microarray technology, antimicrobial-resistance genes in *Salmonella* in preharvest poultry environments unexposed to drugs

- Characterized antibiotic-resistance genes and plasmids in *Salmonella enterica* strains from imported food in collaboration with scientists from the Pacific Regional Laboratory Southwest (PRL-SW)
- Characterized the genetic basis of antimicrobial resistance in *Salmonella enterica* serotypes isolated from food animals
- DNA-sequenced plasmids from multiple *Salmonella enterica* serovar Heidelberg isolates to identify factors contributing to antimicrobial resistance and virulence
- Characterized, at the molecular level, integrons from *Salmonella* strains containing antibiotic-resistance genes
- Provided genetic fingerprint molecular data of *Salmonella* isolates to track outbreak strains for risk-assessment analysis
- Discovered the deletion of regulatory and sensory elements and the insertion of new genetic elements in transposon Tn1546, which is responsible for vancomycin resistance in human clinical isolates of *Enterococcus faecium*
- Developed real-time PCR and multiplex PCR assays for the detection of multiple antibiotic-resistance genes in multidrug resistant bacteria with diverse ecological backgrounds
- Detected and characterized a transporter protein whose activity decreases the susceptibility of *Clostridium perfringens* to fluoroquinolone drugs
- Isolated and characterized nalidixic acid resistant *Klebsiella* spp. from imported seafood. All isolates were also found to harbor *tetC* and *tetD* genes conferring tetracycline resistance
- DNA-sequenced fluoroquinolone-resistant *Pseudomonas* spp. isolated from imported shrimp and found point mutations in *gyrB* and *parC* that mediate resistance to the antibiotic. Fluoroquinolone-resistance genes (*qnrA* and *qnrB*) were also found on the plasmids of the isolates
- Reported on the acetylation, and thus inactivation, of two fluoroquinolone antimicrobial agents, ciprofloxacin and norfloxacin, by an *Escherichia coli* strain isolated from a municipal wastewater treatment plant.

Microbes and Host Interactions

- Isolated and identified bovine intestinal bacteria capable of metabolizing the veterinary antimicrobial drug ceftiofur to metabolites lacking bactericidal activity
- Characterized multiple β -lactamases responsible for the first step in the degradation pathway for the third-generation cephalosporins ceftiofur and ceftriaxone
- Determined the potential of the human-intestinal microbiota to metabolize enrofloxacin
- Determined the amounts of bound fluoroquinolone residue levels on fecal content, using bioassays and analytical chemistry techniques
- Determined the effect of fluoroquinolone exposure on metabolic activities of *Clostridium perfringens*
- Examined the potential of the skin microbiota to metabolize azo dyes

- Determined that probiotic lactobacilli modulate expression of inflammatory genes
- Examined clinical strains of *Staphylococcus aureus*, as well as the type strains of other species of *Staphylococcus*, for the presence of hyaluronidase. All *S. aureus* strains examined possessed at least one hyaluronidase determinant while the type strains of 19 other staphylococcal species did not
- Developed a genital-tract secretion medium for investigating the interactions between toxic-shock syndrome toxin-1 (TSST-1) producing strains of *S. aureus* and *Lactobacillus sp.* for an Office of Women's Health (OWH) project
- Established a virology laboratory at NCTR for studies of foodborne viruses and virus-host interactions.

Environmental Biotechnology

- Conducted studies using omics-based approaches to elucidate mechanisms for the metabolism of high-molecular weight polycyclic-aromatic hydrocarbons (PAH) by *Mycobacterium vanbaalenii* PYR-1
- Performed microarray and high-throughput proteomics experiments to examine the overall biological changes of *M. vanbaalenii*
- Reported on the biotransformation of a toxic environmental contaminant, acridine, to four different metabolites by *M. vanbaalenii*.

Microbiological Surveillance and Diagnostic Support of Research

- During FY 2009, program personnel worked to prevent the introduction of microbial pathogens into NCTR animal colonies by: 1) screening the primate colony for the presence of *Salmonella*, *Shigella*, and *Campylobacter* spp. and endoparasites, 2) sterility testing of various compounds used by NCTR researchers in their experiments, and 3) closely monitoring quarantined animals for the presence of pathogenic microorganisms. Routine monitoring of the animals, environment, food, and water from the breeder colonies was a continuing priority. Program personnel also provided cultures of microorganisms to division, NCTR, and qualified outside researchers.

FY 2010 Plans

Food Safety, Food Biosecurity, and Methods Development

- Conduct DNA-sequencing studies of plasmids from *Salmonella enterica* serovars commonly associated with poultry sources and important causes of invasive human infections
- Develop microbial-centric libraries and data models to enhance data integration, interpretation, and mining in a bioinformatics platform
- Study the mechanisms of pathogenicity and the role of plasmids in non-O157 Shiga toxin-producing *E. coli* (STECs)
- Conduct cytotoxicity studies on non-O157 STECs using various cell lines
- Validate gene-expression data after infection of epithelial cells with *Bacillus anthracis* by real-time PCR

- Identify the key signal transduction pathways involved in epithelial cell stimulation caused by exposure to *B. anthracis*
- Screen strains of *Vibrio* spp. isolated from imported shrimp for virulence genes
- Investigate the potential threat of animal coronaviruses as foodborne pathogens.

Antimicrobial Resistance

- Study the mechanisms of extended spectrum β -lactamases and fluoroquinolone resistance in enteric pathogens from food and veterinary sources
- Study the drug resistance phenotype and genotypes of non-O157 STECs
- Division scientists, in collaboration with CDRH, CBER and Marshfield Clinic, will assess molecular-diagnostic assays as alternative reference methods for premarket evaluation of rapid molecular diagnostic devices for influenza
- Conduct bacterial-conjugation studies to evaluate the impact of antimicrobial exposure on plasmid-transfer efficiency in *Salmonella*
- Division scientists, in collaboration with CFSAN, PRL-SW and Arkansas Regional Laboratories, will continue to investigate the prevalence of antibiotic-resistant *Salmonella* in imported food
- Elucidate the role of new genetic elements in transposon Tn1546, which causes *Enterococcus faecium* to become resistant to high levels of vancomycin, and sequence a 100 kb plasmid that harbors this transposon
- Conduct microarray analysis of fluoroquinolone resistant strains of *Clostridium perfringens*
- Screen multidrug resistant *Vibrio* spp. isolated from imported shrimp for fluoroquinolone and tetracycline-resistance genes
- Screen nalidixic acid resistant *Klebsiella* spp. for quinolone-resistance genes by PCR and DNA sequencing
- Continue studies examining the virulence capacity of staphylococcal hyaluronidases.

Microbes and Host Interactions

- Identify the major metabolites resulting from the degradation of ceftiofur by bovine-intestinal bacteria in pure culture and a bovine microbiota-associated mouse model
- Evaluate the effect of antimicrobial agents on bacteria from the human-intestinal tract for application according to VICH Guideline 36 (Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food—General Approach to Establish a Microbiological Acceptable Daily Intake)
- Initiate studies to investigate the zoonotic and health threats of coronaviruses circulating in adults, children, domestic animals, and wildlife
- Detect the effect of fluoroquinolone exposure on the virulence and survival of *Clostridium perfringens*
- Study the impact of melamine on human-intestinal microbiota

- Continue OWH-funded projects to assess effects and metabolism of azo colorants and nanoscale materials used in women's cosmetics on human-skin microbiota
- Assess the effects of soy isoflavones on vaginal candidiasis
- Assess how probiotic bacteria affect immune-evasive *Salmonella*
- Continue an OWH investigation of probiotics in women's health, examining the inhibitory properties of *Lactobacillus* sp. toward TSST-1 producing strains of *S. aureus* by using an *in vitro* vaginal model and a recently developed genital-tract medium.

Environmental Biotechnology

- Proteomics, genomics, and transcriptomics data will be integrated into the systems-biology context to understand the mechanism of polycyclic-aromatic hydrocarbon (PAH) degradation
- Division scientists, in collaboration with CVM, will seek to isolate microorganisms from the environment that degrade fluoroquinolone drug residues to compounds that no longer select for resistant strains of bacteria.

Microbiological Surveillance and Diagnostic Support of Research

- Continue working to ensure that the research-animal population remains healthy and disease-free.

Contribution to FDA's Strategic Goals

Research in the Division of Microbiology contributes primarily to FDA Strategic Goals 2, 3 and 4.

FDA Strategic Goal 2 (Improve Patient and Consumer Safety)

- Research in food safety, food biosecurity, and methods development supports the regulatory decisions of FDA that ensure the safety of our food supply in the "farm-to-the-fork" continuum.
- Determination of factors that contribute to antimicrobial resistance and virulence in foodborne bacteria will allow development of new intervention strategies to improve the safety of the food supply.
- The metabolism of veterinary antimicrobial compounds by the normal intestinal microbiota of animals may affect the development of antimicrobial resistance. Since FDA sets antimicrobial drug-residue limits in animal products, this knowledge will assist the agency to establish better strategies for antibiotic use.
- Modification of transposon Tn1546 in vancomycin-resistant *Enterococcus faecium*, which causes high mortality in hospitalized patients, will help in understanding the acquisition of resistance and regulation of resistance genes.

FDA Strategic Goal 3 (Increase Access to New Medical and Food Products)

- Drug-resistance data obtained using state-of-the-art technologies will provide FDA with the knowledge base to make strategic regulatory decisions.
- A *Salmonella* Javiana study will provide data for developing an FDA risk-assessment model.

- Different responses in cutaneous and intestinal epithelial cells after handling or consumption of *Bacillus anthracis*-laced food products should help identify molecular markers to enhance the safety of manufactured products.
- Research on coronaviruses (CoV) will increase FDA's preparedness for future CoV outbreaks and for the review of future CoV vaccines and diagnostic tests, as well as for organ transplantation, blood transfusions from asymptomatic carriers, and inactivation of pathogens found in blood products.
- NCTR research on influenza viruses will accelerate the availability of safe, effective, and rapid influenza diagnostic devices.

FDA Strategic Goal 4 (Improve the Quality and Safety of Manufactured Products and the Supply Chain)

- The human-intestinal microflora is known to metabolize food additives, food supplements, and antimicrobial agents. Research will allow FDA to gain a clearer understanding of how drug residues, probiotic products, dietary supplements, and xenobiotic substances affect the intestinal microflora and how microbial changes may affect human health.
- Several recent outbreaks of *Salmonella* in food (vegetables, nuts, and peanut butter) have required quick response by FDA to source-track the organisms. Research on *Salmonella* and other foodborne pathogens will help to improve detection methods.
- The bioinformatics database platform for enteric pathogens integrated in the ArrayTrack™ platform will link data analysis with biological relevance of pathogens and will be available to all FDA regulators and researchers.
- Studies examining vaginal bacterial interactions with probiotic supplements could be extended for FDA assessment of other commercial probiotics.
- Current research will help FDA gain a clearer understanding of how food contaminants affect the intestinal microbiota, how azo dyes and nanoscale materials affect the skin microbiota, and how changes in microbial populations affect human health.
- Biotechnology research on the environmental fate of FDA-regulated drugs is highly relevant to the prevention of microbial antibiotic resistance. If the drug residues can be metabolized to inactive products, they will no longer select for bacterial resistance.
- The environmental-biotechnology research at NCTR can serve as examples for drug manufacturers responsible for documenting environmental fate and toxicity profiles to obtain approval for human or veterinary medical products.

Division of Neurotoxicology Summary of Activities

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Introduction

Fifty-million Americans have a permanent, neurological disability that limits their daily activities. One in three 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 a year. 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 are currently providing scientists with 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 approaches for assessing neurotoxicity 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. Toward this end, specific efforts address several focal areas of fundamental research designed to broadly examine the involvement of: 1) monoamine-neurotransmitter systems as targets for neurotoxicity, 2) mitochondrial dysfunction and oxidative stress as mechanisms of neurotoxicity, 3) the NMDA (N-methyl-D-aspartic acid) receptor complex as a mediator of adult and developmental neurotoxicity, 4) the role of amyloid β -peptide (A β) aggregation in the expression of neurotoxicity, and 5) the role of the blood-brain barrier in neurotoxic processes. 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 strategy employed for achieving these goals involves multidisciplinary approaches

that capitalize on the neurochemistry, molecular neurobiology, neuropathology, neurophysiology, and behavioral expertise of division personnel. The division is expanding capabilities in the area of imaging by adding both microPET and magnetic resonance imaging (MRI) instruments and personnel. In addition, efforts to develop sensitive, high-throughput systems for screening potential neurotoxicants are underway. Other unique features of the division's research capabilities include the ability to: 1) determine chemical concentrations and cellular-level interactions in target tissue, 2) determine changes in gene and protein expression associated with chemical exposures, 3) effect high-throughput, comprehensive cognitive or behavioral assessments, 4) 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, and 5) develop novel histochemical tracers to aid in the evaluation of chemical-induced pathologies.

FY 2009 Accomplishments

Research protocols were implemented or continued to provide data important for the regulatory needs of the agency with respect to pediatric anesthetic agents (including ketamine), the central nervous system stimulants amphetamine and methylphenidate (widely used in the treatment of Attention Deficit Hyperactivity Disorder), and nanoparticles. In addition, analyses of data from a life-time rodent study on the neurotoxic effects of acrylamide continued and several reports on the effects of the chronic administration of pramipexole (PPX), a dopamine D3-receptor agonist, in the nonhuman primate were being readied for publication. This compound is used for the treatment of Parkinson's disease and restless leg syndrome (RLS) in adults and is being considered for pediatric use in the treatment of Tourette's and RLS. Our juvenile nonhuman primate study continues to provide valuable information concerning expected outcomes of chemicals known to interact with specific subcellular targets during development. An additional study using *in vitro* and *in vivo* models was initiated to examine the neuroprotective effects of PPX to better inform regulation of this and similar compounds. It is important to understand the range of PPX effects both as a neuroprotectant and as a neurotoxicant and what mechanisms might be involved in these processes.

In partnership with CDER and NICHD (National Institute of Child Health and Human Development), division staff demonstrated that ketamine-induced neural-cell death in our perinatal monkey cell-culture model is both apoptotic and necrotic in nature. Based on periods of rapid synaptogenesis, the timing of exposures becomes critical when comparing rodent, nonhuman primate, and human-neurotoxic outcomes. *In vivo* nonhuman primate studies have helped to identify developmental periods during which sensitivity to ketamine is greatest and to explore aspects of exposure duration that contribute to toxicity. 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. Studies in rats and mice were conducted to understand the neuroprotective mechanisms of L-carnitine. Regulatory briefings have

helped to determine relevant-data needs and possible labeling changes based on new data generated in the division. In addition, division scientists worked with CDER reviewers to assist the development of guidelines for the assessment of developmental neurotoxicity; neuropathology; and seizures.

Work continued on the evaluation of damage to brain vasculature and meninges after exposure to amphetamines by evaluating changes in vascular-related genes at the transcriptional and translational levels. Thus far, the data indicate that the meninges and surface vasculature of the brain are susceptible to damage when pronounced hyperthermia accompanies amphetamine exposure. Data also suggest that amphetamine- and environmentally-induced hyperthermia differentially alter the expression of genes regulating vascular tone, angiogenesis, and endoplasmic reticulum stress responses in affected meninges and associated vasculature.

Studies on the assessment of human brain/cognitive function using the NCTR Operant Test Battery—the same instrument used in the division's Nonhuman Primate Research Center—continued, primarily in children with depression or anxiety disorder and in children exposed to opiates during the perinatal period. These studies are exemplary of translational neuroscience and highlight the cross-species comparison capabilities within the division.

In support of many of our areas of research, genomic, proteomic, and bioinformatics approaches were developed or enhanced to allow for the identification of gene- and protein-expression profiles associated with neurotoxic events. These included studies on chemically induced mitochondrial dysfunction, where significant increases in the gene expression of a specific uncoupling protein were demonstrated. Identification of such specific events can serve to elucidate mechanisms and provide markers of toxicity. A recently installed microPET imaging device 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. Such ligands will undergo initial assessments in our nonhuman primate models. The suite for housing the complimentary MRI device neared completion and the MRI was delivered.

In collaboration with colleagues at CDER, CDRH, and Wright-Patterson Air Force Base (WPAFB), 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 alteration in the expression of genes associated with apoptosis and oxidative stress. These preliminary data are providing mechanistic information that will help researchers understand the potential risk of nanoparticles to human health.

Studies in the rat peripheral nervous system demonstrated significant sciatic nerve degeneration and nerve conduction blockade after prolonged exposure to the mitochondrial inhibitor, 3-nitropropionic acid (3-NPA). Associated depression of motor nerve conduction velocity improved by acetyl- L-carnitine co-administration. Contrary to other models, peripheral nerve demyelination evoked by 3-NPA is not associated with inflammation.

FY 2010 Plans

Much of the work for the coming year will involve continuation of the efforts mentioned above and focus on specific agency regulatory needs. These include continuing the analyses and interpretation of data from our studies on acrylamide, pediatric anesthetics, nanoparticles, and amphetamines and related compounds (e.g. PPX). Studies focusing on the developmental toxicity of the ubiquitous plasticizer, bisphenol A, and a protocol to assess the efficacy and toxicity of a variety of potential therapeutic agents in a transgenic-mouse model of Alzheimer's disease will begin. Renovations will expand the capacity of the Nonhuman Primate Research Center. The MRI will be functional and initial studies will be completed. Combining the power of MRI with microPET will dramatically increase imaging capabilities and enhance our efforts to describe and define neurotoxic events as they occur over time in living-animal models.

Under a Memorandum of Understanding (MOU) with FDA, National Institute for Standards and Technology (NIST), and National Cancer Institute (NCI), the NCI-established Nanomaterial Characterization Laboratory (NCL) and NCTR will conduct GLP pharmacokinetic studies for several NCL-supplied nanomaterials in nonhuman primates. These studies will generate preclinical data needed for submission to FDA to support INDs (Investigational New Drugs) or NDAs (New Drug Applications) for cancer chemotherapeutic drugs.

Studies to determine the transplacental pharmacokinetics and maternal/fetal plasma concentrations of bisphenol A (BPA) in near-term rhesus macaques are being developed. The pharmacokinetic similarities of rhesus monkeys to humans will provide a foundation for subsequent estimation of the potential exposure of the developing fetus to this agent after maternal exposure.

Phase 1 of the construction of a juvenile monkey nursery within the Nonhuman Primate Research Center is complete, and an additional protocol is being developed to evaluate growth, cognitive, and pubertal development in rhesus monkeys (*Macaca mulatta*) chronically exposed to bisphenol A from birth.

In continued fundamental research into the consequences of mitochondrial dysfunction and oxidative stress, investigations will focus on posttranscriptional and translational regulation occurring during early responses to metabolic stress. cDNA arrays, RT-PCR (reverse transcriptase-polymerase chain reaction), and metabolomic profiles obtained using NMR (nuclear magnetic resonance) technology will be used in attempts to identify biologically significant changes in gene expression that accompany mitochondrial dysfunction. Such analyses will place emphasis on the involvement of apoptotic and inflammatory responses in these processes. To further characterize the rat model of 3-NPA-induced peripheral neuropathy, morphometric and electrophysiological analyses will be undertaken to examine the properties of resveratrol, a naturally occurring neuroprotectant.

To assist the agency with the rapid determination of the neurotoxicity of a vast array of regulated chemicals and food contaminants, the division will begin 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.

Further utilization of omics techniques should allow for the identification of specific genes and pathways involved in the expression of neurotoxicity. Combining state-of-the-art imaging capabilities—microPET and MRI—will provide a new dimension to our abilities to understand adverse neural events. In addition to these specific efforts, division scientists will continue to address several main areas of fundamental research designed to broadly examine the involvement of: 1) monoamine neurotransmitter systems in the development of neurotoxicity and associated vascular damage; 2) mitochondrial dysfunction and oxidative stress as mechanisms of neurotoxicity; 3) the NMDA-receptor complex as a mediator of adult and developmental neurotoxicity; and 4) the role of A β in neurodegenerative processes. 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 colleagues at CDER, CDRH, and WPAFB, division staff plan to study the effects of nanoparticles and carbon nanotubes on the integrity of the blood-brain barrier (BBB). A microvascular endothelial cell culture will be established to model the BBB and allow the study of its permeability to nanoparticles. The toxicity of selected nanoparticles will be studied in both *in vitro* and *in vivo* models.

Contribution to FDA's Strategic Goals

Research in the Division of Neurotoxicology contributes primarily to FDA Strategic Goals 2 and 3.

FDA Strategic Goal 2 (Improve Patient and Consumer Safety)

Ongoing efforts are addressing issues of regulatory concern related to acrylamide (a food contaminant), anesthetic agents (particularly those used in the pediatric setting), and stimulant medications. Research to elucidate the mechanisms surrounding the potential neurotoxicity associated with the pediatric use of anesthetic agents, define sensitive periods of development, explore critical dose-response relationships, and develop protective therapeutic strategies are beginning to inform the agency and clinicians with the knowledge needed to minimize risk and protect public health. Research to determine the risks associated with amphetamine and related compounds—including hemorrhage, hyperthermia, and seizures—will help further clarify the conditions under which these products can be used safely. The development of high-throughput systems for the rapid detection of potentially neurotoxic compounds will help direct subsequent resource utilization in further defining the risks associated with their use.

FDA Strategic Goal 3 (Increase Access to New Medical and Food Products)

Division scientists continue to develop new approaches for the assessment of toxicity. Towards that end, 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 are beginning to provide the agency with the capabilities to get maximal information from invaluable animal models while minimizing the number of animals needed. 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 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.

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 protective effects of new medical and nutritive products. Utilization of *in vitro* brain-cell preparations in our studies on the toxicity of anesthetic compounds is proving to be a valuable tool 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 will be used to help delineate toxic mechanisms and illuminate the potentially efficacy of therapeutic strategies. Mechanistically based approaches, including a zebrafish developmental toxicity screen that is 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 new products.

Division of Personalized Nutrition and Medicine Summary of Activities

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Introduction

The Division of Personalized Nutrition and Medicine (DPNM) 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 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 DPNM 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 branches—Biometry and Biology. The main function of the Biometry branch is to develop biometrical methods for all aspects of FDA's mission, goals, and objectives. A subgroup within the branch analyzes all data from the National

Toxicology Program (NTP). The Biology branch is focusing on the broad areas of pharmacogenomics and nutrigenomics—how individuals respond to drugs and nutrients in foods.

FY 2009 Accomplishments

The Division of Personalized Nutrition and Medicine met major milestones in FY 2009 and laid the foundation for future programs in FY 2010 and beyond.

The National Toxicology Program (NTP) statistician subgroup of the Biometry branch completed 36 statistical reports for 12 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 branch contributed to multiple division, NCTR, and FDA research projects and maintained 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, and 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, and 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 branch is also leading a cross-division program to develop a food-pathogen knowledge base as a part of the agency's Food Protection Plan.

The Biology branch participated in a biomedical-focused, community-based participatory research program (CBPR) in collaboration with the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS) Delta Obesity Prevention Research Unit in Little Rock, Arkansas, and the Boys, Girls, and Adults Community Development Center (BGACDC) in Marvell, Arkansas for a second 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 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 Director of DPNM and five other scientists from Canada, Australia, and Europe.

The division has completed the development of a genomic laboratory focused on whole-chromosome analyses and resequencing of candidate genes involved in polygenic phenotypes. 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.

DPNM also initiated their stem-cell program with the addition of two staff fellows and a support staff person, all of whom have extensive experience in culture techniques. Two protocols on stem-cell research have been initiated in FY 2009. 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 the 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.

Members of DPNM are co-leading the national and international development of web-based nutrient and physical activity assessment tools, software, and databases for biomedical research. DPNM and USDA co-sponsored an interagency (USDA, FDA, and National Institutes of Health) meeting to assess the current state of lifestyle assessments in Beltsville in Spring 2009. This effort led to a second meeting at the European Nutrigenomics Organization meeting in Italy in September. These workshops led to the development of three manuscripts which have been, or are being, submitted for publication.

The division was reviewed by outside consultants and members of the NCTR Scientific Advisory Board in Washington D.C. at a 2-day meeting held in Maryland in August 2009. The Advisory Board was strongly supportive of DPNM activities and plans and provided valuable guidance for further developing its programs.

FY 2010 Plans

DPNM 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 third 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 the Phillips County area.

DPNM is 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 NCTR Healthy Challenge Study is a collaboration with other NCTR scientists in the Divisions of Systems Toxicology, Biochemical Toxicology, Neurotoxicology, Microbiology, and Genetic and Reproductive Toxicology 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 over-the-counter medication acetaminophen, exercise, and the oral-glucose challenge. 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 hosting an international workshop on assessing nutrient intake (calories in) and physical activity levels (calories out) at NCTR in January 2010. This meeting will include members of the USDA-ARS Human Nutrition Center in Beltsville, Maryland, the National Institutes of Health (NIH), CFSAN, academic researchers, and company representatives to discuss the state-of-the-art in assessment tools, databases, and needs to develop a nutritional phenotype database. The results of the workshop will be published and will describe current nutrient intake and physical activity assessment tools, as well as plans to develop comprehensive food-survey websites and databases, along with activity-monitoring software. These tools are much needed not only for research purposes, but when developed further, will have applications in clinics and community-wellness programs.

The Biometry branch will continue focus on the development of: 1) decision models for clinical assignments of patients based on the patient's genomic features and disease phenotypes, 2) methods to identify genomic, proteomic, and metabolomic liver-toxicity biomarkers, and 3) computational algorithms that will efficiently compute adjusted p-values for the large numbers of subsets defined through gene ontology. In addition, the 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.

DPNM's Biometry branch is the lead in developing a protocol for developing 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 Toxicology. 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 Goals

Research in the Division of Personalized Nutrition and Medicine contributes primarily to FDA Strategic Goal 2.

FDA Strategic Goal 2 (Improve Patient and Consumer Safety)

Research in the Biology branch 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 branch collaborates with scientists at NCTR and other FDA Centers by analyzing data with novel risk-assessment algorithms. Specifically, the Biometry branch estimates risks associated with toxic substances and helps set safe-exposure levels that correctly reflect underlying uncertainties. FDA relies on DPNM to: 1) conduct risk assessments for the regulation of specific products and in investigating generic risk-assessment issues, 2) develop mathematical models and computer systems for analyzing pharmacokinetic and pharmacodynamic components of toxic mechanisms, 3) develop classification algorithms for biomedical decision making, including identifying food hazards and assigning patients to drug therapies, 4) develop statistical methods for analyzing genomic, proteomic, metabolomic, and toxicoinformatic data, 5) apply statistical methods to evaluate toxicological, pharmacological, and nutritional concerns, and 6) provide expertise to NCTR scientists on the design, conduct, and analyses of research studies to evaluate the toxicity of regulated products. The Biometry branch is also contributing to FDA's responsibilities to protect the food system in the United States.

Division of Systems Toxicology Summary of Activities

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Introduction

The Division of Systems Toxicology supports the development of new technologies and works to facilitate the integration of scientific data for application to questions that are in direct support of the FDA mission. Six Centers of Excellence comprise the Division of Systems Toxicology: Functional Genomics, Hepatotoxicity, Innovative Technologies, Metabolomics, Proteomics, and Toxicoinformatics. The goals of this division include: 1) to provide technical expertise and guidance for the inclusion of omics and *in silico* data into the review process and within the drug-development process, 2) to identify new, more predictive biomarkers of toxicity, efficacy, prognosis, diagnosis, and disease that will aid in the development and approval of safer and more effective medicines, foods, and medical devices, 3) to identify new approaches to improve food safety, cancer treatment, and medical imaging for less invasive diagnoses, and 4) to improve the bioinformatics capability of the agency. This division continues to initiate collaborations across the FDA regulatory Centers and with academic and pharmaceutical research groups in an effort to improve human health.

The Center for Functional Genomics uses high-information content microarrays in the development of mechanistic and biomarker data 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 genomics issues.

The Center for Hepatotoxicity addresses critical issues related to liver injury by applying a systems-toxicology approach. The goals are to improve the identification of hepatotoxic compounds prior to human exposure and to augment the detection of early signs of injury in humans induced by drugs, chemicals, and disease processes.

The Center for Innovative Technologies uses multi-faceted 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 efforts in computational modeling to improve diagnosis.

The Center for Metabolomics employs multiple metabolomic methods to improve the detection of toxicity and disease in preclinical species and humans.

The Center for Proteomics conducts proteomic research through collaborations with investigators 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.

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

FY 2009 Accomplishments

During FY 2009, division scientists engaged in research addressing a variety of agency issues with special emphasis on areas in biomarker identification, food safety, and bioinformatics. Accomplishments include the following:

- Advances in our understanding of hepatotoxicity
- Formed a Hepatotoxicity Working Group comprised of clinical investigators, individuals from academia and the pharmaceutical industry focused on preclinical studies, and CDER representatives to provide advice and direction for assessing critical preclinical and clinical liver-toxicity issues, such as better methods and biomarkers for identifying idiosyncratic hepatotoxicants
- Completed analysis of data from a pilot systems-toxicology study to develop predictive biomarkers of drug-induced liver injury involved in idiosyncratic liver injury
- Identified sex-based differences in hepatic mitochondrial function that may have implications for observed divergent responses in drug-induced toxicities
- Examined the hepatotoxicity of usnic acid, a dietary supplement
- Produced a compendium of gene-expression profiles in the liver of normal rats and identified the expression patterns of genes associated with age- and sex-related susceptibilities to disease and toxicity
- Identified epigenetic differences in mice that may be responsible for strain-specific susceptibility of mice to nonalcoholic steatohepatitis (NASH)
- Completed the initial phase of the Liver Toxicity Knowledge Base project, including collection of data from literature related to hepatotoxicity, cell-based toxicity assays and microarray experiments, and development of essential bioinformatics tools
- Evaluated effects on hepatotoxic compounds on metabolites in an important metabolic pathway involved in toxicity
- Started a collaborative inter-agency project of *in silico* computational toxicology modeling to predict adverse polypharmacy interactions with Cytochrome p450 enzymes
- Initiated a proof-of-concept project for personalized medicine by conducting an *in silico* study on protein and drug interaction that may explain idiosyncratic hepatotoxicity.

Improved methodology

- Used metabolomic methods to identify novel drug metabolites in several preclinical studies including acetaminophen, valproic acid, and tolcapone
- Continued to advance the science of metabolomics by utilizing ^{13}C glucose flux analyses in preclinical studies and *in vitro* studies
- Enhanced proteomics research capabilities by implementing high-resolution mass spectrometry and laser-induced fluorescence detector
- Started evaluation of key sample preparation factors for quantitative proteomic analysis.

Advance understanding and diagnosis of disease

- Developed accurate computational models for brain-cancer diagnosis using MRS data and started a new collaboration with CDRH
- Characterized protein complexes and modifications potentially involved in cancer development
- Used array-based comparative genomic hybridization (aCGH) to show that chemicals may induce mutations through mitotic recombination in the standard mouse lymphoma cell genetic-toxicity assay.

Improvements in food safety

- Successfully completed a series of validation studies in collaboration with the Arkansas Department of Health for the rapid detection of *E. coli* 0157 in nine food matrices including hamburger, spinach, and chocolate chip cookie dough. The RAPID-B technology proved to be more sensitive and faster than the standard bacterial analytical manual (BAM) methods
- Developed a new RAPID-B assay that detects *Staphylococcus aureus*
- Successfully developed a quantitative FQI (food quality indicator) ammonia sensor that can be used in hen houses to warn of dangerous ammonia levels. Ten thousand prototype sensors were produced as part of an industry-sponsored test.

Provided bioinformatics and data-analysis support to regulatory Centers

- Accomplished 1st phase of the e-submission pilot studies for nonclinical and pharmacogenomics data submission
- Provided infrastructure and expertise to help CDER in data management for two regulatory projects
- Participated as a peer with other FDA Centers in the activities and programs under the auspices of the FDA Bioinformatics Board (BIB) for budgeting, planning, and developing the Janus pilot studies
- Supported the Voluntary eXploratory Data Submission (VXDS) program by analyzing four submissions and participated in the discussion for all other submissions
- Developed a new web-based system, called VISIONS (VXDS/IPRG Status and INformation ON-line system), to facilitate VXDS data management and review

- Completed several new functions in ArrayTrack™ for managing and analyzing the data related to personalized medicine and nutrition, food safety, and predictive toxicology
- Developed a web-based database to manage dietary supplements. The database is tentatively named Electronic Dietary Supplement Compilation (eDISCO).

Continued the work to improve standard approaches in analysis of omics data

- Completed Phase II of the MicroArray Quality Control (MAQC) project and submitted manuscripts summarizing the projects for peer review
- Evaluated the “in-house” data for the MAQC-III project.

FY 2010 Plans

In FY 2010, the Division of Systems Toxicology will continue to emphasize the systems-biology approach for development of predictive biomarkers and mechanistic information for safety assessments of medical products and foods. Additional studies will focus on the development of improved *in silico* modeling approaches for drug safety and medical imaging. To accomplish its mission, the Division of Systems Toxicology 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. Examples include:

- Begin a multi-year, systems-biology study to identify potential biomarkers to improve early detection of idiosyncratic hepatotoxicants
- Identify biomarkers of drug-induced cardiotoxicity
- Study sex- and age-based changes in the genes expressed in the kidney and heart of normal rats focusing on those involved in metabolism and secretion of drugs
- Begin to connect systems-biology data in preclinical studies with imaging biomarkers
- Work with international groups on metabolomics quality-control standards.

Work to improve food safety

- Continue to expand assays using the RAPID-B and OMNIPrint technologies
- Utilize a metabolomics approach to start compiling metabolic signatures of bacterial contamination in milk.

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 technology 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 the e-submission pilot studies for nonclinical and pharmacogenomics data submission
- Continue participation in the review of the pharmacogenomics data submitted through the VXDS program
- Continue to support the FDA bioinformatics modernization plan.

Contribution to FDA's Strategic Goals

Research in the Division of Systems Toxicology contributes to FDA Strategic Goals 1, 2, 3, and 4.

FDA Strategic Goal 1 (Strengthen FDA for Today and Tomorrow)

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.

FDA Strategic Goal 2 (Improve Patient and Consumer Safety)

The entire division is involved with developing potential preclinical and translational safety, efficacy, and disease biomarkers based on new metabolomic, genomic, and proteomic technologies. The unique aspect of this work is the integration of the various datasets coupled with computational biology to get a holistic systems view of medical products and of disease processes. This division is building a Liver Toxicity Knowledge Base to improve reviewer's understanding of safety issues. In addition, new mass-spectrometric and flow-cytometric methods will continue to be developed and validated for the detection of bacteria in food products. There is a continuing effort to develop sophisticated pattern recognition-based algorithms to advance predictive and diagnostic applications.

FDA Strategic Goal 3 (Increase Access to New Medical and Food Products)

The division is developing various bioinformatics tools to support FDA VXDS program, including ArrayTrack™, SNPTrack, and VISIONS. These tools allow the reviewers to easily access the information from both private and public domain—thus enhancing the FDA-review process. Novel computational models are being developed that predict drug safety and efficacy and such new methods will increase the number of safe and effective medical products.

FDA Strategic Goal 4 (Improve the Quality and Safety of Manufactured Products and the Supply Chain)

Scientists in the division are working on methods to detect food spoilage in a commercial setting. This technology is now being commercialized and promises to provide on-the-spot information on the freshness of the food supply.

Division of Veterinary Services Summary of Activities

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Introduction

The Division of Veterinary Services (DVS) provides professional and technical support for all animal-related research projects at NCTR. The division 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 division 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 Division Director is a member of NCTR's Institutional Animal Care and Use Committee (IACUC), serving as Vice-Chair and Attending Veterinarian. The liaison between DVS and the IACUC ensures maximum efficiency in protocol planning and review, provision of the highest quality of animal care and use, and delivery of superior services to the NCTR research community.

DVS 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 division priority. Three veterinarians, two of whom are certified by the American College of Laboratory Animal Medicine (ACLAM) and all of whom hold research degrees in addition to D.V.M.s, 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 attention 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 2009 Accomplishments

Immediate Office

The division provided oversight and management of all NCTR laboratory animal facilities. Division personnel were responsible for breeding, rearing, and 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. The Division Director is NCTR's Attending Veterinarian and the IACUC Vice-Chair. All animal resource needs were managed for all research projects. Division personnel served as government project COTRs (contracting officer's technical representative) for pathology services, 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 DVS 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 evaluation of over-the-counter skin products on the absorption of dermally applied estradiol in mini-pigs. All divisional veterinarians were voting members of the IACUC. To ensure state-of-the-art housing environments for research animals, members of this division played an integral role in planning animal-facility renovation projects, especially the renovation and expansion of the Nonhuman Primate Research Center and the Building 5 Processing Area.

Animal Care/Diet Preparation Services

During FY 2009, contract personnel supported a daily average of 32 experiments. These experiments entailed the daily husbandry services average 4582 rodents, 128 rhesus monkeys, and 2 mini-pigs. 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 and rhesus monkeys, 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 85% of animal care

and diet-preparation staffs are AALAS-certified, and eight members of the animal care management group are Certified Managers of Animal Resources (CMAR). In addition to processing standard irradiated rodent chow (receipt, storage, and delivery), dosed diets, dosed water, and topical creams were prepared to exacting specifications for NTP (National Toxicology Program) experiments. The conversion to irradiated diet from autoclavable diet was completed in FY 2009. Quality-control personnel performed monthly inspections of all animal housing and diet-preparation units, performed hundreds of quality-control audits of animal care and diet-preparation procedures and maintained, updated, and created a large volume of SOPs. 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.

Pathology and Pathology-Related Services

During FY 2009, the Pathology Services group performed the following services:

- Necropsy of 2250 animals; the laboratory processed 38,932 cassettes from trimmed tissue, embedded 34,847 blocks, sectioned 38,483 blocks and produced 33,352 H & E stained slides
- The clinical pathology staff performed different analyses of blood on 2861 animals to include evaluations of hematology (CBCs)—875, chemistries—7443, radioimmunoassays—9314, reticulocytes—354, urinalysis—853, and ELISA (enzyme-linked immunosorbent assay)—5587 as well as collecting and freezing 1153 samples
- Sperm analysis and vaginal cytology on animals for NTP studies.

In addition to routine pathology services, other accomplishments for FY 2009 include:

- Conducted NTP quality assessment and peer review of pathology data for six chronic bioassay studies including rat and mouse studies in acrylamide, glycidamide, and *Aloe vera*
- Authored or co-authored 11 publications in professional journals
- Provided nonroutine services such as digital macrophotography, laser capture microdissection, and image archiving using digital storage of microscopic images at diagnostic resolution
- Immunohistochemical procedures were performed or methods developed for the following procedures: P450scce, C/EBP alpha IHC, Sox-9, GSTP, Tunel, Catalase, ER-alpha, Androgen Receptor, ER-beta, CK10, S100, GMA Plastic
- Draft final or final reports were submitted for seven studies.

FY 2010 Plans

- Continue to support the research mission of NCTR through excellence in animal care, veterinary care, diet preparation, and pathology 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

- Prepare the Animal Care and Use Program Description for the AAALAC site visit
- Coordinate preparation efforts for the AAALAC site visit among NCTR management, research division, animal husbandry, engineering, maintenance, etc. personnel
- Host the AAALAC site visit
- Play a lead role in the recompetition of the Animal Care/Diet Preparation 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 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. DVS, through its support-services functions and research participation, is part of each division's contribution to these goals. DVS also contributes to NCTR's research program through participation in the projects of other divisions as Principal Investigators and Co-Investigators. Several DVS personnel are D.V.M.s or Ph.D.s whose specialties in comparative medicine, veterinary pathology, toxicology, genetics, and biochemistry complement the research teams in all other divisions.

The DVS plays a critical support-services role in NCTR's biomedical research program. DVS personnel interact with individuals from every research division on a daily basis, providing expertise in animal care, diet preparation, laboratory-animal medicine, and pathology. These services are provided by highly trained, skilled, and dedicated individuals whose contributions enhance the quality of the research conducted by NCTR scientists. In addition, DVS oversees the NCTR Laboratory Animal Care and Use Program, which has been accredited by the AAALAC since 1977. This distinction assures Center scientists, FDA, and the American consumer that data generated from animal experiments at NCTR are of the highest integrity.

FY 2009 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, our nation's food supply, cosmetics, and products that emit radiation as well as advancing the 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 Strategic Goal and supports FDA's goals as outlined in the FDA's Strategic Action Plan, which sets forth its long-term strategic goals and objectives. These goals include:

NCTR Research Strategic Goals:

- Goal 1: Advance scientific approaches and tools to promote personalized nutrition and medicine for the public
- Goal 2: Develop science-based best-practice standards, guidance, and tools to incorporate toxicological advancements that improve the regulatory process
- Goal 3: Conduct research and develop strategic technologies to protect the food supply
- Goal 4: Conduct bioinformatics research and development in support of FDA's regulatory mission

FDA Strategic Action Plan Goals:

- Goal 1: Strengthen FDA for Today and Tomorrow
- Goal 2: Improve Patient and Consumer Safety
- Goal 3: Increase Access to New Medical and Food Products
- Goal 4: Improve the Quality and Safety of Manufactured Products and the Supply Chain

NCTR Strategic Goal 1 Advance the scientific approaches and tools to promote personalized nutrition and medicine for the public

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

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

Responsible Division: Neurotoxicology

Collaborating Divisions: Biochemical
Toxicology, Office of the Director

Objectives:

- 1) To determine the post-translational protein modifications in the protein extracts of nigral and striatal tissues in substituted amphetamines and MPTP-treated mice
- 2) To evaluate the effect of various nNOS inhibitors and peroxynitrite decomposition catalysts on the post-translational protein modifications in the protein extracts of nigral and striatal tissues in mice treated with substituted amphetamines and MPTP

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

PI: Beger, Richard D., Ph.D.

Analysis of Blood Pyruvate and Valproic Acid Toxicity in Wistar Han Rats in Response to Dietary Carbohydrate and Calorie Restriction with a High-Fat, Moderate, and Low-Carbohydrate Diet (P00709)

Responsible Division: Systems Toxicology

Collaborating Divisions: Genetic and Reproductive Toxicology, Office of Research, Veterinary Services

Objectives:

- 1) To develop an *in vivo* rat model with lower plasma pyruvate levels by using dietary carbohydrate restriction
- 2) To determine whether pyruvate blood levels in CR rats fed a HF/LC diet are decreased by approximately 30% relative to rats fed a balanced diet
- 3) To determine whether 45% CR Wistar Han rats can adequately survive on a HF/LC diet for several weeks
- 4) To determine whether CR Wistar Han rats fed a HF/LC diet are more susceptible to valproic acid-induced liver injury than rats fed a balanced healthy diet.

PI: Beger, Richard D., Ph.D.

Clinical Metabolomic Biomarkers of Disease and Toxicity (S00643)

Responsible Division: Systems Toxicology

Objective:

To characterize metabolomics signatures found in clinical urine and serum samples seen by 1H NMR and mass spectrometry.

PI: Beger, Richard D., Ph.D.

Preclinical Metabonomic Biomarkers of Toxicity and Disease (E0720401)

Responsible Division: Systems Toxicology

Collaborating Division: Neurotoxicology

Collaborating FDA Center: CDER

Objective:

To examine the utility of metabolomics as an approach to produce predictive models of cardiovascular, renal, neural, and hepatic toxicity.

PI: Beland, Frederick A., Ph.D.

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

Responsible Division: Biochemical
Toxicology

Collaborating FDA Center: CVM

Objective:

To produce data that will put the concern about the potential for benzocaine-induced methemoglobinemia in humans consuming meat from benzocaine treated fish in perspective.

PI: Beland, Frederick A., Ph.D.

Carcinogenicity of Acrylamide and its
Metabolite, Glycidamide, in Rodents
(E0718501)

Responsible Division: Biochemical
Toxicology

Objective:

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

PI: Beland, Frederick A., Ph.D.

DNA Adducts of Tamoxifen (E0701101)

Responsible Division: Biochemical
Toxicology

Objectives:

- 1) To characterize DNA adducts from suspected tamoxifen metabolites
- 2) To develop methods for their detection and quantitation.

PI: Beland, Frederick A., Ph.D.

Genotoxicity and Carcinogenicity of
Acrylamide and its Metabolite,
Glycidamide, in Rodents (E0215001)

External Funding: National Toxicology
Program (IAG)

Responsible Division: Biochemical
Toxicology

Collaborating Division: Genetic and
Reproductive Toxicology

Objective:

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

PI: Beland, Frederick A., Ph.D.

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

External Funding: BG Medicine, Inc.
(CRADA)

Responsible Division: Biochemical
Toxicology

Collaborating Division: Systems
Toxicology

Objective:

To 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.

PI: Beland, Frederick A., Ph.D.

Mechanisms of Nevirapine
Carcinogenicity (E0217101)

External Funding: National Toxicology
Program (IAG)

Responsible Division: Biochemical
Toxicology

Objective:

To determine the mechanisms by which nevirapine induces liver tumors in rats.

PI: Binienda, Zbigniew, K., D.V.M., 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: Office of the Director

Collaborating FDA Center: CFSAN

Objectives:

- 1) To define neurophysiological and neurochemical phenotypes associated with brain exposure to 3-NPA and L-carnitine
- 2) To define changes in patterns of gene expression induced by 3-NPA and L-carnitine in the rat brain
- 3) To assess the attenuation of energy deficits associated with L-carnitine using enzymatic and neurochemical biomarkers of neurotoxicity in the rat model of 3-NPA-induced histotoxic hypoxia
- 4) To establish the relationship between 3-NPA-induced physiological and neurochemical phenotypes and transcriptome profiles in the rat brain model
- 5) To 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:

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: Boudreau, Mary, Ph.D.

A Toxicological Evaluation of Nanoscale Silver Particles in Rodents (E0217001)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division: Office of Research

Objectives:

- 1) To evaluate the effect of size of nanoscale silver particles on plasma protein-binding in blood collected from adult rodents using standard analysis methods to estimate the equilibrium association constant and maximum binding capacity
- 2) To determine the effects of size and dose of nanoscale silver particles on the pharmacokinetic profiles and bioavailability when administered by the oral and intravenous routes in rats, and determine whether the pharmacokinetics of nanoscale silver are the same as silver acetate
- 3) To evaluate the absorption, biodistribution (including the potential to cross the blood-brain barrier), and excretion rates of nanoscale silver particles that differ in size.

PI: Bowyer, John F., Ph.D.

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

Responsible Division: Neurotoxicology

Collaborating Divisions: Biochemical Toxicology, Genetic and Reproductive Toxicology

Objective:

To determine the changes in gene expression that exist prior to the onset of pathology and those that occur during the pathological process and to quantify accompanying behavioral alterations

PI: Chelonis, John, Ph.D.

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

Responsible Division: Neurotoxicology

Collaborating Division: Office of Research

Objective:

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

PI: Chelonis, John, Ph.D.

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

Responsible Division: Neurotoxicology

Objective:

To 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 J., Ph.D.

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

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division: Genetic and Reproductive Toxicology

Collaborating FDA Center: CDER

Objective:

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

PI: Chen, James J., Ph.D.

Evaluating the Statistical Significance of Treatments on a Group of Correlated Genes (E0723601)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division: Genetic and Reproductive Toxicology

Objectives:

- 1) To investigate the true-significance level and power of statistical methods for combining correlated p-values
- 2) To develop adjustments that eliminate or mitigate the deleterious effect of correlations
- 3) To implement computer algorithms that will efficiently compute adjusted p-values for the large numbers of subsets defined through a gene ontology.

PI: Chen, James J., 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

Objectives:

- 1) To find sex-specific high-dimensional biomarkers

- 2) To develop classifiers for each sex using our CERP algorithm as well as several alternative algorithms
- 3) To investigate the improvement in these high-dimensional biomarkers by using the variable importance derived from our classification algorithm to prioritize and combine features
- 4) To find optimal cutoffs to select high-dimensional biomarkers and finalize the classification algorithm
- 5) To assess the performance of sex-specific high-dimensional biomarkers from our classification algorithm by cross-validation to obtain a valid measure of prediction accuracy using publicly available high-dimensional non gender-specific data
- 6) To develop a user-friendly classification software tool which is downloadable from the Internet.

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 Reproductive Toxicology

Collaborating Division: Systems Toxicology

Objectives:

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

- 2) To develop a PCR array containing the primers specifically used to amplify carcinogenesis-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) To define the miRNA biomarker genes associated with carcinogen exposure by prediction of their target genes and determination of their biological functions.

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 (E0217201)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Objective:

To characterize the dose-response for orally administered BPA in the NCTR Sprague-Dawley rat to address the question of adverse effects in rodents near levels of exposure potentially attainable in humans.

PI: Desai, Varsha G., 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 Toxicology

Collaborating Divisions: Genetic and Reproductive Toxicology, Personalized Nutrition and Medicine

Objective:

To elucidate molecular mechanisms of mitochondrial dysfunction that will

address gender-based differences in adverse effects of antiretroviral drugs, such as AZT.

PI: Dobrovolsky, Vasily N., 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 Reproductive Toxicology

Collaborating Division: Systems Toxicology

Collaborating FDA Centers: CDER, CDRH

Objectives:

- 1) To 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
- 2) To use the methods developed in Objective 1 to 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
- 3) To 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 R., Ph.D.

Development of a PBPK/PD Model for Acrylamide (E0721201)

External Funding: University of Maryland (CRADA)

Responsible Division: Biochemical Toxicology

Collaborating Divisions: Genetic and Reproductive Toxicology, Personalized Nutrition and Medicine

Objectives:

- 1) To develop a physiologically based pharmacokinetic/pharmacodynamic model for acrylamide and glycidamide
- 2) To determine mutagenicity of acrylamide and its metabolite glycidamide in Big Blue[®] rats
- 3) To determine the DNA-adduct levels and the extent of mutagenicity of furan and its metabolite cis-2-buten-4-dial in neonatal B6C3F1/TK+/- mice.

PI: Doerge, Daniel R., Ph.D.

Human Studies of Isoflavone Safety and Efficacy (S00607)

Responsible Division: Biochemical Toxicology

Objective:

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

PI: Doerge, Daniel R., Ph.D.

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

External Funding: University of Illinois (CRADA)

Responsible Division: Biochemical Toxicology

Objective:

To evaluate the potential benefits or detrimental effects of dietary phytoestrogens on breast-cancer progression, adipose tissue, and the

brain using well-established laboratory animal models.

PI: Erickson, Bruce D., Ph.D.

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

External Funding: Pfizer, Inc. (CRADA)

Responsible Division: Microbiology

Objectives:

- 1) To evaluate the ability of the bovine intestinal microflora to inactivate ceftiofur using pure culture isolates and mixed fecal cultures
- 2) To identify primary metabolites of ceftiofur degradation
- 3) To isolate ceftiofur-resistant bacteria and determine the primary mechanisms of drug inactivation
- 4) To investigate the metabolic potential of anaerobic fungi isolated from bovine-fecal samples to degrade ceftiofur
- 5) To compare the metabolism of ceftiofur with the human third-generation cephalosporin, ceftriaxone.

PI: Ferguson, Sherry A., Ph.D.

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

Responsible Division: Neurotoxicology

Objective:

To 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 A., Ph.D.

Sex Differences in Drug-Abuse Susceptibility in Methylphenidate-Treated Rats (E0727201)

Responsible Division: Neurotoxicology

Objective:

To determine potential sex differences in substance abuse susceptibility after methylphenidate (Ritalin®) treatment during adolescence.

PI: Ferguson, Sherry A., Ph.D.

Training for Bisphenol A Studies (P00706)

Responsible Division: Neurotoxicology

Objectives:

- 1) To develop the appropriate skills and techniques necessary to conduct subsequent studies of developmental treatment with Bisphenol A by training key personnel, including PIs, technicians, and animal-care personnel
- 2) To develop techniques that include complex behavioral assessments and quantitative volumetric analysis of sexually dimorphic brain regions.

PI: Fu, Peter P., Ph.D.

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

Responsible Division: Biochemical Toxicology

Objective:

To define dose-response curves for benzo[a]pyrene DNA adducts in the A/J mouse lung.

PI: Fuscoe, James C., Ph.D.

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

Responsible Division: Systems Toxicology

Collaborating Division: Genetic and Reproductive Toxicology

Objectives:

- 1) Use the NCTR rat microarray chip to quantitate the relative expression of approximately 4000 genes in the liver of rats at 2, 5, 6, 8, 15, 21, 52, 78, and 104 wks of age
- 2) To verify the relative expression levels by quantitative PCR or Northern analysis.

PI: Fuscoe, James C., Ph.D.

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

Responsible Division: Systems Toxicology

Collaborating Divisions: Genetic and Reproductive Toxicology, Personalized Nutrition and Medicine

Objectives:

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

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: Systems Toxicology

Objectives:

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

- 2) To compare mutual versus species-specific gene-expression response to PPAR- α agonists

- 3) To investigate specific genes regulated by PPAR- α agonists in susceptible species such as rat and mouse compared to human
- 4) To identify novel target genes whose expression has not been previously reported to be affected by PPAR- α agonists
- 5) To determine whether the expression of candidate target genes is PPAR- α 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: Systems Toxicology

Collaborating Division: Genetic and Reproductive Toxicology

Objectives:

- 1) To isolate liver cells 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
- 2) To obtain signature-gene and protein-expression patterns of each cell type for comparison to toxin-induced changes.

PI: Hammons, George J., 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

Objectives:

- 1) To 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) To determine the effect of tobacco smoke on DNMT1, 3a, and 3b expression in liver-cell systems
- 3) To assess the polymorphism frequency identified in DNMT3b in the sample pool included in the study and assess whether it is correlated with expression.

PI: Hansen, Deborah K., Ph.D.

Preliminary Study of to Determine the Physiological and Cardiovascular Effects of *Citrus aurantium* (CA) in Mini-Pigs (P00721)

Responsible Division: Personalized
Nutrition and Medicine

Collaborating Division: Genetic and
Reproductive Toxicology

Objectives:

- 1) To develop procedures for the surgical implantation of telemetry monitors in mini-pigs
- 2) To determine the best method for collection of physiological and cardiovascular variables by telemetry in mini-pigs
- 3) To determine the best method for dosing of mini-pigs with CA extract
- 4) To determine if mini-pigs are a sensitive and reliable animal model for determining the cardiovascular and physiological effects of CA in humans.

PI: Hart, Mark E., 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 (TSST-1) Producing Strains of *Staphylococcus aureus* (E0728601)

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

Responsible Division: Microbiology

Collaborating FDA Center: CFSAN

Objectives:

- 1) To 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 model with recently developed genital-tract secretion medium
- 2) To 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) To isolate and clone the lysostaphin gene into a select group of *lactobacilli*
- 4) To 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 model.

PI: Hart, Mark E., 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: Veterinary Services

Objectives:

- 1) To generate hemagglutinin (HA) surface-presented yeast recombinant avian influenza vaccines
- 2) To characterize humoral and cellular-mediated immune responses of yeast vaccines in mice
- 3) To demonstrate protection of mice from lethal avian-influenza virus through yeast-based immunization.

PI: Hart, Mark E., Ph.D.

Protective Effect of Vaginal *Lactobacillus* Species Against *Staphylococcus Aureus*-Mediated Toxic-Shock Syndrome (E0725501)

Responsible Division: Microbiology

Objective:

To determine whether probiotic administration of *Lactobacillus* can thwart *S. aureus* TSST-1 production if supplemented in women's tampons.

PI: He, Zhen, Ph.D.

Brain Sexual Dimorphic Structures and Sex Hormone-like Compounds (P00710)

Responsible Division: Neurotoxicology

Objective:

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

PI: Hong, Huixiao, Ph.D.

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

Responsible Division: Systems Toxicology

Collaborating Divisions: Personalized Nutrition and Medicine, Z-Tech Corporation

Collaborating FDA Center: CDER

Objective:

To compare the latest methods for analyzing GWAS data with a focus on developing baseline practices using publicly available data sets as well as data sets received through collaborations between FDA and drug sponsors.

PI: Howard, Paul C., Ph.D.

Analytical Assay for Photochemical Generation of Hydroxyl radical (S00728)

Responsible Division: Office of Research

Collaborating Division: Biochemical Toxicology

Objectives:

- 1) To provide support for analysis of the photoactivation of nanomaterials using the •OH/coumarin-3-carboxylic acid assay
- 2) To provide particle-size analysis for all materials being analyzed by •OH method and other nanomaterials used in studies at NCTR and ARL/ORA
- 3) To improve the assay using ultraviolet light diode laser as a replacement to the existing broad-band ultraviolet light A source.

PI: Kaput, James A., Ph.D.

Delta Vitamin Obesity Prevention Summer Camp (E0733001)

Responsible Division: Personalized Nutrition and Medicine

Objectives:

- 1) To 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) To 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) To analyze ancestry through whole genome scans and candidate genes responsive to vitamin intake to associate individual responses with genetic polymorphisms
- 4) To improve the nutrition and genetic education of the participants through lessons taught by local teachers with materials provided by NCTR, USDA, and local UAMS AHEC diabetes educator
- 5) To develop health-economic analyses of the intervention
- 6) To 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: Kaput, James A., Ph.D.

Obesity Prevention Summer Program: Feasibility of Implementing a Multi-Component Obesity Prevention Intervention at a Youth Program in the Mississippi Delta (E0729601)

Responsible Division: Personalized Nutrition and Medicine

Objectives:

- 1) To study the complex interaction of genetic makeup and environment—particularly the food environment
- 2) To test the hypothesis that CBPR (community-based participatory research program), coupled with omics research technologies and healthcare, will produce more reliable scientific data and results and, at the same time, improve the lives of the participants.

PI: Leakey, Julian E., Ph.D.

Studies of Usnic Acid and *Usnea Barbata* Herb in Fischer 344 Rats and B6C3F1 Mice (E0215911)

External Funding: National Toxicology Program (IAG)

Responsible Division: Office of Research

Collaborating Division: Systems Toxicology

Collaborating FDA Center: CFSAN

Objective:

To establish appropriate doses of usnic acid and *Usnea barbata* preparations, administered in feed, in male and female Fischer 344 rats and B6C3f1 mice.

PI: Lyn-Cook, Beverly A., 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)

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

Responsible Division: Associate Director for Regulatory Activities

Collaborating Divisions: Biochemical Toxicology, Office of Research, Personalized Nutrition and Medicine

Collaborating FDA Center: CDER

Objectives:

- 1) To identify polymorphisms in drug-transporter genes identified to be differentially expressed according to gender in human-liver samples
- 2) To correlate polymorphism frequencies in males and females to gene expression
- 3) To 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
- 4) To evaluate effects of polymorphisms in transporter genes on uptake and clearance of chemotherapeutic drugs in a functional assay.

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

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

Responsible Division: Associate Director for Regulatory Activities

Collaborating Divisions: Biochemical Toxicology, Personalized Nutrition and Medicine, Systems Toxicology

Objectives:

- 1) To identify sex differences in the gene expression of drug transporters known to be involved in transport of chemotherapeutic drugs and with hepatic expression in human-liver tissues
- 2) To evaluate sex-related hepatic drug-transport function 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
- 3) To characterize the relationships between transporter-gene expression and uptake or excretion of chemotherapeutic drugs defined with the sandwich model and transporter-transfected cell lines
- 4) To evaluate the effects of sex hormones on hepatic-transporter gene expression in human-cancer cell lines and sandwich-cultured hepatocytes

- 5) To identify and validate novel transporter-drug correlations using a chemogenomic approach followed by cytotoxicity and drug-uptake studies in cell lines overexpressing specific transporter genes
- 6) To 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
- 7) To develop guidelines and recommendations for clinical-trial design and analysis of sex differences in new drug applications.

PI: Lyn-Cook, Beverly A., 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 Divisions: Biochemical Toxicology, Neurotoxicology, Personalized Nutrition and Medicine, Veterinary Services

Objective:

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

PI: Mckinzie, Page B., Ph.D.

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 Reproductive Toxicology

Objective:

To use 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.

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 Reproductive Toxicology

Objectives:

- 1) To 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) To use model mutagens, *N*-ethyl-*N*-nitrosourea (ENU) and cyclophosphamide (CP) to investigate the potential utility of the *TCR* gene-mutation assay using isolated spleen lymphocytes derived from treated Fisher 344 rats
- 3) To 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 M., 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 Reproductive Toxicology

Collaborating Division: Systems Toxicology

Objectives:

- 1) To determine if the L5178Y/TK+/- MLA adequately detects both aneuploidy and mitotic recombination
- 2) To 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) To determine the fundamental genetic mechanism(s) causing the small and large colony thymidine kinase mutant phenotypes.

PI: Morris, Suzanne M., 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 Reproductive Toxicology

Collaborating Divisions: Personalized Nutrition and Medicine, Systems Toxicology

Objectives:

- 1) To determine the effect of mutation in the *p53* tumor suppressor gene on gene-expression profiles in young and aged mice

- 2) To 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

Collaborating Divisions: Associate Director for Regulatory Activities, Biochemical Toxicology, Systems Toxicology

Objectives:

- 1) To 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) To characterize molecular mechanisms of sex hormones 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) To measure sex-hormone modulation of aspirin effect on platelet aggregation and its related biomarkers using human platelet precursor cells
- 4) To identify sex-hormone modulation of aspirin actions in human endothelial and epithelial cell lines, by measuring prostacyclin dynamics and aspirin-targeting enzymes expression

- 5) To evaluate sex-hormone modulation of response to aspirin in apolipoprotein 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: Systems Toxicology

Objectives:

- 1) To identify microRNA biomarkers of adipogenesis process
- 2) To investigate the effect of micronutrients on microRNA expression during the differentiation process of mesenchymal stem cells into adipocytes.

PI: Parsons, Barbara L., Ph.D.

Cancer Mutations as Biomarkers of Cancer Risk (E0726501)

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Division: Personalized Nutrition and Medicine

Objectives:

- 1) To develop the information necessary for the rational use of oncogene mutations as quantitative biomarkers of cancer risk
- 2) To 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) To validate a streamlined allele-specific competitive blocker PCR (ACP-PCR) methodology
- 4) To develop the methodology necessary to measure oncogene MF (mutant fraction) in cell-free DNA isolated from plasma
- 5) To convey to the regulatory risk-assessment community the regulatory significance of the data regarding tumor-associated mutations.

PI: Parsons, Barbara L., 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 Reproductive Toxicology

Collaborating Division: Neurotoxicology

Objectives:

- 1) To further develop, evaluate, and disseminate an NCTR-developed method, allele-specific competitive blocker-PCR (ACB-PCR)
- 2) To determine if 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: Parsons, Barbara L., Ph.D.

Measurement of Cancer-Associated Gene Mutation in Colon Tumor and Nontumor Tissue (E0716001)

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Division: Neurotoxicology

Objectives:

- 1) To determine *K-ras* codon 12 GGT→GAT and GGT→GTT mutant

frequencies in colonic ACF, adenomas, and carcinomas first by DNA sequencing and, if mutation is not detected, then by ACP-PCR

- 2) To determine *K-ras* codon 12 GGT→GAT and GGT→GTT mutant frequencies in tumor margin samples and tumor-distant, normal-appearing colonic epithelium from colon-cancer patients

- 3) To determine *K-ras* codon 12 GGT→GAT and GGT→GTT mutant frequencies in autopsy samples of colonic epithelium from colon disease-free individuals.

PI: Paule, Merle G., Ph.D.

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

Responsible Division: Neurotoxicology

Objective:

To measure aspects of learning, short-term-memory and attention, motivation, time perception, and color and position discrimination.

PI: Paule, Merle G., Ph.D.

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

External Funding: University of Arkansas for Medical Sciences (CRADA)

Responsible Division: Neurotoxicology

Objectives:

- 1) To develop and implement a comprehensive assessment of all levels of the neuraxis to determine CNS deficits due to balance disorder and vertigo
- 2) To develop and assess strategies to restore those deficits.

PI: Pogribny, Igor P., Ph.D.

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

External Funding: National Cancer Institute/DNA Hypomethylation (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Divisions: Genetic and Reproductive Toxicology, Office of the Director, Systems Toxicology

Objectives:

- 1) To determine if the temporal alterations in genomic methylation profile in preneoplastic-liver tissue observed in the folate/methyl-deficient model of rat endogenous hepatocarcinogenesis also occur in other carcinogenesis model
- 2) To identify genes that are consistently up-regulated or down-regulated in target tissue during the promotion stage of carcinogenesis
- 3) To 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: Rafii, Fatemeh, Ph.D.

Biotransformation of Isoflavonoid Phytoestrogens by Colonic Microfloras of Experimental Animals (E0724401)

Responsible Division: Microbiology

Collaborating Divisions: Biochemical Toxicology, Systems Toxicology

Objective:

To use fecal samples of monkeys and rodents to find out if the metabolites

produced by intestinal microfloras of experimental animals exposed to phytoestrogens are the same as those of humans or whether the animal-colonic bacteria metabolize them to different compounds.

PI: Schmued, Laurence C., Ph.D.

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

Responsible Division: Neurotoxicology

Objectives:

- 1) To 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, an inhibitor of metabolic respiration and metamphetamine, a dopamine agonist) on each of the above mentioned vascular elements such as perivascular pericytes, vascular lumen and perivascular sheath
- 2) To characterize the response of certain vascular elements to neurotoxic insults
- 3) To provide fluorescent and bright-field labeling at the vascular lumen.

PI: Schmued, Laurence C., Ph.D.

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

Responsible Division: Neurotoxicology

Objective:

To test the hypothesis that AD 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.

PI: Sonko, Bakary, Ph.D.

Evaluation of Glycolysis and TCA Fluxes in MPTP-Treated C57BL Mouse Model of Parkinson's Disease (PD) (E0732601)

Responsible Division: Systems Toxicology

Collaborating Division: Neurotoxicology

Objectives:

- 1) To determine fluxes of ¹³C-glucose in the glycolysis pathway and through the TCA cycle in MPTP C57BL mouse model of PD
- 2) Use the data to estimate the contributions of glycolysis and TCA cycle pathways to energy metabolism in the model
- 3) To identify potential energy metabolic biomarkers of PD in this setting.

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

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

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

Responsible Division: Biochemical Toxicology

Collaborating Division: Associate Director for Regulatory Activities

Collaborating FDA Center: CDER

Objectives:

- 1) To characterize UGT mRNA expression in normal and malignant human-breast tissues isolated from the same donor and from different donors
- 2) To identify polymorphisms in those *UGT* genes that show significant inter-individual differences in UGT mRNA expression in all breast tissues

- 3) To determine the methylation profile of those UGTs identified in objective 2 and correlate it to UGT expression
- 4) To determine the effects of polymorphisms in UGT genes on glucuronidation of E2, 4-OH-E1, and 4-hydroxy-Tamoxifen 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 Toxicology

Objectives:

- 1) To apply metabolomic methods to investigate a drug-metabolite profile in urine samples from preclinical studies
- 2) To determine the excretion kinetics of the drug-*N*-acetyl-cysteine conjugates and S-adenosylmethionine
- 3) To investigate mercapturic acids profile using a highly sensitive and selective constant neural-loss technique developed on a triple quadrupole mass spectrometer.

PI: Tong, Weida, Ph.D.

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

Responsible Division: Systems Toxicology

Collaborating Divisions: Genetic and Reproductive Toxicology, Z-Tech Corporation

Objectives:

- 1) Liver Ontology (LO)—To develop a LO that characterizes liver pathology and toxicity
- 2) Gene-Expression Data—To collect the existing gene-expression data

- 3) Text Mining—To conduct text mining on more than 13 millions abstracts in PubMed and other public resources with an emphasis on liver-related data and to establish the association between the liver specific entities
- 4) Known Data—To assemble the substantial knowledge available in public domains on liver toxicity, including genes/proteins pathways/networks, and chemicals/drugs in such a way that it can be integrated with other information in LTKB and effectively mined
- 5) Experiment—To conduct gene-expression studies on well-understood and characterized hepatic and nonhepatic compounds
- 6) LTKB—To establish liver toxicity-related regulatory networks and genes/proteins/pathways/chemicals/disease associations.

PI: Tong, Weida, Ph.D.

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

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

Responsible Division: Systems Toxicology

Collaborating FDA Center: CDER

Objectives:

- 1) 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
- 2) To further augment the collection of the genomic data from public resources and through collaborations
- 3) To develop a standard data curation model for the sex-biased DILI in

ArrayTrack™ to manage the collected data

- 4) To 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.

PI: Wagner, Robert D., Ph.D.

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

Responsible Division: Microbiology

Objectives:

- 1) To ascertain how VEC respond at the molecular level to contact with *C. albicans*
- 2) To establish whether probiotic *lactobacilli* have an effect on VEC resistance to *C. albicans*, and how that effect is mediated
- 3) To establish if estrogen has an influence on these processes.

PI: Wang, Cheng, Ph.D.

Assessment of Ketamine in the Developing Nonhuman Primate (E0718901)

Responsible Division: Neurotoxicology

Collaborating Divisions: Biochemical

Toxicology, Bionetics Site

Management, Office of the Director

Collaborating FDA Center: CDER

Objectives:

- 1) To determine, using neurohistochemical approaches, if, and at what developmental stages, ketamine exposure increases neuronal apoptosis/proliferation

- 2) To determine, using neurohistochemical approaches, the dose-response for ketamine to produce apoptosis at the most sensitive developmental stage
- 3) To determine the reversibility or permanence of the response using behavioral, imaging, and neurohistochemical approaches
- 4) To determine, at the most sensitive stage and dose, genomic and proteomic responses to ketamine treatment.
- 3) To identify, adapt, develop, and standardize appropriate 2-D protein separation techniques
- 4) To integrate results of above objectives to provide front-end components of a functional proteomics facility.

PI: Word, Beverly R.

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

Responsible Division: Associate Director for Regulatory Activities

Objectives:

- 1) To determine the effect of cigarette smoke condensate on DNA methylation of several genes in lung cells
- 2) To 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.

PI: Yu, Li-Rong, Ph.D.

Methods for Support of a Functional Proteomics Facility at NCTR (E0713501)

Responsible Division: Systems Toxicology

Objectives:

- 1) To establish and standardize for routine-use procedures for whole cell and subcellular-organellar isolation for a variety of tissues
- 2) To develop and standardize specific and sensitive markers of cell type and organellar purity and yield

NCTR Strategic Goal 2

Develop science-based best-practice standards, guidance, and tools to incorporate toxicological advancements that improve the regulatory process

PI: Ahn, Young, Ph.D.

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

Responsible Division: Microbiology

Collaborating FDA Center: CVM

Objectives:

- 1) To develop the methodology to determine if antimicrobial-agent residues bound to fecal contents are microbiologically active
- 2) To 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) To determine the potential of the intestinal microbiota to metabolize antimicrobial residues.

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 Reproductive Toxicology

Collaborating FDA Center: CFSAN

Objective:

To 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 Manganese (Mn) Nanoparticles in PC-12 Cells and in Mice (E0725701)

Responsible Division: Neurotoxicology

Objectives:

- 1) To evaluate the neurotoxicity of different size manganese nanoparticles using PC-12 cultured cells
- 2) To determine if *in vitro* exposure to manganese nanoparticles selectively induces specific genomic changes in PC-12 cultured cells using oligonucleotide microarrays
- 3) To determine if multiple doses of Mn-nanoparticles produce reactive-oxygen species, alterations in lipid peroxidation and/or changes in antioxidant enzymes and levels of glutathione in various regions of the mouse brain
- 4) To determine if single or multiple doses of manganese nanoparticles induce specific genomic changes in various regions of the mouse brain using oligonucleotide microarrays
- 5) To determine if single or multiple doses of Mn-nanoparticles produce significant changes in neurotransmitter concentrations in various regions of the mouse brain
- 6) To determine if single or multiple doses of Mn-nanoparticles produce significant changes in the formation of 3-nitrotyrosine in various regions of the mouse brain

- 7) To 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)

Responsible Division: Neurotoxicology

Objectives:

- 1) To evaluate the neurotoxicity of different sizes of Ag-nanoparticles using cultured PC-12 cells
- 2) To determine if *in vitro* exposure to Ag-nanoparticles selectively induces specific genomic changes in cultured PC-12 cells using microarrays
- 3) To determine if single or multiple doses of Ag-nanoparticles produce reactive-oxygen species, alterations in lipid peroxidation and/or changes in antioxidant enzymes and glutathione levels in the rat brain
- 4) To determine if single or multiple doses of Ag-nanoparticles induce specific genomic changes in the rat brain as indicated with microarrays
- 5) To determine if single or multiple doses of Ag-nanoparticles produce significant changes in neurotransmitter concentrations in the brain in rat
- 6) To determine if single or multiple doses of Ag-nanoparticles produce significant changes in the formation of 3-nitrotyrosine (3-NT) in the rat brain
- 7) To determine if multiple doses of Ag-nanoparticles produce morphological alterations in, brain, or other visceral organs of the rat.

PI: Ali, Syed, Ph.D.

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

Responsible Division: Neurotoxicology

Collaborating Division: Office of the Director

Objectives:

- 1) To develop a primate model of PD using the chemical neurotoxin, MPTP
- 2) To implant microelectrodes within the subthalamus through stereotaxic guidance for DBS to:
 - 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 and this data will be compared with data from controls
 - study patterns of tremor and dyskinesia after DBS treatment in a PD/MPTP animal model and data will be compared within each animal as its own control
- 3) To evaluate brain neurochemistry, which includes the neurotransmitters dopamine, serotonin, and their metabolites, oxidative stress markers such as reactive-oxygen species, formation of 3-nitrotyrosine, antioxidant enzyme activities, gene-expression, transcription factors associated with dopaminergic neurodegeneration, and post-mortem brain pathology using histochemical techniques.

PI: Beland, Frederick A., 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: Genetic and Reproductive Toxicology

Objective:

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

PI: Beland, Frederick A., Ph.D.

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

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Objective:

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

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 Divisions: Office of Research, Regulatory Compliance and Risk Management

Collaborating FDA Center: CFSAN

Objective:

To 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 F., Ph.D.

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

Responsible Division: Neurotoxicology

Objectives:

- 1) To evaluate the effects of both acute and chronic amphetamine (AMPH) exposure on the vasculature of the rat brain
- 2) To examine vasculature within the parenchyma of three brain regions (the striatum, parietal cortex and the combined piriform and amygdaloid nuclear cortices) where AMPH-induced neurodegeneration can occur
- 3) To look at the effects of AMPH on the vasculature associated with pial and arachnoid membranes and vasculature of the choroid plexus.

PI: Buzatu, Dan A., Ph.D.

Analysis of Proton Magnetic Resonance Spectroscopy Data Using a Distributed Artificial Neural Network (E0719501)

Responsible Division: Systems Toxicology

Collaborating Division: Personalized Nutrition and Medicine

Objective:

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

PI: Cerniglia, Carl E., Ph.D.

Proteomic Approaches to Elucidate Biodegradative Pathways (E0711801)

Responsible Division: Microbiology

Collaborating Division: Z-Tech Corporation

Objectives:

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

PI: Chen, Huizhong, Ph.D.

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

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

Responsible Division: Microbiology

Collaborating Division: Personalized Nutrition and Medicine

Objectives:

- 1) To evaluate the metabolism and effect of color additives used in cosmetics on the skin microbiota and potential to adversely affect women's health
- 2) To assess the degradability of the synthetic azo colorants in cosmetics by skin bacteria
- 3) To identify and quantify the potential carcinogenic and toxic aromatic amines in the metabolites
- 4) To elucidate the role of the microflora with potential genotoxic effects of cosmetic azo dyes on women's health
- 5) To determine physicochemical properties of the azo dye degrading enzymes from the skin bacteria
- 6) To establish a standardized assay to determine the reductive capacity of the skin microflora on the azo colorants.

PI: Chen, James J., Ph.D.

Optimal Tree-Based Ensemble Methods for Class Prediction (E0722101)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Divisions: Systems Toxicology, Z-Tech Corporation

Objectives:

- 1) To build on the novel NCTR-developed Decision Forest classification model to produce an ensemble of decision trees, each constructed from a different set of predictors, by statistically pruning to optimal size using cross-validation
- 2) To use Monte Carlo simulation techniques to compare the performance of the proposed Decision Forest classifiers to the performance of a single optimal decision tree.

PI: Chen, James J., Ph.D.

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

Responsible Division: Personalized Nutrition and Medicine

Collaborating FDA Centers: CDER, CDRH, CFSAN

Objective:

To conduct cancer and noncancer risk assessments of FDA-regulated products to assist in establishing safest conditions of exposure to toxic substance.

PI: Delclos, Kenneth, Ph.D.

Di(2-ethylhexyl)phthalate (DEHP) Toxicokinetics in Neonatal Male Rhesus Monkeys Following Intravenous and Oral Dosing (E0216001)

External Funding: National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center: CDRH

Objectives:

- 1) To quantify the metabolism and disposition of multiple, single-intravenous doses of DEHP administered to male rhesus monkeys during the first 12 postnatal weeks
- 2) To quantify the metabolism and disposition of multiple, single-oral doses of DEHP administered to male rhesus monkeys during the first 12 postnatal weeks
- 3) To use the results of this work to evaluate the feasibility and utility of a subchronic toxicity study of DEHP
- 4) To utilize blood and testicular tissue from the infant monkeys to establish methods to be utilized in the subchronic study and/or estimate variability in the endpoints to aid in determining the number of animals that will be required in each dose group for a subchronic study.

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

Objectives:

- 1) To demonstrate that the cystic kidney disease is decreased in incidence and/or severity in rats fed soy-containing diets
- 2) To evaluate the renal toxicity of dietary DEHP in developing rats maintained on a soy-free diet
- 3) To evaluate potential early markers of renal cystogenesis in *p*-nonylphenol-

and DEHP-treated rats and their modulation by soy-containing diets

- 4) To evaluate the roles of modulation of antioxidant defenses and cyclooxygenase activities in the protective effect of soy against *p*-nonylphenol and, if demonstrated, DEHP-induced renal toxicity
- 5) To assess the effect of diet on hepatic, testicular, and lung toxicity of DEHP.

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 Centers: CBER, CDRH

Objectives:

- 1) To 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 affect the metabolic profile of DEHP
- 2) To examine DEHP metabolism in neonatal rodents dosed intravenously and orally and relate this metabolism to biological effects.

PI: Dobrovolsky, Vasily N., 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
Reproductive Toxicology

Collaborating Division: Personalized
Nutrition and Medicine

Objectives:

- 1) To 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
- 2) To develop flow-cytometric methods for the detection of hematopoietic cells with mutations in the *PIG-A* gene in C57Bl/6 mice.

PI: Doerge, Daniel R., Ph.D.

Ketamine Pharmacokinetics in Children
(E0726201)

External Funding: University of
Arkansas for Medical Sciences (CRADA)

Responsible Division: Biochemical
Toxicology

Collaborating Division: Neurotoxicology

Objective:

To develop and validate a sensitive LC/MS/MS method to quantify the enantiomers of ketamine and nor-ketamine in plasma from children dosed with racemic ketamine during surgical procedures.

PI: Doerge, Daniel R., 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: CDRH

Objectives:

- 1) To determine Bisphenol A (BPA) pharmacokinetics at low dose
- 2) To measure free and conjugated forms of BPA separately
- 3) To use deuterium-labeled BPA to avoid issues of background contamination
- 4) To use LC/MS/MS for sensitivity and selectivity of measurement
- 5) To determine complete rat data set for blood, tissue, and excreta across stages of development (pregnant females, fetuses, neonates)
- 6) To determine BPA pharmacokinetics from oral and intravenous administration in pregnant, lactating, and nonpregnant female rats and neonatal rats
- 7) To determine plasma and urinary pharmacokinetic data in neonatal and adult monkeys
- 8) To use the new pharmacokinetic data in conjunction with literature data from experimental animals and humans to build a PBPK model for BPA.

PI: Ferguson, Sherry A., Ph.D.

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

Responsible Division: Neurotoxicology

Objectives:

To 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 P., Ph.D.

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

Responsible Division: Biochemical Toxicology

Collaborating FDA Center: CFSAN

Objectives:

1) Microsomal Metabolism Mediated Studies

- To 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
- To 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

- To 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)
- To use 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: Fu, Peter P., 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: CFSAN

Objectives:

1) Chemical Reactions

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

- To determine whether or not nanomaterials enhance or inhibit free-radical formation, mediated by microsomal metabolism, in a nanoparticle size-dependent manner
- To 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

- To 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.

- 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: Gamboa Da Costa, Goncalo J., Ph.D.

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

Responsible Division: Biochemical Toxicology

Objective:

To investigate the nephrotoxic effect of a seven-day co-exposure to melamine and cyanuric acid in Fischer 344 rats.

PI: Gopee, Neera V., D.V.M., Ph.D., DABT

Evaluating the Effects of Over-the-Counter Skin Products, such as Sunscreen, on the Absorption of Dermally Applied Estradiol, in an *In Vitro* and *In Vivo* Model (E0730401)

Responsible Division: Veterinary Services

Collaborating Division: Office of Research, Systems Toxicology

Collaborating FDA Center: CDER

Objectives:

1. To investigate the pig as an animal model that will allow the measurement of systemic estradiol, when applied dermally
2. To use the animal model to mimic the clinically reported effects of sunscreen application on estradiol absorption from topically applied estradiol products

3. To evaluate factors, such as components in sunscreens or time of application, on the rate and extent of estradiol absorption from dermally applied products

4. To develop an *in vitro* system study and determine the individual components or combination of components in sunscreens responsible for the enhancement in the absorption of estradiol from topically applied estradiol products

5. To use this *in vitro* model to evaluate factors that may impact absorption of estradiol from dermally applied products.

PI: Hansen, Deborah K., Ph.D.

Developmental Toxicity of Bitter Orange in Rats (E0214701)

External Funding: National Toxicology Program (IAG)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division: Genetic and Reproductive Toxicology

Collaborating FDA Center(s): CFSAN

Objective:

To determine potential developmental toxicity of synthetic synephrine and *Citrus aurantium* extract in rats.

PI: Hansen, Deborah K., Ph.D.

Physiological Effects of Bitter Orange in Rats (E0214901)

External Funding: National Toxicology Program (IAG)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Divisions: Biochemical
Toxicology, Genetic and Reproductive
Toxicology, TPA Pathology Contract

Collaborating FDA Center: CFSAN

Objective:

To determine potential physiological effects of synthetic synephrine as well as an extract from the botanical *Citrus aurantium* alone and in combination with caffeine in rats.

PI: Howard, Paul C., Ph.D.

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

Responsible Division: Office of Research

Collaborating Division: Biochemical
Toxicology

Objectives:

- 1) To determine the chemicals in tattoo pigments and their metabolism *in vitro*
- 2) To develop methodology for tattooing SKH-1 hairless mice in a quantitative and reproducible manner
- 3) To determine the extent of inflammation induced by the implanted pigment and determine the time of recovery following tattooing
- 4) To 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) To determine if tattoo pigments are photocarcinogenic in the SKH-1 hairless mouse using simulated-solar light.

PI: Howard, Paul C., Ph.D.

NCTR/ORR Nanotechnology Core Facility (S00714) and (S00715)

Responsible Division: Office of Research

Objectives:

- 1) To support the needs of NCTR to characterize nanoscale materials used in toxicology tests and to detect these materials in biological samples
- 2) To support the needs of ARL-ORA to detect and characterize nanoscale materials in FDA-regulated products
- 3) To support the needs of ARL-ORA to detect and characterize nanoscale materials in FDA-regulated products.

PI: Howard, Paul C., Ph.D.

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

External Funding: National Toxicology Program (IAG)

Responsible Division: Office of Research

Collaborating Division(s): Veterinary
Services

Objectives:

To determine the immunogenicity of permanent makeup inks using a modified lymph-node-proliferation assay protocol.

PI: Leahey, Julian E., Ph.D.

Maintenance of the Transgenic p16/p19(-/-) Haplodeficient [NCTR Strain Code, 7V] Breeding Colony for (E0216301)

External Funding: National Toxicology Program (IAG)

Responsible Division: Office of Research

Objective:

To provide support to maintain the p16/p19(-/-) breeding colony [NCTR code, 7V] at NCTR for use in future NTP protocol development.

PI: Leakey, Julian E., Ph.D.

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

External Funding: National Toxicology Program (IAG)

Responsible Division: Office of Research

Objective:

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

PI: Leakey, Julian E., 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: Office of Research

Objectives:

- 1) To investigate the potential toxicity of chondroitin sulfate and glucosamine, administered by oral gavage in male rats
- 2) To determine whether subchronic exposure of glucosamine or chondroitin sulfate potentiate the pathological effects of noninsulin-dependent diabetes in obese diabetic rats.

PI: Leakey, Julian E., Ph.D.

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

External Funding: National Toxicology Program (IAG)

Responsible Division: Office of Research

Objective:

To evaluate the subchronic hepatotoxicity of *Usnea* Lichen in male and female Fischer 344 rats and B6C3F mice.

PI: Leakey, Julian E., Ph.D.

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

External Funding: National Toxicology Program (IAG)

Responsible Division: Office of Research

Collaborating Division: Biochemical Toxicology

Objective:

To evaluate the potential toxicity and carcinogenicity of perinatal and chronic exposures to AIDS drugs, Zidovudine and Lamivudine in C57BL/6(N5)trp53 (+/-) haplodeficient F1 transgenic mice.

PI: Manjanatha, Mugimane G., Ph.D.

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

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Divisions: Biochemical Toxicology, Office of the Director, Personalized Nutrition and Medicine

Objectives:

- 1) To determine the metabolites of methylphenidate at early times after exposure in B6C3F1 mice to compare the major metabolites in the human, monkey, and mouse
- 2) To determine the plasma levels of methylphenidate and its major metabolites in the B6C3F1 mouse after 28 days of exposure
- 3) To determine the effect of exposure to methylphenidate on body and organ weights of the B6C3F1 mouse after 28 days of exposure
- 4) To determine if long-term exposure to methylphenidate results in a dose-responsive increase in the liver c11

gene mutant frequency of Big Blue[®] mouse

- 5) To determine the pharmacokinetics of methylphenidate and its major metabolite, ritalinic acid, in B6C3Fa mice.

PI: Moore, Martha M., Ph.D.

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

External Funding: Centers for Disease Control (IAG)

Responsible Division: Genetic and Reproductive Toxicology

Objective:

To 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: Moore, Martha M., Ph.D.

Genetic-Toxicology Evaluations in Support of FDA Centers for Evaluating Substances for their Genotoxic Potential (S00677)

Responsible Division: Genetic and Reproductive Toxicology

Objectives:

To provide direct research to FDA Centers.

PI: Morris, Suzanne M., Ph.D.

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

External Funding: National Institutes of Health/National Institute for Child Health and Human Development (IAG)

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Divisions: Biochemical Toxicology, Bionetics Site Management, Neurotoxicology, Office of the Director, Personalized Nutrition and Medicine

Objectives:

- 1) To determine the baseline frequency of measures of genetic damage in a population of juvenile rhesus monkeys
- 2) To 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) To determine if chronic exposure to methylphenidate results in measurable effects on the behavior of juvenile rhesus monkeys utilizing the NCTR Operant Test Battery
- 4) To determine the plasma concentration of methylphenidate and its major metabolite, ritalinic acid, during the chronic exposure of juvenile rhesus monkeys to the drug.

PI: Morris, Suzanne M., Ph.D.

Evaluation of Growth and Pubertal Development in Male Rhesus Monkeys (*Macaca mulatta*) Chronically Exposed to Methylphenidate Hydrochloride (MPH) (E0728701)

External Funding: National Institute for Child Health and Human Development (IAG)

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Divisions: Biochemical Toxicology, Neurotoxicology, Office of the Director, Personalized Nutrition and Medicine, Veterinary Services

Objective:

To evaluate pharmacokinetics and operant behavior testing changes in post-pubertal rhesus monkeys.

PI: Parsons, Barbara L., Ph.D.

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

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Division: Office of Research

Objectives:

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

PI: Patterson, Tucker A., Ph.D.

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)

External Funding: Boehringer Ingelheim Pharmaceuticals Inc. (CRADA)

Responsible Division: Neurotoxicology

Objectives:

- 1)To establish acquisition curves for several operant behaviors in juvenile

- rhesus monkeys during chronic oral exposure to pramipexole and vehicle
- 2)To determine whether such exposure results in any significant changes in the acquisition and performance of these operant and other observable behaviors
- 3)To determine whether such exposure results in any significant changes in clinical chemistry or ophthalmic parameters
- 4)To determine plasma distribution profiles and concentrations of pramipexole at various stages of chronic exposure
- 5)To conduct standard postmortem toxicological investigations
- 6)To conduct a focused neuropathological evaluation.

PI: Schnackenberg, Bradley, Ph.D.

Neuroprotective Effects of Pramipexole Against Methamphetamine- and MPTP-Induced Cytotoxicity in *In Vitro* and *In Vivo* Models (E0732001)

Responsible Division: Neurotoxicology

Objectives:

- 1)To determine if pramipexole is neuroprotective against MPTP/MPP+ and/or methamphetamine cytotoxicity in primary rat cortical cultures
- 2)To determine if pramipexole is neuroprotective *in vivo* using a mouse model of MPTP/MPP+ and methamphetamine-induced neurotoxicity.

PI: Shi, Leming, Ph.D.

MicroArray Quality Control (MAQC) Project Database (S00691)

Responsible Division: Systems Toxicology

Objectives:

- 1)To create a database that will be a collection of microarray data sets

and analysis results provided by MAQC participants

- 2) To serve as a critical mechanism for MAQC participants to share and exchange data in developing predictive models.

PI: Shi, Leming, Ph.D.

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

Responsible Division: Systems Toxicology

Collaborating Division: Personalized Nutrition and Medicine

Objectives:

- 1) To assess the reliability of microarray-based predictive models (classifiers) for clinical (diagnosis, prognosis, and treatment outcome) and preclinical (toxicogenomics) applications
- 2) To make consensus recommendations to the microarray community the critical component of personalized medicine
- 3) To facilitate the appropriate application of microarray data in the discovery, development, and review of FDA-regulated products.

PI: Sutherland, John B., Ph.D.

Microbial Degradation of Fluoroquinolone Antimicrobial Agents (E0722701)

Responsible Division: Microbiology

Collaborating Divisions: Biochemical Toxicology, Bionetics Site Management, Personalized Nutrition and Medicine

Objective:

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

PI: Tolleson, William H., Ph.D.

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

Responsible Division: Biochemical Toxicology

Collaborating Division: Office of Research

Objectives:

- 1) To characterize photochemical DNA damage in the skin of TP-ras/ink-4a mice exposed to UVA+UVB radiation
- 2) To determine whether cutaneous malignant melanoma can be induced in neonatal TP-ras (+) ink4a (+/-) transgenic mice using UVA+UVB radiation
- 3) To identify photochemically induced mutations within the ink4a/p16/CDKN2A and p53 loci in tumor tissues
- 4) To 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: Tong, Weida, Ph.D.

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

Responsible Division: Systems Toxicology

Collaborating Division: Office of the Director

Objective:

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.

PI: Wang, Cheng, Ph.D.

Assessment of Gaseous Anesthetics in the Developing Nonhuman Primate (E0728501)

Responsible Division: Neurotoxicology

Collaborating Division: Office of the Director

Objectives:

- 1) To evaluate dose-response effects of gaseous anesthetics to determine if:
 - prolonged exposure to nitrous oxide or isoflurane alone will result in an increase in neuronal cell death
 - combinations of nitrous oxide and isoflurane will prevent or enhance each other's effects on the developing nonhuman primate
- 2) To 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) To determine, using noninvasive imaging techniques [High resolution dedicated positron emission tomography and MRI], 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
- 4) To identify potential underlying mechanisms that could link alteration of mitochondrial function and elevation of reactive-oxygen species to gaseous anesthetic-induced neuronal cell death.

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: Bionetics Site Management, Neurotoxicology, Office of the Director

Objectives:

- 1) To 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
- 2) To elucidate the relationship between apoptosis identifying ligands (specific tracers) and subsequent behavioral deficits.

NCTR Strategic Goal 3

Conduct research and develop strategic technologies to protect the food supply

PI: Beland, Frederick A., Ph.D.

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

Responsible Division: Biochemical Toxicology

Objective:

To determine the effect of urinary pH upon the renal toxicities elicited by a combined exposure of melamine and cyanuric acid.

PI: Buzatu, Dan A., 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 Toxicology

Collaborating Division: Microbiology

Collaborating FDA Center: ORA

Objective:

To conduct a FERN (Food Emergency Response Network) level-one validation for LITMUS Rapid Identification of Bacterial Pathogens (RAPID-B) screening of viable pathogens in food.

PI: Buzatu, Dan A., Ph.D.

The Development of Novel Nanotube-Based Technologies That Benefit Public Health, Protect the Public, Produce High-Efficiency Separations and Filtration, and Improve Energetic-Material Therapeutics (E0720501)

Responsible Division: Systems Toxicology

Collaborating Division(s): Biochemical Toxicology

Objectives:

- 1) To develop novel technologies for the filtration of chemical and biological hazards from air, water, blood, and other media
- 2) To develop technologies that protect public health or otherwise benefit the public
- 3) To develop novel nanotube/monoclonal antibody-based cancer therapies.

PI: Chen, James J., Ph.D.

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

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division: Microbiology, Systems Toxicology

Objective:

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

PI: Doerge, Daniel R., 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: Genetic and Reproductive Toxicology

Objectives:

- 1) To develop and validate LC-ES/MS/MS assays to quantify the major furan-derived DNA adducts in liver, the major furan-derived hemoglobin adduct(s), and the major furan-derived urinary glutathione-derived metabolite
- 2) To 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) To determine concentration of furan in irradiated NIH-31 diet using headspace-GC/MS
- 4) To determine toxicokinetics of furan in male and female Fischer 344 rats following exposure by single gavage administration using headspace-GC/MS
- 5) To combine all data to construct a PBPK model for future use in determining carcinogenic risks from human exposure to furan through the diet
- 6) To determine mutagenicity of furan in liver *in vivo* using male Big Blue[®] rats
- 7) To determine the dose-response relationships for furan-mediated hepatotoxicity and cell proliferation in liver of male and female Fischer 344 rats
- 8) To determine effects of furan on methylation status in rat liver and kidney DNA and histones as epigenetic changes related to carcinogenic process.

PI: Gamboa Da Costa, Goncalo J., 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 Divisions: Microbiology, Systems Toxicology

Collaborating FDA Centers: CFSAN, CDRH, CVM

Objectives:

- 1) To conduct a pharmacokinetic 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 pre-formed salt
- 2) To determine the NOAEL of a combined exposure to melamine and cyanuric acid in F344/N rats for 28 and 90 days
- 3) To investigate the occurrence of early metabonomic and proteomic biomarkers of melamine + cyanuric acid-induced nephrotoxicity obtainable by noninvasive methods;
- 4) To 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: Khan, Ashraf A., Ph.D.

Molecular Characterization of *Salmonella* spp. and *Vibrio* spp. Isolated from Seafood and Development of Microarray Detection Method (E0720801)

Responsible Division: Microbiology

Collaborating FDA Center: ORA

Objective:

To characterize representative isolates of *Salmonella* and *Vibrio* spp. by molecular techniques, such as pulsed-field gel electrophoresis, multilocus sequencing, ERIC (enterobacterial repetitive intergenic consensus), and REP-PCR (repetitive extragenic palindromic-PCR) methods.

PI: Khan, Saeed A., Ph.D.

The Survival of *Bacillus Anthracis* in Processed Liquid Eggs (E0725101)

Responsible Division: Microbiology

Objectives:

- 1) To determine the lag phase duration, growth rate, and maximum population density of *B. anthracis* Sterne strain at different temperatures used for storing and cooking liquid eggs
- 2) To identify the inactivation kinetics of spores of Sterne strain at different temperatures.

PI: Melchior, William B., Ph.D.

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

Responsible Division: Biochemical Toxicology

Objectives:

- 1) To develop the precise materials and methods needed to perform the proposed assays
- 2) To prove that the assays work simply, rapidly, and reliably
- 3) To prove that the assays function as desired in real-world situations, such as with contaminated food stuffs.

PI: Melvin, Cathy, Ph.D.

Clostridium-Botulinum Toxin Bioassay–
Determination of Human Health Hazard
in Regulatory-Food Samples (E0725901)

Responsible Division: Veterinary Services

Collaborating Division: Regulatory

Compliance and Risk Management

Collaborating FDA Center: ORA

Objective:

To conduct a mouse bioassay to detect *clostridium*-botulinum toxins in food or other sources that may affect human health.

PI: Melvin, Cathy, Ph.D.

Paralytic Shellfish Toxin Bioassay–
Determination of Human Health Hazard
(E0725801)

Responsible Division: Veterinary Services

Collaborating Division: Regulatory

Compliance and Risk Management

Collaborating FDA Center: ORA

Objective:

To conduct a mouse bioassay to detect paralytic-shellfish toxins in food or other sources that may affect human health.

PI: Nawaz, Mohamed S., Ph.D.

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

Responsible Division: Microbiology

Collaborating FDA Centers: CVM, ORA

Objectives:

- 1) To isolate and identify fluoroquinolone-resistant Gram negative bacteria from shrimp imported from different countries
- 2) To determine the molecular characterization of fluoroquinolone-resistant determinants
- 3) To molecular type fluoroquinolone-resistant bacteria.

PI: Nayak, Rajesh R., Ph.D.

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

Responsible Division: Microbiology

Objectives:

- 1) To 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 guidelines
- 2) To determine the variation in genetic clonality among the drug-resistance genotypes by fingerprints the bacteria using the CDC's PulseNet pulsed-field gel electrophoresis protocol
- 3) To identify the genes in the multiple antibiotic region of the *Salmonella* Genomic Island class 1 integron gene cassettes in the resistant phenotypes
- 4) To detect antimicrobial-resistance genes in select multi drug-resistant Javiana isolates by a PCR-based and microarray biochip methodologies.

PI: Paule, Merle G., Ph.D.

Developmental Neurotoxicity Assessment of Acrylamide in Rats (E0215101)

External Funding: National Toxicology Program (IAG)

Responsible Division: Neurotoxicology

Collaborating Divisions: Biochemical Toxicology, Office of the Director

Objective:

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

PI: Schnackenberg, Laura, Ph.D.

Metabolomic Signatures of Bacterial Contamination in Milk as a Model System (E0732501)

Responsible Division: Systems Toxicology

Collaborating Division: Microbiology

Collaborating FDA Center: ORA

Objectives:

To evaluate metabolomic signatures resulting from microbial (spoilage and pathogenic) activities in milk, which is a well-defined but complex matrix.

PI: Tolleson, William H., Ph.D.

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

Responsible Division: Biochemical Toxicology

Collaborating Division: Systems Toxicology

Objective:

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

PI: Tolleson, William H., Ph.D.

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

Responsible Division: Biochemical Toxicology

Collaborating FDA Center: CFSAN

Objectives:

- 1) To identify cleaning/sanitizing treatments that result in elimination and/or inactivation of protein toxins (abrin and ricin) on food-contact surfaces
- 2) To identify surrogate(s) that can be used to study chemical inactivation of abrin or ricin

- 3) To 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 H., Ph.D.

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

Responsible Division: Biochemical Toxicology

Collaborating FDA Center: CFSAN

Objectives:

- 1) Measure forward-rate constants for thermal denaturation of ricin and abrin at seven temperatures and three buffer combinations by monitoring the quenching of intrinsic protein (tryptophan) fluorescence (EX295, EM340) in a thermostatted spectrofluorimeter
- 2) To select T_m for toxin proteins and measure reverse-rate constants (protein renaturation) at one temperature and one buffer combination
- 3) To calculate K_{eq} and ΔG from ratio of rates
- 4) To determine $T \Delta S$ from ΔG and ΔH
- 5) To determine the influence of solvent pH on isothermal toxin folding/unfolding equilibria
- 6) To 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 D., 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

Objectives:

1. To 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. To use pathway-focused gene-expression profiles generated from real-time 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. To 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. To 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. To compare the probiotic-treated and untreated mice for expression of dendritic cell, macrophage, and intestinal epithelial cell (IEC)-derived cytokines.

NCTR Strategic Goal 4

Conduct bioinformatics research and development in support of FDA's regulatory mission

PI: Patterson, Tucker A., Ph.D.

Gender-Specific Gene-Expression Analysis and Cross-Mapping Differentially Expressed Genes to Specific Chromosomal Locations in the Male and Female Rat (E0730901)

Responsible Division: Neurotoxicology

Objectives:

- 1) To find sex-specific gene-expression differences in four different regions of the rat brain
- 2) To determine if specific biological pathways are associated with differentially expressed genes.

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)

Responsible Division: Systems Toxicology

Objectives:

- 1) To use data received from CDER drug-review offices and ArrayTrack™ to analyze data and send results back to CDER collaborators
- 2) To develop the functionality in ArrayTrack™ to facilitate the review of nonmicroarray PGx data as relevant to the Critical Path Initiative
- 3) To develop new modules in ArrayTrack™ to review proteomics and metabolomics data and data from genome wide association studies
- 4) To develop modules in ArrayTrack™ to allow electronic data submission in the VGDS/VXDS program.

PI: Tong, Weida, Ph.D.

Janus (BIB) (S00699)

Responsible Division: Systems Toxicology

Collaborating Division: Z-Tech Corporation

Objective:

To integrate NCTR's ArrayTrack™ software with Janus to provide omics data capability and enable electronic data submission and review.

FY 2009 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 2009**.

1. Arias, H., Santamaria, A., and Ali, S.F. (2009). Pharmacological and neurotoxicological actions mediated by bupropion and diethylpropion. *International Review of Neurobiology*. 88:223-239.
Responsible Division: Neurotoxicology
2. Baek, S., Kweon, O., Kim, S., Baek, D., Chen, J.J., and Cerniglia, C.E. (2009). ClassRHO: A platform for classification of bacterial rieske non-heme iron ring-hydroxylating oxygenases. *Journal of Microbiological Methods*. 76(3):307-309.
Responsible Division: Personalized Nutrition and Medicine
Co-Author Division: Microbiology
3. Beger, R., Hansen, D.K., Schnackenberg, L., Cross, B.M., Fatollahi, J.J., Lagunero, F.T., Sarnyai, Z., and Boros, L.G. (2009). Single valproic acid treatment inhibits glycogen and RNA ribose turnover while disrupting glucose-derived cholesterol synthesis in liver as revealed by the [U-13C6]-D-glucose tracer in mice. *Metabolomics*. 5:336-345.
Responsible Division: Genetic and Reproductive Toxicology
Co-Author Division: Systems Toxicology
4. Beilke, L.D., Aleksunes, L.M., Holland, R.D., Besselsen, D.G., Beger, R., Klaassen, C.D., and Cherrington, N.J. (2009). Car-mediated changes in bile acid composition contributes to hepatoprotection from LCA-induced liver injury in mice. *Drug Metabolism and Disposition*. 37(5):1035-1045.
Responsible Division: Systems Toxicology
5. Boctor, S.Y. and Ferguson, S.A. (2009). 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*. e-pub.
Co-Author Division: Neurotoxicology
6. Boctor, S.Y. and Ferguson, S.A. (2009). Neonatal NMDA receptor antagonist treatments have no effects on prepulse inhibition of postnatal day 25 Sprague-Dawley rats. *Neurotoxicology*. 30:151-154.
Responsible Division: Neurotoxicology

7. Bowyer, J.F. , Latendresse, J.R., Delongchamp, R.R., Warbritton, A.R., Thomas, M., Divine, B., and Doerge, D.R. (2009). The mRNA expression and histological integrity in rat forebrain motor and sensory regions are minimally affected by acrylamide exposure through drinking water. *Toxicology and Applied Pharmacology*. 240(3):401-411.
Responsible Division: Neurotoxicology
Co-Author Divisions: Biochemical Toxicology
8. Bushel, P.R., Nielsen, D., and Tong, W. (2009). Proceedings of the First International Conference on Toxicogenomics Integrated with Environmental Sciences (TIES-2007). *BMC Proceedings*. 3(Suppl 2):S1.
Responsible Division: Systems Toxicology
9. Cao, Y., Calafat, A.M., Doerge, D.R., Umbach, D., Bernbaum, J.C., Twaddle, N.C., Ye, X., and Rogan, W.J. (2009). Isoflavones in urine, saliva and blood of infants: data from a pilot study on the estrogenic activity of soy formula. *Journal of Exposure Science and Environmental Epidemiology*. 19(2):223-234.
Responsible Division: Biochemical Toxicology
Co-Author Division: Genetic and Reproductive Toxicology
10. Chamberlain, P.L., Cerniglia, C.E., and Mclean, J. Narasin. *70th Joint FAO/WHO Expert Committee on Food Additives*.
Responsible Division: Microbiology
11. Chen, H., Xu, H., Heinze, T.M., and Cerniglia, C.E. (2009). Decolorization of water and oil-soluble azo dyes by *Lactobacillus acidophilus* and *Lactobacillus fermentum*. *Journal of Industrial Microbiology & Biotechnology*. e-pub.
Responsible Division: Microbiology
Co-Author Division: Biochemical Toxicology
12. Chen, T., Heflich, R.H., Moore, M., and Mei, N. (2009). Differential mutagenicity of aflatoxin B(1) in liver of neonatal and adult mice. *Environmental Molecular Mutagenesis*. e-pub.
Responsible Division: Genetic and Reproductive Toxicology
13. Delclos, K.B. , Weis, C.C., Bucci, T.J., Olson, G.R., Mellick, P.W., Sadovova, N.V., Latendresse, J.R., Thorn, B.T., and Newbold, R. (2009). Overlapping but distinct effects of genistein and ethinyl estradiol (EE(2)) in female Sprague-Dawley rats in multigenerational reproductive and chronic toxicity studies. *Reproductive Toxicology*. 27(2):117-132.
Responsible Division: Biochemical Toxicology
Co-Author Divisions: Office of Research and Division of Personalized Nutrition and Medicine

14. Dobrovolsky, V.N., Boctor, S.Y., Twaddle, N.C., Doerge, D.R., Bishop, M.E., Manjanatha, M., Kimoto, T., Miura, D., Heflich, R.H., and Ferguson, S.A. (2009). Flow cytometric detection of PIG-A mutant red blood cells using an erythroid-specific antibody: application of the method for evaluating the *in vivo* genotoxicity of methylphenidate in adolescent rats. *Environmental and Molecular Mutagenesis*. e-pub.
Responsible Division: Genetic and Reproductive Toxicology
Co-Author Divisions: Biochemical Toxicology and Neurotoxicology
15. Dobrovolsky, V.N., Shaddock, J.G., Mittelstaedt, R.A., Manjanatha, M., Miura, D., Uchikawa, M., Mattison, D., and Morris, S.M. (2009). Evaluation of *Macaca mulatta* as a model for genotoxicity studies. *Mutation Research*. 673(1):21-28.
Responsible Division: Genetic and Reproductive Toxicology
16. Espandiari, P., Rosenzweig, B., Zhang, J., Zhou, Y., Schnackenberg, L., Vaidya, V.S., Goering, P.L., Brown, R.P., Bonventre, J.V., Mahjoob, K., Holland, R.D., Beger, R.D., Thompson, K., Hanig, J., and Sadrieh, N. (2009). Age-related differences in susceptibility to cisplatin-induced renal toxicity. *Journal of Applied Toxicology*. e-pub.
Responsible Division: Systems Toxicology
17. Fan, X., Fang, H., Hong, H., Perkins, R., Shi, L., and Tong, W. (2008). Correlation analysis of external RNA controls reveals its utility for assessment of microarray assay. *Analytical Biochemistry*. 385:203-207.
Responsible Division: Systems Toxicology
Co-Author Division: Z-Tech Corporation
18. Fan, X., Shi, L., Fang, H., Harris, S., Perkins, R., and Tong, W. (2009). Investigation of reproducibility of differentially expressed genes in DNA microarrays through statistical simulation. *BMC Proceedings*. 3(Suppl 2):S4.
Responsible Division: Systems Toxicology
Co-Author Division: Z-Tech Corporation
19. Fang, H., Harris, S.C., Su, Z., Chen, M., Qian, F., Shi, L., Arasappan, D., Ge, W., Fan, X., Hong, H., Xu, Z., Turner, S.A., Bishop, M.E., Jackson, M.A., Perkins, R.G., and Tong, W. (2009). FDA bioinformatics tool for public use—ArrayTrack™. *Regulatory Research Perspectives*. 8(1).
Responsible Division: Systems Toxicology
Co-Author Divisions: Genetic and Reproductive Toxicology and Z-Tech Corporation
20. Fang, J. and Beland, F.A. (2009). Long-term exposure to zidovudine delays cell cycle progression, induces apoptosis, and decreases telomerase activity in human hepatocytes. *Toxicological Sciences*. 111(1):120-30.
Responsible Division: Biochemical Toxicology

21. Feng, J., Heinze, T.M., Xu, H., Cerniglia, C.E., and Chen, H. (2009). Evidence for significantly enhancing reduction of azo dyes in *Escherichia coli* by expressed cytoplasmic azoreductase (AzoA) of *Enterococcus faecalis*. *Protein & Peptide Letters*. e-pub.
Responsible Division: Microbiology
Co-Author Division: Biochemical Toxicology
22. Ferguson, S.A. and Boctor, S.Y. (2009). Use of food wafers for multiple daily oral treatments in young rats. *Journal of the American Association of Laboratory Animal Science*. 48:292-295.
Responsible Division: Neurotoxicology
23. Ferguson, S.A., Delclos, K.B. , Newbold, R.R. a Flynn, K.M. (2009). Few effects of multi-generational dietary exposure to genistein or nonylphenol on sodium solution in male and female Sprague-Dawley rats. *Neurotoxicology and Teratology*. 31:143-148.
Responsible Division: Neurotoxicology
24. Ferguson, S.A., Garey, J.D., Smith, M.E., and Paule, M.G. (2008). Pre and postnatal acrylamide treatment has few effects on preweaning behaviors and developmental landmarks in rats. *Neurotoxicology and Teratology*.
Responsible Division: Neurotoxicology
25. Ferguson, S.A., Gopee, N., Paule, M.G., and Howard, P. (2009). Female mini-pig performance of temporal response differentiation, incremental repeated acquisition, and progressive ration operant tasks. *Behavioural Processes*. 80:28-34.
Responsible Division: Neurotoxicology
26. Foley, S.L., Lynne, A.M., and Nayak, R.R. (2009). Molecular typing methodologies for microbial source tracking and epidemiological investigations of Gram-negative bacterial foodborne pathogens. *Infection, Genetics and Evolution*. 9:430-440.
Responsible Division: Microbiology
27. Foley, S.L., Lynne, A.M., and Nayak, R.R. Molecular typing methods of enterobacteriaceae: *Escherichia coli*, *Yersinia*, *Salmonella* and *Shigella*. Book chapter in *Molecular Typing in Bacterial Infections*.
Responsible Division: Microbiology
28. Fu, P.P., Chiang, H.M., Xia, Q., Chen, T., Chen, B.H., Yin, J., Wen, K.C., Lin, G., and Yu, H. (2009). Quality assurance and safety of herbal dietary supplements. *Journal of Environmental Science and Health, Part C*. 27:91-119.
Responsible Division: Biochemical Toxicology
Co-Author Division: Genetic and Reproductive Toxicology

29. Gambus, A., van Deursen, F., Polychronopoulos, D., Foltman, M., Jones, R.C., Edmondson, R.D., Calzada, A., and Labib, K. (2009). A key role for Ctf4 in coupling the MCM2-7 helicase to DNA polymerase alpha within the eukaryotic replisome. *EMBO*. 28(19):2992-3004.
Responsible Division: Systems Toxicology
30. Gao, Y., Holland, R.D., and Yu, L. (2009). Quantitative proteomics for drug toxicity. *Briefings in Functional Genomics and Proteomics*. 8(2):158-166.
Responsible Division: Systems Toxicology
31. Garey, J. and Paule, M.G. (2009). Effects of chronic oral acrylamide exposure on incremental repeated acquisition (learning) task performance in Fischer 344 rats. *Neurotoxicology and Teratology*. e-pub.
Responsible Division: Neurotoxicology
32. George, N. and Chen, J.J. Batch effect estimation of microarray platforms with analysis of variance. *Sources and Solutions*.
Responsible Division: Personalized Nutrition and Medicine
33. Gilbert, K.M., Przybyla-zawislak, B.D., Pumford, N.R., Han, T., Fuscoe, J., Schnackenberg, L., Holland, R.D., Macmillan-Crow, L., Doss, J.C., and Blossom, S.J. (2009). Delineating liver events in trichloroethylene-induced autoimmune hepatitis. *Chemical Research in Toxicology*. 22:626-632.
Responsible Division: Systems Toxicology
Co-Author Divisions: Neurotoxicology and Toxicologic Pathology Associates
34. Gopee, N., Roberts, D.W., Webb, P., Cozart, C., Siitonen, P.H., Latendresse, J.R., Warbritton, A.R., Yu, W.W., Colvin, V.L., Walker, N.J., and Howard, P. (2009). Quantitative determination of skin penetration of PEG-coated CdSe quantum dots in dermabraded but not intact SKH-1 hairless mouse skin. *Toxicological Sciences*. 111(1):37-48.
Responsible Division: Biochemical Toxicology
Co-Author Divisions: Regulatory Compliance and Risk Management, Toxicologic Pathology Associates
35. Guo, L., Li, Q., Xia, Q., Dial, S.L., Chan, P.C., and Fu, P.P. (2009). Analysis of gene expression changes of drug metabolizing enzymes in the livers of F344 rats following oral treatment with kava extract. *Food and Chemical Toxicology*. 47(2):433-442.
Responsible Division: Systems Toxicology
Co-Author Division: Biochemical Toxicology
36. Guo, L., Mei, N., Liao, W., Chan, P.C., and Fu, P.P. Ginkgo biloba extract-induced gene expression changes in xenobiotics metabolism and Myc-centered network. *OMICS: A Journal of Integrative Biology*.
Responsible Division: Systems Toxicology
Co-Author Divisions: Biochemical Toxicology and Genetic and Reproductive Toxicology

37. Guo, L., Shi, Q., Fang, J., Mei, N., Ali, A.A., Lewis, S.M., Leahey, J.E., and Frankos, V.H. (2008). Review of usnic acid and *Usnea barbata* toxicity. *Journal of Environmental Science and Health, Part C*. 26:317-338.
Responsible Division: Systems Toxicology
Co-Author Divisions: Biochemical Toxicology, Genetic and Reproductive Toxicology and Office of Research
38. Harel, L., Costa, B., Tcherpakov, M., Zapatka, M., Oberthuer, A., Hansford, L.M., Vojvodic, M., Levy, Z., Chen, Z., Lee, F.S., Avigad, S., Yaniv, I., Shi, L., Eils, R., Fischer, M., Brors, B., Kaplan, D.R., and Fainzilber, M. (2009). CCM2 mediates death signaling by the TrkA receptor tyrosine kinase. *Neuron*. 63:585-591.
Responsible Division: Systems Toxicology
39. He, Z. (2009). Fluorogold induces persistent neurological deficits and circling behavior in mice over-expressing human mutant tau. *Current Neurovascular Research*. 6(1):54-61.
Responsible Division: Neurotoxicology
40. Hong, H. , Shi, L. , Fuscoe, J. , Goodsaid, F.M. , Mendrick, D., and Tong, W. Potential sources of spurious associations and batch effects in genome-wide association studies. Book Chapter in *Batch Effect and Experimental Shift in Microarray Analysis: Sources and Solutions*.
Responsible Division: Systems Toxicology
Co-Author Division: Personalized Nutrition and Medicine
41. Hong, H., Hong, Q., and Tong, W. (2009). Accurate prediction and recognition of subfamilies of G protein-coupled receptors from amino acid sequences. *The 2009 International Conference on Bioinformatics and Computational Biology*. 3-9.
Responsible Division: Systems Toxicology
42. Hong, H., Hong, Q., Perkins, R.G., Shi, L., Fang, H., Su, Z., Dragan, Y.P., Fuscoe, J., and Tong, W. The accurate prediction of protein family from amino acid sequence by measuring features of sequence fragments. *Journal of Computational Biology*.
Responsible Division: Systems Toxicology
Co-Author Division: Z-Tech Corporation
43. Hong, H., Perry, G., Sorgel, F., Gedela, S., and Akulapalli, S. (2009). Editorial: Journal of Bioequivalence & Bioavailability. *Journal of Bioequivalence & Availability*. 1(1):001-002.
Responsible Division: Systems Toxicology
44. Hong, H., Teitel, C.H., James, L.P., Tong, W., Hinson, J.A., Fuscoe, J., and Dragan, Y.P. (2008). SELDI-based proteomic determination of hepatic biomarkers of mouse serum following acetaminophen administration. *Journal of Proteomics and Bioinformatics*. 1(8):424-436.
Responsible Division: Systems Toxicology

45. Il'Yasova, D., McCarthy, B.J., Erdal, S., Shimek, J., Goldstein, J., Doerge, D.R., Myers, S.R., Vineis, P., Wishnok, J.S., Swenberg, J., Bigner, D.D., and Davis, F.G. (2009). Human exposure to selected animal neurocarcinogens: a biomarker-based assessment and implications for brain tumor epidemiology. *Journal of Toxicology and Environmental Health, Part B*. 12:175-187.
Responsible Division: Biochemical Toxicology
Co-Author Division: Neurotoxicology
46. Jeong, S., Cerniglia, C.E., and Greenlees, K. Avilamycin. *70th Joint FAO/Who Expert Committee on Food Additives*.
Responsible Division: Microbiology
47. Joseph, A., Lee, T., Moland, C.L., Branham, W.S., Fuscoe, J., Leahey, J.E., Allaben, W.T., Lewis, S.M., Ali, A.A., and Desai, V.G. (2009). Effect of (+)- usnic acid on mitochondrial functions as measured by mitochondria-specific oligonucleotide microarray in liver of B6C3F1 mice. *Mitochondrion*. 9:149-158.
Responsible Division: Systems Toxicology
Co-Author Divisions: Office of Research and Division of Personalized Nutrition and Medicine
48. Jung, C.M., Heinze, T.M., Schnackenberg, L., Mullis, L., Elkins, S.A., Elkins, C., Steele, R.S., and Sutherland, J.B. (2009). Interaction of dietary resveratrol with animal-associated bacteria. *FEMS Microbiology Letters*. 297:266-273.
Responsible Division: Microbiology
Co-Author Divisions: Biochemical Toxicology and Systems Toxicology
49. Kaput, J. and Ning, B. (2009). Nutrigenomics for pet nutrition and medicine. *Compendium for Continuing Education for the Practicing Veterinarian*. 31:40-45.
Responsible Division: Personalized Nutrition and Medicine
50. Kaput, J., Chen, J.J. , and Slikker, W. (2009). Personalized nutrition and medicine in perinatal development. Book chapter in *Developmental Toxicology, 3rd Edition*. Edited by D. Hansen, D and B. Abbott. Published by Informa Healthcare USA. 123-144.
Responsible Division: Personalized Nutrition and Medicine
Co-Author Division: Office of the Director
51. Kaput, J., Cotton, R.G., Hardman, L., Watson, M., Al-Aqeel Al, Al-Aama J.Y., Al-Mulla, F., Alonso, S., Aretz, S., Auerbach, A.D., Bapat, B., Bernstein, I.T., Bhak, J., Bleoo, S.L., Blöcker, H., Brenner, S.E., Burn, J., Bustamante, M., Calzone, R., Cambon-Thomsen, A., Cargill, M., Carrera, P., Cavedon, L., Cho, Y.S., Chung, Y.J., Claustres, M., Cutting, G., Dalgleish, R., den Dunnen, J.T., Díaz, C., Dobrowolski, S., dos Santos, M.R., Ekong, R., Flanagan, S.B., Flicek, P., Furukawa, Y., Genuardi, M., Ghang, H., Golubenko, M.V., Greenblatt, M.S., Hamosh, A., Hancock, J.M., Hardison, R., Harrison, T.M., Hoffmann, R., Horaitis, R., Howard, H.J., Barash, C.I., Izagirre, N., Jung, J., Kojima, T., Laradi, S., Lee, Y.S., Lee, J.Y., Gil-da-Silva-Lopes, V.L., Macrae, F.A., Maglott, D., Marafie, M.J., Marsh, S.G., Matsubara, Y., Messiaen, L.M.,

Möslein, G., Netea, M.G., Norton, M.L., Oefner, P.J., Oetting, W.S., O'Leary, J.C., de Ramirez, A.M., Paalman, M.H., Parboosingh, J., Patrinos, G.P., Perozzi, G., Phillips, I.R., Povey, S., Prasad, S., Qi, M., Quin, D.J., Ramesar, R.S., Richards, C.S., Savige, J., Scheible, D.G., Scott, R.J., Seminara, D., Shephard, E.A., Sijmons, R.H., Smith, T.D., Sobrido, M.J., Tanaka, T., Tanaka, S.V., Taylor, G.R., Teague, J., Töpel, T., Ullman-Cullere, M., Utsunomiya, J., van Kranen, H.J., Vihinen, M., Webb, E., Weber, T.K., Yeager, M., Yeom, Y.I., Yim, S.H., and Yoo, H.S. (2009). Planning the human variome project: the Spain report. *Human Mutation*. 30:496-510.

Responsible Division: Personalized Nutrition and Medicine

52. Kashimshetty, R., Desai, V.G., Kale, V.M., Lee, T., Moland, C.L., Branham, W.S., New, L.S., Chan, E.C., Younis, H., and Boelsterli, U.A. (2009). Underlying mitochondrial dysfunction triggers flutamide-induced oxidative liver injury in a mouse model idiosyncratic drug toxicity. *Toxicology and Applied Pharmacology*. 238(2):150-159.

Responsible Division: Systems Toxicology

Co-Author Division: Personalized Nutrition and Medicine

53. Khan, A.A., Ponce, E.R., Nawaz, M.S., Cheng, C., Khan, J.A., and Summage-West, C.V. (2009). Identification and characterization of class 1 integron resistance gene cassettes among *Salmonella* strains isolated from imported seafood. *Applied and Environmental Microbiology*. 75(4):1192-1196.

Responsible Division: Microbiology

54. Khan, S.A., Sung, K., Nawaz, M.S., Cerniglia, C.E., Tamplin, M.L., Phillips, R.W., and Kelley, L.C. (2009). The survivability of *Bacillus anthracis* (Sterne strain) in processed liquid eggs. *Food Microbiology*. 26:123-127.

Responsible Division: Microbiology

55. Kim, S., Kweon, O., and Cerniglia, C.E. (2009). Proteomic applications to elucidate bacterial aromatic hydrocarbon metabolic pathways. *Current Opinion in Microbiology*. 12:301-309.

Responsible Division: Microbiology

56. Kodell, R.L., Pearce, B.A., Baek, S., Moon, H., Ahn, H., Young, J.F., and Chen, J.J. (2009). A model-free ensemble method for class prediction with application to biomedical decision making. *Artificial Intelligence in Medicine*. 46(3):267-276.

Responsible Division: Personalized Nutrition and Medicine

Co-Author Division: Office of Information Technology

57. Latendresse, J.R., Bucci, T.J., Olson, G.R., Mellick, P.W., Weis, C.C., Thorn, B.T., Newbold, R., and Delclos, K.B. (2009). Genistein and ethinyl estradiol dietary exposure in multigenerational and chronic studies induce similar proliferative lesions in mammary gland of male Sprague-Dawley rats. *Reproductive Toxicology*. 28(3):342-353.

Responsible Division: Biochemical Toxicology

Co-Author Divisions: Office of Research, Personalized Nutrition and Medicine,
Toxicologic Pathology Associates

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Glossary of Acronyms and Abbreviations

This glossary is provided to assist you in interpreting acronyms, abbreviations, and phrases you encounter while reading this publication. This is not meant to take the place of standard language or scientific dictionaries, which should be referred to if any short form of 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
aCGH	array-based comparative genomic hybridization
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
AZT	zidovudine or azidothymidine
BAM	bacterial analytical manual
BBB	blood-brain barrier
BPA	Bisphenol A
CA	<i>Citrus aurantium</i>
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
CERP	Classification by Ensembles from Random Partitions
CFSAN	Center for Food Safety and Applied Nutrition, FDA

Acronym/ Abbreviation	Name
CMAR	Certified Managers of Animal Resources
CNS	central nervous system
COTR	contracting officer's technical representative
CoV	coronaviruses
CP	cyclophosphamide
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
DGRT	Division of Genetic and Reproductive Toxicology
DHHS	Department of Health and Human Services
DILI	drug-induced liver injury
DNMT	DNA methyltransferase
DPNM	Division of Personalized Nutrition and Medicine
DVS	Division of Veterinary Services
eDISCO	Electronic Dietary Supplement COmpilation
ENU	<i>N</i> -ethyl- <i>N</i> -nitrosourea
EPA	Environmental Protection Agency
ERIC	enterobacterial repetitive intergenic consensus
ESR	electron spin resonance
FDA	Food and Drug Administration
FERN	Food Emergency Response Network
FQI	food quality indicator
GABA	gamma-aminobutyric acid
GC-MS	gas chromatography-mass spectrometry
GGT	guanine guanine thymidine
GLP	good laboratory practice
GST	glutathione S-transferase
GTT	guanine thymidine thymidine
GWAS	Genome-Wide Association Study
HF/LC	high-fat/low-carbohydrate
HIV	human immunodeficiency virus
HMA	human microbiota-associated

Acronym/ Abbreviation	Name
HPLC	high-performance liquid chromatography
IACUC	Institutional Animal Care and Use Committee
IAG	Interagency Agreement
ICCVAM	Interagency Coordination Committee on the Validation of Alternative Methods
IEC	intestinal epithelial cell
IHC	immunohistochemical
<i>in silico</i>	modeled on a computer
<i>in situ</i>	in place; localized and confined to one area
<i>in vitro</i>	in animal models
<i>in vivo</i>	in cell cultures
IND	Investigational New Drug
ISH	<i>in situ</i> 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
MEHP	mono(2-ethylhexyl)phthalate
miRNA	microRNA
MLA	mouse lymphoma assay
MOU	memorandum of understanding
MPH	methylphenidate hydrochloride
MPP+	1-methyl-4-phenylpyridinium
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
NCI	National Cancer Institute
NCL	Nanomaterial Characterization Laboratory
NCTR	National Center for Toxicological Research, FDA
NDA	New Drug Application
NICHD	National Institute of Child Health and Human Development

Acronym/ Abbreviation	Name
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIST	National Institute for Standards and Technology
NMDA	n-methyl-d-aspartate
NMR	nuclear magnetic resonance
nNOS	neuronal nitric oxide synthase
NOAEL	no observable adverse effect level
NP	<i>p</i> -nonylphenol
NTP	National Toxicology Program
ORA	Office of Regulatory Affairs, FDA
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
PIG-A	phosphatidylinositol glycan anchor biosynthesis, class A
PPAR	peroxisome proliferator-activated receptor
PPX	Pramipexole
PRL	prolactin
PRL-SW	Pacific Regional Laboratory Southwest
RAPID-B	Rapid Identification of Bacterial Pathogens
REP-PCR	repetitive extragenic palindromic-PCR
RLS	restless leg syndrome
ROS	reactive-oxygen species
RT-PCR	reverse transcriptase-polymerase chain reaction
SAB	Science Advisory Board
SHLC	sex hormone-like compound
SLE	systemic lupus erythematosus (lupus)
SNP	single nucleotide polymorphism
SOP	standard operating procedure
SSL	simulated-solar light
STEC	Shiga toxin producing <i>Escherichia coli</i>
TCA	tricarboxylic acid cycle

Acronym/ Abbreviation	Name
TCR	T-cell receptor
TK	thymidine kinase
TSST-1	toxic-shock syndrome toxin-1
UAMS AHEC	University of Arkansas for Medical Sciences Arkansas Area Health Education Centers
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

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