Research Accomplishments and Plans

NCTR

National Center for Toxicological Research

FDA

DHHS

FY 2007 - FY 2008

Preface

The National Center for Toxicological Research (NCTR) is an important research component of the U.S. Food and Drug Administration (FDA) that plays a critical role in the FDA's and the Department of Health and Human Services' (DHHS) mission to promote and protect 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 international and intragovernmental cooperation 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, coexists with the Arkansas Regional Laboratory comprising the Jefferson Laboratories of the FDA.

NCTR conducts FDA mission-critical, peer-reviewed, critical path (translational) research targeted to develop a scientifically sound basis for regulatory decisions and reduce risks associated with FDA-regulated products. This research is aimed at evaluating the biological effects of potentially toxic chemicals or microorganisms, defining the complex mechanisms that govern their toxicity, understanding critical biological events in the expression of toxicity, and developing methods to improve assessment of human exposure, susceptibility, and risk. NCTR's research efforts are primarily directed at supporting the 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, quantitative risk assessment, transgenics (mimicking responses in animal models by insertion or ablation of toxicologically relevant genes into a test animal or tissue culture), neurotoxicology, microbiology, chemistry, and genetic or reproductive/ developmental toxicology. Using its strengths in methods development, statistics, analytical chemistry, and spectroscopy, NCTR has developed and is standardizing new technologies, such as genomics, proteomics, metabonomics, and nanotechnology, to identify and characterize early biomarkers of toxicity using quantitative risk-assessment methods. In addition, 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. 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 in other FDA Centers as well as in other government agencies, academia, and industry. One such example is the use of ArrayTrack™, a software tool developed at NCTR to store, analyze, and interpret DNA microarray data. This tool is being used by several FDA regulatory Centers in assessing phamacogenomic 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 Division of Personalized Nutrition and Medicine has been established and a new NCTR/FDA Bioimaging Center is under development to provide noninvasive, translatable biomarkers for safety assessment. In addition to methods and standards development, NCTR conducts safety assessment of compounds nominated to the FDA for evaluation under an agreement with the National Institute of Environmental Health Sciences/National Toxicology Program. All of the studies conducted at NCTR are intimately associated with Secretary Mike Leavitt's goals of advancing scientific and biomedical research and development related to health and human services and protecting the public from infectious, occupational, environmental, and terrorist threats.

William Slikker, Jr., Ph.D.

Director, NCTR

Vision/Mission

Vision

NCTR is 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 international and intragovernmental cooperation to improve and protect public health and enhance the quality of life for the American people.

Mission

NCTR conducts peer-reviewed scientific research and provides expert technical advice and training that enable FDA to make sound science-based regulatory decisions and improve the health of the American people. The research is focused towards FDA's goals: 1) to understand critical biological events in the expression of toxicity, and 2) to develop and characterize methods, and incorporate new technologies to improve the assessment of human exposure, susceptibility, and risk.

NCTR is dedicated to supporting the FDA mission to protect and promote public health:

- NCTR provides innovative and interdisciplinary research that promotes personal and public health.
- NCTR develops novel translational research approaches to provide the FDA/DHHS with sound scientific infrastructure and multidisciplinary scientific expertise targeted towards addressing critical Agency/Department public-health needs.
- NCTR partners with scientists across FDA, other government agencies, industry, and academia to strengthen the scientific foundations vital to developing sound regulatory policy.
- NCTR establishes national and international collaborations and promotes innovation among government, industry, and academic partners, leveraging resources in order to promote the international standardization and global harmonization of regulatory science.

Research Structure

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

The NCTR scientific core is the study of biochemical and molecular markers of health status using a systems-biology approach and emerging technologies, personalized nutrition and drug therapy to enhance human health, bioinformatics, and predictive technology development, neurotoxicology to include use of imaging techniques, risk-assessment methods, statistical modeling, and development of microbiological and chemical rapid-detection technologies to assure food safety and biosecurity. The NCTR research divisions work together in a seamless effort to support the Food and Drug Administration's (FDA's) mission to rapidly bring safe and efficacious products to the market and to reduce the risk of adverse-health effects from products on the market. The divisions include:

- Division of Biochemical Toxicology
- Division of Genetic and Reproductive Toxicology
- Division of Microbiology
- Division of Neurotoxicology
- Division of Personalized Nutrition and Medicine
- Division of Systems Toxicology
- Division of Veterinary Services

Science Advisory Board

Function

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

FY 2007 Accomplishments

On July 24-25, 2007, the NCTR Division of Microbiology's Research and Surveillance Programs were reviewed by a subcommittee of the NCTR SAB that includes FDA-liaison members. The review subcommittee included scientists from government, industry, and academia and was chaired by Dr. Anthony L. Pometto, Professor, Department of Food Science and Human Nutrition, Iowa State University. Representatives from CFSAN, CVM, CBER, and ORA participated in the review. Dr. Carl E. Cerniglia, Director of the Division of Microbiology, presented an overview of the research program, and the Division research scientists described their current research interests.

On December 5, 2007, the site visit report noted that the Division of Microbiology is to be complimented for its expertise and diverse research collaborations supporting FDA initiatives, such as antimicrobial resistance, food safety and biosecurity, gastrointestinal and host interactions, environmental biotechnology, and overall surveillance in support of the broader NCTR research community. They reported that the research activities within the Division are relevant to the FDA Strategic Plan and maintain strong linkages with the other FDA Centers and ORA Laboratories.

A site visit team from the NCTR Science Advisory Board is scheduled to perform an in-depth analysis of research programs within the Division of Biochemical Toxicology on April 29-30, 2008. The site visit team, will be led by Board Chair, Dr. James Popp, and involve subject experts from academia, industry, NIH, and each FDA product-line Centers and ORA. The Division will present results and future plans from studies on risk evaluation of food, drugs, and cosmetics. A report of the site visit committee will be prepared.

The report of both sites visits will be formally presented at the full NCTR Science Advisory Board meeting to be held at NCTR on August 12-13, 2008.

SAB Membership Roster

1. **Chairman**
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8. Consumer Representative—Vacant

Division of Biochemical Toxicology
Summary of Activities

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Introduction

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

FY 2007 Accomplishments

A major emphasis within the Division is to conduct research on compounds nominated by the FDA for evaluation by the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP). This focus reflects the fact that NCTR has superb animal facilities supported by a multidisciplinary staff of scientists with strong mechanistic research experience—giving the Center the capability to conduct subchronic and chronic toxicological assessments in a rigorous manner to address FDA's needs. These studies currently serve as the benchmark by which toxicological assessments are made by the 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 2007, Division investigators continued chronic bioassays on acrylamide, a carcinogen found in many baked and fried foods, in response to an NTP nomination by the Center for Food Safety and Applied Nutrition (CFSAN). The bioassays assess the carcinogenicity of glycidamide, a genotoxic metabolite of acrylamide. The assessment involves administering acrylamide and glycidamide to newborn mice to determine if infants are particularly susceptible to their carcinogenic properties. Division investigators are also conducting mechanistic studies on acrylamide and glycidamide, including toxicokinetics, DNA and hemoglobin adduct dosimetry, and *in vivo* mutagenesis assays to complement the two-year bioassays. The results have demonstrated a linkage between circulating biomarkers of acrylamide exposure, rodent toxicokinetics, and tissue DNA adducts. This linkage was used to develop a physiologically based pharmacokinetic/pharmacodynamic (PB-PK/PD) model to predict

human DNA-adduct levels associated with the dietary consumption of acrylamide. In response to another NTP nomination, Division investigators continued conducting two-year chronic bioassays on *Aloe vera,* a widely used dietary supplement. To assess the potential toxicities associated with *Aloe vera* ingestion, research scientists examined the expression of cyclooxygenases, alterations in DNA methylation patterns, and the ability of *Aloe vera* polysaccharides to alter the growth of colonic bacteria that ferment nonstarch polysaccharides.

Division investigators completed and successfully defended reports on multigeneration and chronic studies of ethinyl estradiol. The results indicate that this endocrine-disrupting chemical has subtle multigeneration effects. As part of the investigations into endocrine-disrupting agents, an NTP report on the multigeneration *p*-nonylphenol study was drafted. Experiments were also conducted to evaluate the effect of diet upon the renal and testicular toxicity of di-(2-ethylhexyl)phthalate (DEHP) and *p*-nonylphenol, chemicals present in medical devices and plastic packaging materials. The results underline the need for careful consideration of the diets used when conducting toxicological studies for safety assessments.

In response to a nomination by the Center for Biologics Evaluation and Research (CBER), and supported by the Center for Devices and Radiological Health (CDRH), Division investigators initiated studies on the toxicokinetics of intravenous and oral DEHP in neonatal male rhesus monkeys as a prelude to a subchronic toxicity study. 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.

In other studies, high-performance liquid chromatography (HPLC) tandem mass-spectrometry methods were developed to detect and quantify DNA adducts from pyrrolizidine alkaloids, which are hepatotoxic and tumorigenic phytochemicals present in herbal products, including herbal dietary supplements sold in the United States. In collaboration with investigators at the Environmental Protection Agency (EPA), Division staff developed HPLC tandem mass-spectrometry methods for the determination of unstable DNA adducts from the ubiquitous carcinogen benzo[*a*]pyrene.

An area of concern to the FDA, in particular CFSAN, is the potential toxicity of cosmetic ingredients due to their interaction with light. To address this concern, NCTR, in collaboration with NIEHS/NTP, established the NCTR Center for Phototoxicology located within the Division. During FY 2007, evaluations were completed on the toxicity and cocarcinogenicity of topically applied *Aloe vera*, and draft reports were initiated on the photococarcinogenesis studies of retinyl palmitate. Studies were continued to assess the phototoxicity of nanoscale titanium dioxide, a component of certain sunscreens and other cosmetic products. The results indicated that the regional lymph nodes and liver in mice are the primary sentinel organs for the dermal penetration of topically applied nanoscale materials. They further indicated that nanoparticles do not penetrate intact skin, but do penetrate skin compromised by dermabrasion. In other studies, the cocarcinogenicity of tattoo pigments was investigated.

Antiretroviral drugs are being used to prevent the mother-to-child transmission of human immunodeficiency virus type-1 (HIV-1), the virus responsible for acquired immunodeficiency syndrome (AIDS). While effective in preventing viral transmission, the long-term consequences of perinatal exposure to these drugs are presently unknown. During FY 2007, Division investigators completed a bioassay to assess the effects of transplacental exposure to the antiretroviral drugs zidovudine and lamivudine in combination with nevirapine and nelfinavir. In addition, a bioassay was continued to assess the carcinogenic potential of the drugs administered transplacentally and neonatally. In further studies, Division investigators evaluated the effects of antiretroviral drugs on cell-cycle kinetics.

In FY 2007, chemists in the Division participated in an Agency-wide task force responsible for investigating the pet food contamination with melamine and derivatives. Division members synthesized isotopically labeled standards of melamine and cyanuric acid for use in the development and validation of HPLC-MS methodologies. These standards were provided to a number of FDA and other government laboratories.

During FY 2007, Division investigators continued to apply a robust cell-based functional assay to assess the bioterrorism agents ricin and abrin. This method is being applied to FDA-regulated foods to determine the residual biological activity of ricin and abrin in heat-treated infant formula, fruit juices, and yogurt cultures. Investigators are developing a sensitive polymerase chain reaction (PCR)-based assay compatible with food samples to detect the catalytic activity of ricin or similar ribosome-inhibiting proteins, including abrin, modeccin, Shiga toxin, viscumin (mistletoe lectin I), and volkensin. In addition, collaborations were established with investigators at CFSAN to develop, test, and apply biochemical and biological assays for additional bioterrorism agents, including staphylococcus enterotoxins, which could potentially be found in food products.

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 2007, Division investigators determined that epigenetic changes, such as alterations in DNA methylation and histone modification, play an important role in the mechanism of genotoxic and nongenotoxic hepatocarcinogenesis, and that these changes may be used as biomarkers for carcinogenic potential.

FY 2008 Plans

In FY 2008, Division investigators will complete and defend the final NTP reports on the multigenerational reproductive effects of *p*-nonylphenol. Draft pathology reports on the chronic two-year bioassays of *Aloe vera*, acrylamide, and glycidamide will be completed, as will the newborn-mouse assay comparing the carcinogenicity of acrylamide and glycidamide. Chronic studies will continue to determine the effects of transplacental and neonatal exposure to zidovudine and lamivudine in combination with nevirapine and nelfinavir. Studies to assess the pharmacokinetics and toxicities of the food contaminant furan will be initiated.

Investigators associated with the NCTR Center for Phototoxicology will complete and defend the final NTP report on the cocarcinogenicity of *Aloe vera* and continue to study

the interaction of light with tattoo pigments. Specifically, photocarcinogenesis studies will continue on various tattoo inks using full-spectrum simulated-solar light. Investigation will continue to determine if tattoo inks can elicit an immune response—either directly or through metabolism or photoactivation. Experiments will also continue to investigate potential dermal penetration and toxic properties of nanoscale materials. Studies are being planned to continue the characterization of transgenic-mouse models for photocarcinogenesis, with an emphasis on the induction of cutaneous and ocular melanoma. Experiments will be initiated to determine the toxicities associated with exposure to nanosilver and nanogold particles.

In collaboration with investigators at the EPA, Division staff will apply HPLC tandem mass-spectrometry methods to assess the levels of stable and unstable DNA adducts in mice administered benzo[*a*]pyrene. 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. In addition, studies will continue to determine the pharmacokinetics of methylphenidate (Ritalin) in monkeys and mice.

In collaboration with investigators in the NCTR's Division of Systems Toxicology, experiments will continue to apply omics techniques to determine biomarkers of liver toxicity. In additional experiments, Division investigators will initiate studies to investigate the toxicity of melamine in combination with cyanuric acid. Division personnel will also collaborate with investigators at the National Center for Food Safety and Technology (NCFST) to measure thermodynamic constants for thermal inactivation of bioterrorism agents ricin and abrin under conditions found in foods and compare the potencies of detergents and chemical sanitizing agents to inactivate or eliminate these bioterrorism agents contaminating 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 collaborated with investigators in NCTR's Division of Systems Toxicology to use new omics techniques to determine the potential for drugs to cause liver toxicity. Division investigators collaborated with other FDA laboratories in the development of melamine-testing methodologies in seafood, the study of melamine accumulation in fish, and the implementation of a seafood testing program for melamine contamination.

Division investigators have developed new techniques that have improved the scientific capabilities of the Agency. These include HPLC coupled with tandem mass-spectrometric methods to assess pharmacokinetic and toxicokinetic parameters of chemicals and drugs of interest to the 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 a neurotoxicant that was recently identified in baked and fried starchy foods, notably French fries, potato chips, bread, coffee, and many other consumer food products. Evaluations are also being conducted on Aloe vera, a natural product that is 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 emphasizes toxicological assessments of chemicals found in cosmetic products. These chemicals include alpha-hydroxy acids, beta-hydroxy acids, Aloe vera, retinyl palmitate, and nanoparticles. As part of the effort to assess potential toxicities associated with topical agents, Division investigators are evaluating potential risks associated with permanent make-up and tattoos, which are being used by an increasing proportion of the U.S. population.

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 regarding genetic and reproductive/developmental toxicology. 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 the FDA Centers, other government agencies, and other organizations. They also are active participants in the FDA Genetic Toxicology Network, the CDER Genetic Toxicology Network, and other interagency workgroups.

The Division research is divided into four research areas: 1) genetic-toxicology research addresses the development of methods to assess the potential for chemicals to negatively impact human-genetic material or the function of the genetic material; 2) reproductive/ developmental toxicology focuses on methods to understand normal human development and how chemicals might alter normal development; 3) dietary research primarily focuses on the potential hazards of dietary supplements; and 4) omics research, coupled with the more traditional approaches, is being used to improve the ability of FDA to incorporate new and powerful technologies into regulatory decision-making.

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

FY 2007 Accomplishments

In FY 2007, 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 DGRT research accomplishments include:

1. Developed a series of allele competitive blocker-polymerase chain reaction (ACB-PCR) genotypic-selection methods that can directly measure specific mutations in genes involved in tumor induction when they occur in as few as one cell in 100,000 cells. These assays were used to measure mutations in tumors resulting from solar-light exposure and in mice treated with 4-aminobiphenyl. These studies, conducted in collaboration with the NCTR Center for Phototoxicity, have shown that the skin tumors resulting from solar exposure had relatively high frequencies of *p53* mutations, indicating that while this mutation was not the initiating event for the tumor, it was important for tumor formation.

2. Continued two studies evaluating the presence of *p53* mutations in colon cancer from both mice and humans.

3. Continued a study in collaboration with researchers in NCTR's Division of Biochemical Toxicology and investigators in the Carcinogenesis Division, National Health and Environmental Effects Research Laboratory of the Environmental Protection Agency (EPA) that addresses the shape of the dose-response curve for low-dose exposure to carcinogens. NCTR research using this ACB-PCR technology indicates that this approach provides the opportunity to detect the rare mutations involved in the etiology of cancer prior to the development of the actual visible tumor. 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.

4. Completed studies that provide insight into the mutagenic potential of comfrey (a herbal medicine) and riddelliine (a herbal tea in certain regions of the world).

5. Completed studies showing that azathioprine (an immunosuppressant) does not cause a selection for sperm that carry a mutation in the hypoxanthine guanine phosophoribosyl transferase gene (*hprt*). This provides assurance that male humans taking this drug might have an increased risk of sperm causing a deficiency in the *hprt* gene.

6. Continued a comprehensive study for assessing 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 Deficient Hyperactivity Disorder.

7. Initiated 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.

FY 2008 Plans

1. A new approach for using the quantitative analysis of *in vivo* mutation data to inform the mode-of-action assessment for carcinogens has been developed. This approach will be evaluated for its utility in a Cooperative Research and Development Agreement (CRADA) with the Toxicology Excellence for Risk Assessment (TERA) and an informal collaboration with Environ, International.

2. DGRT scientists will continue studies applying the new genotypic-selection technology measuring specific rare mutations in cancer-causing genes. The collaboration with EPA to apply this technology to understand the shape of the dose-response curve at low dose will be completed. Under a CRADA with CIIT, this technology is being applied to develop biologically based dose-response models for carcinogens. A new emphasis will be placed on developing this technology to use as a biomarker to identify potential carcinogens—thus providing an alternative to the two-year cancer bioassay. In addition, efforts will be made to make the technology more rapid and easy to conduct.

3. DGRT scientists will continue to investigate the possibility of using the new genotypic-selection technology to determine the number of specific tumor mutations still present following the treatment of tumors with cancer chemotherapeutics. Because this technology can detect these cancer biomarkers when they are present at a low frequency, it should be possible to use this approach to evaluate the efficacy of cancer treatment. The technology could readily determine whether a particular treatment is effective for a particular patient—thus providing a personalized-medicine approach to evaluating the efficacy of cancer therapy.

4. DGRT scientist will continue research to develop and characterize the new *PIG-A* assay. Once developed and characterized, this assay will be applicable for use in human-clinical trials to assess the potential for mutagenic activity.

5. In collaboration with scientists in the Division of Biochemical Toxicology, the AIDS studies to model the use of antiretroviral drug combinations to prevent the transmission of the virus from HIV-pregnant women to their children will continue. Human-clinical data suggest that a major target for the toxicity of AIDS therapeutic agents is the mitochondria, and studies will be conducted to evaluate the long-term effects of perinatal treatments to mice on mitochondrial-DNA copy number and mutation.

6. The Division's collaborative study with the NCTR's Division of Neurotoxicology to assess the potential for methylphenidate to induce mutagenic damage will be completed.

7. In direct response to an FDA need, Division scientists will develop a new research program using the comet assay. This assay measures the ability of chemicals to induce DNA-strand breakage and is used internationally for hazard identification. The results of this project will provide answers to questions that need to be addressed concerning the appropriate parameters for conducting the assay and will be used to help develop the guidance for conducting the assay.

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 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. The FDA requires that petitioners provide data evaluating the potential genetic toxicity of their products as a part of the product-approval process. Because genetic damage is believed to be important in tumor development, this information is used as a part of the evaluation of suspected carcinogens. Regulatory decisions are based not only on the identification of potentially genotoxic substances, but also on an understanding of their mode-of-action. Research within the Division 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.

FDA's Strategic Goal 2 (Improve Patient and Consumer Safety)
Reproductive/developmental toxicology is important to the Agency because one of the difficult challenges facing the FDA is the identification and regulation of chemicals, food additives, and biological therapies that may produce birth defects. Such defects affect 7% of humans at birth, another 7% have low birth weights, and at least 25% of pregnancies end in spontaneous abortion. The Division specializes in research to understand how toxicants may induce birth defects such as neural-tube defects. Current research addresses the role that folic acid plays in the normal closure of the neural tube. This research supports current thinking that diet and toxicants may be important in producing certain birth defects.

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

Division of Microbiology
Summary of Activities

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Introduction

The Division of Microbiology at NCTR 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 within NCTR and FDA. The Microbiology Division has the staff and facilities to help address the scientific challenges encountered by FDA and other government organizations. Examples of the research within the Division and collaborative research with scientists from other NCTR Divisions, FDA Centers, academic institutions, and other federal agencies are described below. Projects are based on FDA priorities and programmatic expertise. The research program is divided into five areas: 1) food safety, food biosecurity, and methods development; 2) antimicrobial resistance; 3) gastrointestinal microbiology and host interactions; 4) environmental biotechnology; and 5) microbiological surveillance and diagnostic support of research.

FY 2007 Accomplishments

Food Safety, Food Biosecurity, and Methods Development

Scientists in the Division of Microbiology completed epidemiological studies to source-track and delineate horizontal-transmission pathways of *Salmonella* serovars in a poultry-production environment. The studies indicated that *Salmonella*, originating from birds and other environmental sources, was resistant to multiple drugs even though the bird-flocks were treated with antimicrobials, and stringent biosecurity measures were implemented in the facility. The studies concluded that after initial colonization of *Salmonella* Heidelberg in the turkey flocks during the brooding period, these bacteria cross-contaminated other birds, drinkers, and litter samples by direct and/or indirect contact and transmitted across pens during the 20- to 22-week production cycle.

In collaboration with CVM, Marshfield Clinic Research Foundation and North Dakota State University, researchers in the Division of Microbiology recently completed a study to molecularly characterize *S.* Heidelberg isolates from turkey-associated sources (farm, veterinary-diagnostic facilities, abattoirs, and retail meats) using antimicrobial-susceptibility testing, pulsed-field gel electrophoresis, and plasmid analysis. The researchers found that 42% percent of the isolates were resistant to at least one of the 15 antimicrobial agents tested and 4% of the isolates were resistant to eight or more antimicrobial agents.

Between 2001 and 2005, 210 *Salmonella* isolates representing 64 different serovars were isolated from seafood samples imported into the U.S. from 26 different countries. These isolates were tested for their susceptibility to 16 antibiotics of veterinary and clinical importance. Twenty-five percent of the *Salmonella* strains were resistant to at least one

antibiotic tested. Several *Salmonella* strains were highly resistant to multiple antibiotics, including trimethoprim-sulfamethoxazole, streptomycin, ampicillin, kanamycin, nalidixic acids, and tetracycline. The pulsed-field gel electrophoresis and plasmid analysis showed that strains are genetically distinct and also have variant class-1 integrons. The integrons from several multidrug-resistant *Salmonella* strains were characterized, and the sequences were submitted to GenBank to compare the prevalence of antibiotic resistance in *Salmonella* strains from food.

Fifty-two tetracycline-resistant *Citrobacter* spp. and 61 tetracycline-resistant *E. coli* were isolated from farm-raised catfish samples. All isolates were resistant to multiple antibiotics. PCR (polymerase chain reaction) protocols detected the presence of several tetracycline-resistant genes (*tet*) in the genomic DNA from all isolates. Integrons were amplified and sequenced from the DNA of the isolates. Sequence analysis indicated these integrons contained inserts encoding trimethoprim-resistant genes (*dhfr*) and aminoglycoside adenyltransferase (*aadA*1) that encodes resistance to streptomycin and spectinomycin. Results indicate that the use of tetracycline and Romet-30® (sulfadimethoxine-ormetoprim) antibiotics may have aided the selection of bacteria resistant to these antibiotics.

Division scientists completed research for an Interagency Agreement (IAG) with the United States Department of Agriculture (USDA) and the Department of Homeland Security for a project on the survivability of *Bacillus anthracis* stored at various temperatures in processed liquid egg media (whole egg, egg yolk, and egg white). At -20°C, the spore counts remained unchanged for 2-3 weeks in all the media, whereas at 5°, 10°, and 15°C, the spore titer declined by 60%. When stored at temperatures between 20° and 40° C, robust growth was observed in the whole egg and egg yolk containing 10% sugar. However, there was no apparent growth in egg yolk containing 10% salt.

Antimicrobial Resistance

Division of Microbiology scientists previously showed that live microbial feed additives could act as vectors for the transfer of antimicrobial-drug resistance to endogenous bacteria in human consumers of contaminated poultry products. Division scientists have now evaluated the genetic-transfer mechanism and determined that the *Lactococcus lactis* was able to transfer *vanA*, *vanB*, and *vanC* genes to *S. aureus*. This is one of the first reports of multiple vancomycin-resistance genes originally found in different *Enterococcus* species to be present in another bacterial genus.

The Division is evaluating the effects of fluoroquinolones on resistance development in bacteria from the human intestinal tract. Experiments using *Clostridium perfringens,* a commensal colonic bacterium and food pathogen, have shown that in addition to changes that occur in the fluoroquinolone target as the result of exposure to fluoroquinolones, fluoroquinolones may exert other changes resulting in enhanced production of toxins that are virulence factors for some strains.

Microbiology scientists, in collaboration with scientists in the Division of Systems Toxicology, have developed proteomic approaches to identify and characterize the protein profiles of *Staphylococcus aureus*.

Gastrointestinal Microbiology and Host Interactions

The Division of Microbiology research staff has expertise and long-standing interest in assessing risks to the gastrointestinal microflora of humans when antimicrobial compounds are ingested in food residues, probiotics, and dietary supplements. Division scientists provide guidance and expert advice to FDA, other national regulatory agencies, and the World Health Organization on the potential human-health risks associated with the use of antimicrobial agents, competitive-exclusion products, probiotics, and dietary supplements in veterinary and human-clinical medicine.

Division of Microbiology scientists developed an experimental tissue-culture system to investigate the effects of low concentrations of antimicrobial drugs on the barrier effect of the intestinal microbiota against *Salmonella*. Results of this work suggest that the barrier effect (colonization resistance) is often more sensitive to low antibiotic concentrations than has been shown with minimum inhibitory concentration MIC-based assays. This work provides a potential new tool in determining acceptable daily-intake limits of antibiotic residues.

Division scientists are participating in a Cooperative Research and Development Agreement (CRADA) with Pfizer Animal Health to study the degradation of the veterinary antimicrobial ceftiofur by the normal bovine-intestinal microflora. Division microbiologists isolated and characterized bacteria from bovine intestinal tracts that degrade the third-generation cephalosporin drug ceftiofur. These bacteria, mainly *Bacillus* spp. and *Bacteroides* spp., produced beta lactamase enzymes that are involved in the enzymatic pathways that degrade ceftiofur.

An FDA Office of Women's Health (OWH) project is currently ongoing to investigate the protective effect and potential probiotic useage of *Lactobacillus* for *Staphylococcus aureus*-mediated infection. In FY 2007, Division scientists developed a medium that simulates vaginal secretions and assessed 30-40 strains of *Lactobacillus* for their ability to either inhibit growth or inhibit the production of toxic-shock syndrome toxin-1 (TSST-1) from *S. aureus* MN8, the prototypic TSST-1 producing strain of *S. aureus*.

Another new field for investigation in the Division of Microbiology is determining the critical role that the normal microflora of the human skin and the human intestine has in the metabolism of tattoo pigments and topically applied colorants and azo dyes in food. A study on the enzymatic mechanisms of azo-dye degradation by skin and intestinal microflora, and a survey on metabolism of azo dyes by predominant skin and intestinal microorganisms has been performed.

Environmental Biotechnology

Human exposure to polycyclic aromatic hydrocarbons (PAHs) in the environment could occur from the charcoal grilling of meat and bioaccumulation in fish. Division scientists identified 194 genes responsible for the degradation of PAHs in the PAH-degrading *Mycobacterium*, then analyzed the genes using microarray methods and determined the functional roles of genes and enzymes involved in PAH-degradation pathways. Additionally, 28 genes involved in the tricarboxylic-acid cycle were identified. Division scientists identified complete metabolic pathways for pyrene and fluoranthene in *M. vanbaalenii* PYR-1. The results were combined with genomic, metabolic, and proteomic

data at the systems-biology level for the understanding of PAH-degradation in the environment.

Research on the degradation of antimicrobial agents, such as fluoroquinolones, to biologically inactive products continues to be a major emphasis in the Division of Microbiology. Bacteria were isolated from wastewater on a medium containing norfloxacin. Of the isolates that were highly resistant to five fluoroquinolones, one *Escherichia coli* strain had a ciprofloxacin-acetylating gene. This bacterium also had resistance mutations in the gyrase A and topoisomerase IV genes. HPLC (high-performance liquid chromatography) and MS (mass spectrometry) showed that it inactivated both ciprofloxacin and norfloxacin by N-acetylation.

Microbiological Surveillance and Diagnostic Support of Research

The continuing primary mission of the Surveillance and Diagnostic program is to provide the assurance that NCTR research data is not compromised by the use of infected or unhealthy experimental animals. During FY 2007, program personnel worked to prevent the introduction of microbial pathogens into NCTR animal colonies by: 1) screening the primate colony for the presence of *Campylobacter* spp., 2) closely monitoring quarantined Lean Zucker Rats for the presence of *Staphylococcus aureus*, and 3) testing the animal quarantine area for the presence of *Pseudomonas aeruginosa*. Routine monitoring of the animals, environment, food, and water from the breeder colonies was a continuing priority.

FY 2008 Plans

Food Safety, Food Biosecurity, and Methods Development

1. In collaboration with CVM, USDA, and Arkansas Public Health Laboratory, researchers in the Division of Microbiology are currently studying the microbial genetics of *Salmonella* Javiana from clinical, animal, and food-related outbreak populations in the United States. Researchers propose to study the genetic diversity of these bacteria, analyze patterns of antimicrobial-susceptibility profiles, and investigate mechanisms of drug resistance.
2. Enterohemorrhagic *E. coli* (*E. coli* O157:H7) has been implicated in several foodborne illnesses and outbreaks associated with roast beef, vegetables, salad bars, retail meats, raw milk, and person-to-person transmissions. In collaboration with the Arkansas Public Health Laboratory, CVM, and Marshfield Clinic Research Foundation, researchers in the Division of Microbiology have initiated preliminary studies to investigate the mechanisms of antimicrobial resistance in both nonpathogenic and *E. coli* O157:H7 and H7-negative strains isolated from veterinary diagnostics and human origin.
3. Infections in humans caused by *Salmonella* and *Escherichia* species continually cause serious infections and illnesses each year in the United States. A new method will be developed to monitor new and, as yet, uncharacterized antibiotic-resistance phenotypes in field isolates. This project is in collaboration with CVM and ORA.

4. Division scientists will collaborate with ORA on the isolation and characterization of fluoroquinolone-resistant bacteria from imported seafood sold in supermarkets.
5. The Division will continue working on a project in collaboration with USDA on the survivability of *Bacillus anthracis.* Division scientists will analyze the effect of egg white added to beef and milk on the survival of *B. anthracis* Sterne, *Salmonella*, *stapylococci*, *enterococci*, and *E. coli*.
6. The Division plans to study host-pathogen interactions, especially the expression of host and pathogen genes, by microarray analysis using human intestinal epithelial cells and foodborne pathogens as model systems.

Antimicrobial Resistance

1. Division scientists will analyze the role of *orf29* and *orf30* on vancomycin resistance by their insert ional inactivation individually or simultaneously.
2. *Aeromonas* spp. isolates obtained from CVM will be analyzed by pulsed-field gel electrophoresis (PFGE), polymerase chain reaction (PCR), and other molecular biology-based typing methods. The Division will conduct conjugation experiments to determine the rate of *tet* gene transferability from *tet*-resistant *Aeromonas* spp. to *tet*-sensitive *E. coli.* Similar studies will be done with *tet*-resistant *Citrobacter* isolates.
3. The Division will continue evaluation of the effect of fluoroquinolones on resistance development in bacteria from the human intestinal tract and analyze the physiological changes that occur in these bacteria as the result of exposure to antimicrobial agents.
4. The study of efflux-mediated drug resistance will move into a new phase this year with the development of a Critical Path-focused protocol that addresses methodologies to detect active intrinsic-resistance mechanisms in foodborne and veterinary pathogens of *Enterobacteriaceae*. This project will have interactions involving CVM and ORA.
5. Microbiology scientists will continue to investigate the interaction of *S. aureus* and influenza in animal models of respiratory disease in collaboration with St. Jude Children's Research Hospital.
6. Microbiology scientists will collaborate with the University of Arkansas in Fayetteville to examine the efficacy of yeast-derived vaccines for the prevention of avian influenza in humans.

Gastrointestinal Microbiology and Host Interactions

1. The Division of Microbiology will continue to explore the potential for bacteria in competitive exclusion and other probiotic products to transfer potentially hazardous genes to human gastrointestinal tract-associated bacteria.
2. In FY 2008, a proposal was approved by the OWH to conduct a study of gene-expression responses in vaginal epithelial cells stimulated by the yeast *Candida albicans* and probiotic lactobacilli.
3. The recent discovery by Division immunologists that probiotic bacteria can block an immunoinhibitory effect of *Salmonella* on T-cells in mice has led to a new

study of changes in the expression of signal-transduction genes in mucosal-immune tissues of mice orally challenged with *Salmonella* and probiotic bacteria.

4. Microbiology scientists will continue to evaluate the inhibitory properties of *Lactobacillus* for TSST-1-producing strains of *S. aureus*.
5. *Lactobacillus* microarrays will be used to address questions involving efflux-pump function along with various resistance-type studies.
6. The Division will analyze degradation products of azo dyes by skin and intestinal microorganisms, using specific enzymatic treatments, HPLC, and LC-MS/GC-MS methods.
7. Microbiology scientists will continue to use DNA probes and antibodies from *E. faecalis* and *S. aureus* azoreductases for screening similar genes in skin and intestinal microflora to determine the distribution of the azoreductase genes among predominant bacteria and the enzyme expression levels in these microorganisms. The Division of Microbiology recently received funding from OWH to perform assessment of effects and metabolism of synthetic azo colorants used in women's cosmetics on human-skin microbiota.
8. As part of our ongoing CRADA with Pfizer Animal Health, the Division will focus on the specific mechanisms of ceftiofur degradation by isolated bovine intestinal bacteria.

Environmental Biotechnology

1. The Division scientists will determine, using gene probes and microarray technology, the fate of PAHs and PAH-catabolic gene expression in a variety of soil systems.
2. Microbiology scientists will obtain samples from wastewater treatment plants to screen for bacteria that degrade or transform fluoroquinolones and identify those microorganisms that degrade fluoroquinolones.

Microbiological Surveillance and Diagnostic Support of Research

In FY 2008, the Microbiological Surveillance and Diagnostic Support group will validate the automated microbial-identification systems currently in use and increase the use of PCR and other molecular diagnostic and microbial identification systems.

Contribution to FDA's Strategic Goals

The Division of Microbiology staff is conducting a number of projects in conjunction with other FDA Centers to provide critical research that supports FDA's Strategic Goals 2 and 4.

FDA Strategic Goal 2 (Improve Patient and Consumer Safety)

Food safety and biodefense are important considerations to the FDA and national security of the United States. The research goals in Food Safety, Food Biosecurity, and Methods Development area of the Division of Microbiology are to conduct scientific research that will supplement the regulatory decision of the FDA to ensure the safety of our food supply in the "farm-to-the-fork" continuum. The studies conducted in this research area highlight the use of molecular methodologies such as the pulsed-field gel electrophoresis and plasmid typing to source-track the origin and dissemination of pathogenic and drug

resistance bacteria from different food animal, veterinary, or clinical origin. Researchers have used statistical analysis to analyze the variation in the genetic clonality among the strains and serotypes using complex algorithms.

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

Research is being conducted in the Division of Microbiology on the importance of human intestinal microflora in the metabolism and conversion of food additives, food supplements, and antimicrobial agents. These approaches 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 changes in this population may affect human health.

Environmental biotechnology research being conducted in the Division of Microbiology on the environmental fate of FDA-regulated drugs is highly relevant to the prevention of microbial antibiotic resistance. Before a veterinary drug can be granted approval for marketing, FDA requires that the drug be subjected to environmental risk assessment. If the drug residues can be metabolized to inactive products, they will no longer select for bacterial resistance. The research conducted by the Division of Microbiology is increasing our understanding of the metabolism of these compounds and will further help FDA understand effects on the environment.

Division of Neurotoxicology
Summary of Activities

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Introduction

Approximately twenty-five percent of all Americans will suffer a brain-related disorder during their lifetime. Brain and other nervous system-related disorders account for more hospitalizations than any other major disease group in the United States. The estimated annual cost to the national economy for treatment, rehabilitation, and related consequences is thought to far exceed $400 billion. Known and suspected causes of brain-related disorders include exposures to chemicals such as therapeutic drugs, food additives, food products, cosmetic ingredients, pesticides, and naturally occurring substances. Recent advances in biomedical research are currently providing our scientists with a variety of new tools with which to better study and understand the etiology of brain-related disorders and the mechanisms associated with chemically induced neurotoxicity and to further reduce the risks associated with neurotoxic events.

The number of potential neurotoxicants that require FDA regulation is estimated to be in the thousands. Thus, identifying methods and approaches for assessing neurotoxicity is critical for the development of guidelines applicable for the assessment of neurotoxic risk. It is clear that chemicals that are known and suspected causes of brain-related disorders are vital to the national economy and our quality-of-life. Therefore, the challenge is to determine at what doses and under what conditions these compounds can be used 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. Towards this end, specific research efforts address three main 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; and 3) the NMDA (N-methyl-D-aspartic acid) receptor complex as a mediator of adult and developmental neurotoxicity. 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 has been to employ multidisciplinary approaches that capitalize on the neurochemistry, molecular neurobiology, neuropathology, neurophysiology, and behavioral expertise of Division personnel. The Division is in the process of expanding capabilities in the area of imaging by adding both MicroPET and magnetic resonance imaging (MRI) instruments and personnel. 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/behavioral assessments; and 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.

FY 2007 Accomplishments

Research protocols were implemented or continued to provide data important for the regulatory needs of the Agency with respect to acrylamide (a ubiquitous food contaminant), pediatric anesthetics agents (including ketamine), the central nervous system stimulants amphetamine and methylphenidate (widely used in the treatment of Attention Deficit Hyperactivity Disorder), and nanoparticles. Under a CRADA a unique assessment of the effects of chronic exposure to the dopamine D3 receptor agonist, pramipexole, was begun in our pediatric nonhuman-primate model. This compound is being considered for use in the treatment of Tourette's Disorder and Restless Leg Syndrome. Such studies provide invaluable information concerning expected outcomes of chemicals known to interact with specific cellular entities during development.

In partnership with colleagues at 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 outcome. *In vivo* nonhuman-primate studies have helped to identify developmental periods during which sensitivity to ketamine is greatest and also to explore aspects of exposure duration that contribute to toxicity. Importantly, use of the monkey and a similar rodent model are beginning to help identify compounds that may be able to prevent or ameliorate anesthetic-induced neurotoxicity. Regulatory briefings have helped to determine relevant data needs and possible labeling changes based on these new data.

Division staff played a critical role in the FDA Microarray Quality Control (MAQC) project, which established quality-control metrics and thresholds for objectively assessing the performance achievable by different microarray platforms and evaluating the merits and limitations of various data-analysis methods. The MAQC project involved six FDA Centers, major providers of microarray platforms and RNA samples, the Environmental Protection Agency (EPA), National Institute of Standards and Technology (NIST), academic laboratories, and other stakeholders. The MAQC project is helping improve microarray technology and foster its proper applications in the discovery, development, and review of FDA-regulated products.

Studies employing new stains developed by Division staff [Fluoro-Ruby (an anterograde tracer) and Fluoro-Jade C (a marker of dying nerve cells)] continued to help elucidate mechanisms-of-action of several neurotoxicants including the amphetamines. The technique of prelabeling axons and terminals with Fluoro-Ruby prior to insult and then subsequent staining with Fluoro-Jade C to identify frank degeneration appears to be applicable to all types of neurotoxicants that damage axons and terminals. In addition, regionally specific gene-expression platforms are under development to aid in

determining which genes might be involved in the production of neurotoxicity within a specific brain region.

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 behavioral laboratory—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. For example, in studies on chemically induced mitochondrial dysfunction, 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 MicroPET imaging device was acquired and will provide the opportunity to follow such events noninvasively in longitudinal fashion, providing time-course information on lesion development, severity, and recovery.

FY 2008 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 studies on acrylamide, pediatric anesthetics, nanoparticles, and amphetamines and related compounds. A comprehensive protocol 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 be developed. Building renovations will expand the capacity of the Nonhuman Primate Research Center and provide housing for a new MRI-imaging device, which is slated to become operational early next fiscal year. Combining the power of MRI with our MicroPET capabilities will dramatically increase our ability to describe and define neurotoxic events as they occur over time in living animal models.

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.

Further utilization of omics techniques should allow for the identification of specific genes and pathways involved in the expression of neurotoxicity and incorporation of 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 three main 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; and 3) the NMDA-receptor complex as a mediator of adult and developmental neurotoxicity.

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)
Substantial effort is being made to specifically address issues of regulatory concern around acrylamide, a food contaminant, anesthetic agents (particularly those used in the pediatric setting), and stimulant medications. Division research to elucidate the mechanisms surrounding the neurotoxicity associated with the pediatric use of anesthetic agents, define sensitive periods of development, explore critical dose-response relationships, and develop protective therapeutic strategies will provide clinicians with the knowledge needed to minimize risk and protect public health.

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, development of state-of-the-art imaging capabilities will provide opportunities to monitor the onset of toxic responses and to delve further into their mechanisms and time course. These imaging resources will 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 continues to provide training for undergraduate and graduate students, postdoctoral fellows, and visiting scientists—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 potentially beneficial therapeutic strategies. Mechanistically based approaches are being 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 make-up.

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

The overall goals of the DPNM are to develop and implement research strategies that account for genetic, environmental, and cultural diversity that influence expression of genetic make-ups and produce knowledge for improving personal and public health. These overarching goals will be met with three 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 the data from omics and environmental assessments that will result in evidence-based and validated biomedical decision-making.

The Division has two branches—Biometry and Molecular Epidemiology. The main function of the Biometry branch is to develop biometrical methods for all aspects of the FDA's mission, goals, and objectives. A subgroup within the Division analyzes all data from the National Toxicology Program (NTP). The Molecular Epidemiology branch has focused on differences that occur between men and women in the metabolism of drugs, particularly those involved in chemotherapy.

FY 2007 Accomplishments

Following the reorganization of the Biometry and Risk Assessment Division and the Molecular Epidemiology Division into the Division of Personalized Nutrition and Medicine in October 2006, the first permanent director arrived in November 2007. The Director's background is in the emerging discipline of nutrigenomics, a foundational concept for personalizing nutrition and medicine. As the new leadership in the Division began formulating the Division's research plans, members of the two branches met major milestones in FY 2007.

The NTP subgroup, which is housed within the Biometry branch, completed 18 statistical reports for six different NTP protocols. This staff also provided statistical support, 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 four research projects and maintained communications with the scientists on risk-assessment methodology in the Interagency Risk Assessment Consortium. The research efforts focused on analyzing microarray data, developing 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 Molecular Epidemiology branch received funding from the Office of Women's Health (OWH) and is analyzing those drugs with large (e.g., aspirin) and relatively narrow therapeutic ranges (e.g., chemotherapeutic drugs). Numerous studies have shown that women experience more frequent and more severe adverse reactions to 5-fluorouracil, doxorubicin, and cisplatin. To assess gender differences in chemotherapeutic toxicity, investigators have profiled drug-transporter genes through microarray and real-time PCR (polymerase chain reaction). They have identified differences in expression of 20 specific transporter genes—uptake genes in the solute-carrier gene class and efflux genes in the ATP (adenosine 5'-triphosphate) binding cassettes transporter gene classes—according to gender. An *in vitro* sandwich human-hepatocyte model was used to show that females had a slower clearance rate than males. Individual differences in expression within gender were also detected suggesting that some individuals may be at a greater risk than others regardless of gender. Studies were initiated to examine whether estrogens and androgens would directly or indirectly regulate genes involved in metabolizing aspirin. DPNM investigators also analyzed genes involved in methylating DNA, a process which contributes to all major diseases, including some birth defects.

FY 2008 Plans

The DPNM is focusing on developing a medium-to-high throughput resequencing and genotyping facility with state-of-the-art epigenetic analyses, organized by a laboratory information management system (LIMS) for sample tracking and data acquisition. The Division is enlarging its human studies through collaboration with the United States Department of Agriculture (USDA) Agricultural Research Service (USDA-ARS) Delta

Nutrition Intervention Research Initiative, which will extend the capabilities of both government agencies by linking community-based participatory research (CBPR) with omics laboratory analyses. CBPR may bridge population-based research strategies to the individual. The community partner in this collaboration is in Marvell, Arkansas. Researchers at the University of Alberta's Institute for Health Economics are collaborating with DPNM to conduct a cost-benefit analysis of its research and intervention studies in Marvell.

DPNM is collaborating with physicians and scientists at the Michigan Metabolomics and Obesity Center and the University of Illinois at Chicago on Type 2 diabetes and is contributing to the design of a Phase III clinical trial of islet-cell transplantation for treating Type 1 diabetes. DPNM intends to collaborate with other clinical-research programs in the Little Rock area as well as others nationally and internationally. DPNM is also taking a leadership role in the harmonization of research strategies for chronic diseases, analyses of micronutrient requirements in the world's population groups, and the development of the Nutrigenomics Society.

To accomplish the ambitious agenda of discovering the paths to personalized nutrition and medicine, the DPNM is collaborating with other NCTR scientists in the Divisions of Systems Toxicology, Biochemical Toxicology, Neurotoxicology, and Genetic and Reproductive Toxicology who are experts in transcriptomic, proteomic, and metabolomic concepts and instrumentation. A unique partnership with experts in the Division of Microbiology will extend the nutrigenomics approach to include analyses of gut microflora, which is known to influence nutrient availability. Laboratory animal models for analyzing gene-nutrient interactions during maternity, early development, and in healthy animals genetically susceptible to chronic diseases resulting from unbalanced nutrition are being developed in collaborations with the Division of Veterinary Services and the NTP.

The Biometry branch will 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.

The Molecular Epidemiology branch will continue its effort to analyze differences in metabolism due to gender. One major project is to analyze expression of genes involved in drug transport. Studies are currently planned to determine if the individual differences in expression may be correlated to specific polymorphisms in the transporter genes. In addition, investigators are measuring whether sex hormones modulate the effects of aspirin on platelet aggregation, its related biomarkers (COX-1, COX-2, PGE2, TXA2, and LTB4), prostacyclin dynamics (PGE2, TXA2, and LTB4), and aspirin-targeting

enzymes (COXs, NOSs, and LOX) expression. Since individual women and men differ, these studies contribute to developing knowledge of personalized medicine.

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 Molecular Epidemiology 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 gender and individual genetic make-up. 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 of the Division 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 the 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.

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 integration of data from multiple technology platforms for scientific application to questions associated with the FDA's Critical Path Initiative that are in direct support of the FDA mission. Six Centers of Excellence comprise the Division of Systems Toxicology, including the Centers for Functional Genomics, Proteomics, Metabolomics, Toxicoinformatics, Hepatotoxicity, and Chemistry. The emphasis of this Division is to provide technical expertise and guidance for the inclusion of omics and *in silico* data into the review process. The goal of the Division is to use proof-of-concept protocols to identify new, more predictive biomarkers of toxicity, prognosis, diagnosis, and disease that will aid in the development and approval of safer and more effective medicines.

The Center for Functional Genomics uses high-information content microarrays in the development of mechanistic and biomarker data for improved safety assessments. Whole-genome commercial arrays, as well as in-house fabricated custom microarrays, show great promise in drug-safety evaluation, and FDA is actively encouraging this new technology. Major efforts include: 1) identifying sources of technical variability in microarray experiments and developing quality assurance/quality-control (QA/QC) procedures to help ensure that microarray data submitted to FDA are of sound quality, and 2) development and application of new high-throughput tools.

The Center for Proteomics continues to develop methods to help clarify the biological mechanisms associated with toxicity using mass spectrometry-based proteomics. Initial work focused on improvements in throughput and sensitivity of mass spectrometry-based proteomic experiments, as well as the development of bioinformatics to handle the large volume of data generated.

The Center for Metabolomics was established to aid in the assessment of preclinical and clinical safety issues as part of an FDA-wide biomarkers-development effort. This Center has initiated active collaborations within NCTR, across FDA, and with academic and pharmaceutical research groups. This research effort is an important component of the FDA's Critical Path Initiative.

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. The goal of the toxicoinformatics group is to develop methods for the analysis and integration of omics (genomics, transcriptomics, proteomics, and metabolomics) datasets.

The Hepatotoxicology group develops and applies a systems-toxicology approach to the analysis of questions in liver toxicity based on an integrated cell, molecular, transcriptomic, proteomic, and metabolomic platform. Biomarker profiles are generated for more effective assessment of risk for acute toxicity and liver-cancer development. Development of new methods for identifying and limiting the effect of agents to cause liver toxicity is of great importance in support of the FDA's Critical Path Initiative and the long-term impact on public health.

The Chemistry group has a program in mass spectrometry-based analyses in counterterrorism, as well as a significant effort in sensor and nanotube technology. In addition, the group provides computational—including artificial intelligence—approaches to predictive toxicology.

FY 2007 Accomplishments

During FY 2007, Division scientists engaged in research addressing a variety of Agency issues with special emphasis on Critical Path, food safety, and bioinformatics. Accomplishments include the following:

- Participated in the development of the Metabolomics Standards Initiative (MSI)
- Developed potential biomarkers for acute renal failure during cardiac bypass surgery in children, preclinical liver-toxicity biomarkers for acetaminophen exposures, and preclinical pediatric nephrotoxicity biomarkers for cisplatin
- Applied metabolomics methodologies to develop new preclinical biomarkers for drug-induced liver injury
- Conducted mechanism studies of hepatic effects of antidiabetic drugs, hypolipidemic drugs, and dietary supplements
- Identified a mechanism-of-action for nongenotoxic and genotoxic rodent carcinogens
- Determined changes in gene-expression profiles of drug-metabolizing genes caused by liver toxins and other compounds
- Investigated the role of mitochondrial function underlying gender-associated differences in adverse effects to zidovudine (AZT), an anti-HIV drug
- Evaluated the involvement of mitochondria in possible muscle toxicity due to AZT exposure
- Identified mitochondria-associated genes whose expression is altered by usnic acid, a weight-loss dietary supplement
- Implemented new Affymetrix-based gene-expression system
- Continued development of quality-control methods for microarrays
- Expanded the functionality of ArrayTrack™ to warehouse, visualize, analyze, and interpret data from diverse omics technology as well as clinical and nonclinical data
- Provided ArrayTrack™ training to FDA reviewers and scientists as well as conducting additional training courses at: 1) an international conference, 2) University of Arkansas at Little Rock, 3) University of Medicine and Dentistry of New Jersey, and 4) Colorado State University

- Integrated ArrayTrack™ into the Voluntary Genomic Data Submission (VGDS) program with training for reviewers, analysis of submitted data, and formal discussions between NCTR, CDER, and pharmaceutical sponsors
- Conducted the electronic data submission pilots for both regulatory nonclinical data submission and pharmacogenomics data submission programs
- Participated in the development of the *Pharmacogenomic Data Submissions Guidance for Industry* document
- Provided general support to NCTR in omics-data management and analysis
- Started Phase II of the MicroArray Quality Control (MAQC) project to help define best practices for developing predictive signatures based on omics profiles
- Developed novel computational models that predict: 1) toxicity of dioxin-like compounds and many other endocrine disruptors in food, and 2) catastrophic idiosyncratic drug side-effects such as hERG receptor inhibition and agranulocytosis

FY 2008 Plans

In FY 2008, 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. To accomplish its mission, the Division of Systems Toxicology will:

- Continue development of an integrated, state-of-the-art omics platform consisting of microarray, NMR (nuclear magnetic resonance)- and MS (mass spectrometry)-based metabolomic, lipidomic, and proteomic signatures that can perform analyses of compounds of interest to FDA
- Provide technical expertise to FDA in genomic, proteomic, and metabolomic interpretation and guidance
- Continue to utilize a series of liver and renal toxins to demonstrate the utility of integrated omics analyses
- Continue to develop a systems-toxicology approach to the integrative analysis of omics data with conventional toxicology assessments
- Continue to develop computational models of biological activity, toxicity, and biomarker-pattern identification
- Continue to develop nanotechnology and sensor-technology efforts
- Use high-throughput PCR-based arrays to determine changes in gene-expression profiles of drug metabolizing genes caused by liver toxins and other compounds
- Develop a bioinformatics infrastructure, called SNPTrack, for genotyping-data management, analysis, and interpretation
- Develop an electronic data submission environment for the VGDS program
- Develop a liver toxicity knowledge base that fills a data gap in FDA for drug review
- Develop mass spectrometric-based proteomic platforms for robust protein identification with high sensitivity and dynamic range
- Develop technologies for proteome-wide identification of protein modifications

- Address drug-safety issues using proteomics technology by identifying protein biomarkers (and mechanisms) of disease state, drug response, and toxicity
- Continue development of Food Quality Indicators as useful indicators of food spoilage
- Continue development of RAPID-B (Rapid Identification of Bacterial Pathogens) for the detection of pathogenic food contaminants
- Continue development of computational models for prediction of drug side-effects.

Contribution to FDA's Strategic Goals

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

FDA Strategic Goal 2 (Improve Patient and Consumer Safety)
The entire Division is involved with developing potential preclinical and translational safety biomarkers based on new metabolomic, genomic, and proteomic technologies. Potential safety biomarkers for liver and kidney injury were developed. The unique aspect of this work is our integration of the various datasets to get a holistic systems view of the safety problems caused by medical products. A novel genomics tool, MitoChip, was developed and validated for detecting early toxicities associated with mitochondrial dysfunction. Understanding these new technologies strengthens the science that supports product safety. In addition, new mass-spectrometric and flow-cytometric methods were developed and validated for the detection of bacteria in food products. In addition, the Chemistry staff is continuing to develop sophisticated pattern recognition-based algorithms that use advanced noninvasive imaging scans for the accurate diagnosis and characterization of brain and breast cancers. Such methods will improve tumor diagnosis and has the potential to enable early noninvasive screening.

FDA Strategic Goal 3 (Increase Access to New Medical and Food Products)
Members of the Centers for Functional Genomics and Toxicoinformatics actively participated in developing and writing the science-based companion Pharmacogenomic Data Submissions Guidance for Industry. This guidance was based upon experiences gained in the VGDS effort, in which the centers for Toxicoinformatics, Functional Genomics, Proteomics, and Metabolomics helped analyze genomics data submitted by industry. Our efforts with leading the community-wide MAQC project also resulted in best practices for microarrays and were instrumental in the development of a guidance document. In addition, the ArrayTrack™ software, developed by our Toxicoinformatics team for the management, analysis, and interpretation of microarray data, was used in the VGDS process and has now been made publicly available to ensure that the scientific practice at FDA for pharmacogenomics is consistent with that of the scientific community. Finally, a second phase of the MAQC project is establishing best practices in developing predictive signatures from microarray data. All these contributions strengthen the scientific foundation of FDA's regulatory mission. In addition, collaborations were developed with government agencies, foreign government agencies, industry, and academia to develop better tools for safety prediction.

Novel computational models were developed that predict: 1) toxicity of dioxin-like compounds and many other endocrine disruptors in food and 2) catastrophic idiosyncratic drug side-effects such as hHERG receptor inhibition and agranulocytosis. Such new computational methods will increase the number of safe and effective new medical products.

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

The Chemistry group has invented and optimized a colorimetric detector that indicates food spoilage in a commercial setting. This technology is now being commercialized and promises to provide consumers with 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 the Center's Animal Care and Use Program, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). Included within the division are contracted services for animal husbandry, veterinary care, diet preparation, and pathology. This workforce is stable, highly trained and skilled, and boasts a high percentage of certified employees in their respective disciplines.

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

DVS oversees the operation of four 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), are charged with ensuring that healthy animals are available for research projects, providing veterinary care if needed, training of research staff, and participating in projects requiring veterinary expertise. These veterinarians share emergency-call duty during 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 containing a specific pathogen-free barrier work environment. The majority of dosed diets, 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.

The Pathology Services group provides support to NCTR investigators to include necropsy and routine histopathology as well as Molecular Pathology, Immunohistochemistry, Clinical Pathology, and other nonroutine services such as digital macrophotography, laser capture microdissection, and image archiving using digital storage of microscopic images at diagnostic resolution. The staff includes a professional team of pathologists and specialists in molecular and toxicologic pathology, a medical technologist, and American Society for Clinical Pathology (ASCP)-certified technical support staff.

FY 2007 Accomplishments

Immediate Office

The Division provided oversight and management of all laboratory animal facilities at NCTR. Division personnel were responsible for breeding, rearing, and/or acquiring and quarantining all experimental animals used on-site. Personnel submitted annual reports assuring compliance with federal regulations and National Institutes of Health guidelines relative to our Animal Care and Use Program and participated in semi-annual program reviews, facility inspections, and experimental protocol reviews as part of the NCTR IACUC proceedings. All animal resource needs were managed for all research projects. Division personnel served as government project officers for pathology services, animal care and diet preparation services, and rodent bedding contracts for the Center. This arrangement assured coordination of activities and provided essential input associated with IAG and CRADA development, initiation, and completion.

The Division hosted the AAALAC International site visit in June 2007, which consisted of two days of intense scrutiny of NCTR's Animal Care and Use Program by three highly qualified laboratory animal science professionals. Division personnel prepared the "Animal Care and Use Program Description," a requisite document submitted to AAALAC in advance of the site visit. In November 2007, NCTR was again granted continued full-accreditation status by AAALAC International.

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. 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. The rodent breeding colony was successfully transferred to a modern facility and housed in state-of-the-art ventilated caging systems. After eradicating *Helicobacter hepaticus* from the rodent breeding colony in FY 2006, the program was extended to all conventionally housed mice in FY 2007 resulting in elimination of the pathogen from all mouse groups by mid-year.

Animal Care/Diet Preparation Services

During FY 2007, contract personnel supported a daily average of 32 experiments. These experiments entailed the daily husbandry services for an average 6600 rodents and 147

rhesus monkeys. In addition, housing, animal care, and technical services were provided for 14 minipigs for a three-month period. A variety of technical procedures were performed on many experiments, including tattooing, tumor palpations, biological sample collections, injections, oral gavage (including neonatal mice), behavior assessments on rats and rhesus monkeys, application of topical-dosed creams, rodent breeding operations, quarantine of rodents and rhesus monkeys, physical and pregnancy examinations of rhesus monkeys, microchip implantations, anesthesia of animals, and humane euthanasia. An on-going 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 rodent chow (autoclaving, packaging, and delivery), dosed diets, dosed water, and topical creams were prepared in a barrier facility to exacting specifications for NTP experiments. A higher percentage of irradiated rodent diet was used in FY 2007, an intentional change designed to decrease costs and improve feed quality. 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 2007, the Molecular Pathology group performed the following immunohistochemical (IHC) services:

- Routine histopathology on 4,427 animals including the scheduled necropsies of 14 minipigs and 56 nonhuman primates—not routine species for NCTR
- Blood analysis on 2714 animals including evaluations of hematology, chemistries, RIAs, reticulocytes, spinal fluid, urinalysis, and platelet isolation

In addition to routine pathology services, other accomplishments for FY 2007 include:

- Conducted NTP quality assessment and peer review of pathology data for perinatal carcinogenicity of the AZT/3TC/NVP combination and retinyl palmitate
- Provided nonroutine services such as digital macrophotography, laser capture microdissection, and image archiving using digital storage of microscopic images at diagnostic resolution
- Developed procedures for processing of multiple tissues from several transgenic mice using two different fixatives
- Developed procedures for harvesting cartilage and synovial fluid from the joints, specific areas of the skeletal system, and the gross trimming of the stifle joints and decalcification using EDTA (ethylene diamine tetra-acetic acid) to allow for future immunohistochemistry
- Developed whole-body perfusion techniques for nonhuman primates
- Modified Aperio Image Analysis algorithms for use with Aperio ScanScope to generate high-resolution digital images

- Developed a fully functional bar-coding system that will allow the labeling of vials that are snap-frozen and stored in a -80º C. freezer
- Prepared tissue microarray blocks and slides using 1.0, 1.5, and 2.0 mm core sizes
- Detected oncoproteins, oncosupressor proteins, cell-cycle and apoptosis-associated proteins, tumor markers, lymphoid markers, hormones and hormone receptors, growth factors and their receptors, and cell-type markers for immunohistochemistry protocols
- Performed *in situ* hybridization

FY 2008 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
- Play an active role in the expansion and renovation of the nonhuman-primate facility
- Expand and improve the environmental enrichment program for nonhuman primates
- 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
- Conduct quality assessment and pathology working group for combination drugs for AIDS

Contribution to FDA's Strategic Goals

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

FY 2007 Ongoing Research Projects

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

PI: Ali, Syed, 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 Division(s): Biochemical Toxicology, Office of the Director

Objective(s):

1) To determine the post-translational protein modifications in the protein extracts of nigral and striatal tissues in substituted amphetamines and MPTP-treated mice
2) To evalulate 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 immuno-precipitation 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: Ali, Syed, Ph.D.

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

Responsible Division: Neurotoxicology

Objective(s):

1) To evaluate the neurotoxicity of different sized 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 (catalase, superoxide dismutase, glutathione peroxidase), 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, an *in vivo* biomarker for oxidative stress, 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.

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

External Funding:
University of Arkansas at Fayetteville (CRADA)

Responsible Division: Neurotoxicology

Collaborating Division(s): Bionetics Site Management, Office of the Director

Objective(s):

1) To develop a primate model of Parkinson's disease (PD) using the chemical neurotoxin, MPTP
2) To implant microelectrodes within the subthalamus through stereotaxic guidance for deep brain stimulation (DBS) to:
   a) monitor and analyze patterns of tremor and dyskinesia in the PD/MPTP animals wirelessly using smart wireless sensors developed by the University of Arkansas at Fayetteville, Arkansas. Data gathered in this phase will be compared with data from controls
   b) study patterns of tremor and dyskinesia after DBS treatment in a PD/MPTP animal model. Data will be compared in this phase within each animal as its own control
3) To evaluate brain neurochemistry, which includes the neurotransmitters dopamine, serotonin, and their metabolites, oxidative stress markers such as reactive oxygen species (ROS), formation of 3-nitrotyrosine (3-NT), antioxidant enzyme activities, gene-expression, transcription factors associated with dopaminergic neurodegeneration, and post-mortem brain pathology using histochemical techniques

PI: Baek, Songjoon, Ph.D.

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

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Systems Toxicology, Z-Tech Corporation

Collaborating FDA Center(s): CBER

Objective(s):

1) To build on the novel Decision Forest classification model developed at NCTR 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. A primary area of application is the classification of subjects into risk categories in class-prediction problems occurring with genomics and proteomics data

PI: Beger, Richard, Ph.D.

Biomarkers of Liver Disease and Toxicity (E0718801)

Responsible Division: Systems Toxicology

Objective(s):

To develop biomarker profiles for normal individuals and those with liver diseases or toxicity.

PI: Beger, Richard, Ph.D.

Preclinical Metabonomic Biomarkers of Toxicity and Disease (E0720401)

Responsible Division: Systems Toxicology

Collaborating Division(s): Neurotoxicology

Collaborating FDA Center(s): CDER

Objective(s):

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

PI: Beland, Frederick, Ph.D.

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

Responsible Division: Biochemical Toxicology

Objective(s):

To compare the carcinogenicity of acrylamide and its metabolite glycidamide in $B_6C_3F_1$ mice treated neonatally.

PI: Beland, Frederick, Ph.D.

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

External Funding:
EPA/National Health and Environmental Effects Research Laboratory (IAG)

Responsible Division: Biochemical Toxicology

Objective(s):

To define dose-response curves for benzo[a]pyren DNA adducts in the A/J mouse lung utilizing the application of HPLC-ES-MS/MS methodologies developed at NCTR.

PI: Beland, Frederick, Ph.D.

DNA Adducts of Tamoxifen (E0701101)

Responsible Division: Biochemical Toxicology

Objective(s):

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

PI: Beland, Frederick, Ph.D.

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

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Compound Nominated By: CFSAN

Objective(s):

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

PI: Beland, Frederick, Ph.D.

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

External Funding:
BG Medicine, Inc. (CRADA)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Systems Toxicology

Objective(s):

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. Emphasis will be placed upon drugs that do not demonstrate "classical" signs of liver toxicity during preclinical stages of drug development.

PI: Binienda, Zbigniew, 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(s): Office of the Director

Collaborating FDA Center(s): CFSAN

Objective(s):

1) To define neurophysiological and neurochemical phenotypes associated with brain exposure to 3-NPA (3-nitropropionic acid or methamphetamine) 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 F344 Rat and the $B_6C_3F_1$ Mouse Administered *Aloe Vera* Plant Constituents in the Drinking Water (E0214201)

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Compound Nominated By: CFSAN

Objective(s):

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

standardized preparations of *Aloe vera* to explore the limits of safety for the *Aloe vera* leaf constituents present in commercial products.

PI: Bowyer, John, Ph.D.

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

Responsible Division: Neurotoxicology

Collaborating Division(s): Personalized Nutrition and Medicine, Systems Toxicology, Z-Tech Corporation

Objective(s):

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

PI: Chen, Huizhong, Ph.D.

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

Responsible Division: Microbiology

Collaborating Division(s): Biochemical Toxicology

Objective(s):

The research will focus on metabolic capacity and enzyme expression in human skin microflora. The objectives are:

1) Biodegradation and bioconversion of pigments used for tattooing and permanent make-up pigments
2) To determine the effects of the skin microflora on tattoo and topically applied dyes that reside in the dermis
3) To isolate, clone, and over-express genes encoding for azoreductases and nitroreductases, which are able to decolorize the pigments
4) To determine physicochemical properties of the purified native enzymes from the bacteria and/or the expressed recombinant enzymes cloned in *E. coli*
5) To elucidate the role of the microflora with potential genotoxic effects of tattoo and permanent make-up pigments

PI: Chen, Huizhong, Ph.D.

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

Responsible Division: Microbiology

Objective(s):

1) To develop a rapid method for quantification of intestinal bacteria
2) To perform qualitative analysis of the communities for several major genera and discover the species which are noncultivated
3) To isolate and identify the bacterial species from probiotics used for human or animal health
4) To develop a microarray method for the detection of intestinal bacteria

PI: Chen, James, Ph.D.

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

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Genetic and Reproductive Toxicology

Collaborating FDA Center(s): CDER

Objective(s):

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

PI: Chen, Tao, Ph.D.

Comparison of Mutation Induction and Types of Mutations in the *cII* Gene of Big Blue®® Mice Treated with Carcinogens as Neonates and Adults (E0709001)

Responsible Division: Genetic and Reproductive Toxicology

Objective(s):

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

PI: Chen, Tao, Ph.D.

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

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Division(s): Biochemical Toxicology, Systems Toxicology

Objective(s):

1) To treat Big Blue® rats subchronically with riddelliine, AA, and comfrey using procedures appropriate for tumor induction
2) To analyze DNA-adduct formation in the target tissues for carcinogenesis and in spleen lymphocytes
3) To determine the *cII* mutant frequencies and the types of *cII* mutations in the target tissues of treated rats
4) To determine global gene-expression patterns in the target and surrogate tissues of treated rats
5) To correlate gene-expression patterns with DNA-adduct formation and mutation induction in treated rats

PI: Chen, Tao, 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(s): Systems Toxicology

Objective(s):

1) To determine if the L5178Y/TK+/- Mouse Lymphoma Assay 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: Chou, Ming, Ph.D.

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

Responsible Division: Biochemical Toxicology

Objective(s):

1) To characterize the structures of the eight DHP-derived DNA adducts
2) To study metabolism of retronecine-based pyrrolizidine alkaloids, heliotridine-based pyrrolizidine alkaloids, otonecine-based pyrrolizidine alkaloids, and pyrrolizidine alkaloid N-oxides by liver microsomes of F344 rats, B6C3F, mice, and humans of both sexes, and compare metabolism profiles
3) To study the DNA-adduct formation *in vitro* (from liver microsomal metabolism of the pyrrolizidine alkaloids described above in the presence of calf thymus DNA) and *in vivo* and determine whether or not the same set of DHP-derived DNA adducts is formed
4) To determine whether or not the levels of DHP-derived DNA adducts from different types of necine-based pyrrolizidine alkaloids

formed in target tissues (liver) are significantly higher than those in nontarget tissues

5) To determine whether or not pyrrolizidine alkaloid N-oxides can be metabolized by rat and mouse liver microsomes to the parent pyrrolizidine alkaloids and whether or not DHP-derived DNA adducts are formed in significant amounts both *in vivo* and *in vitro*
6) To determine whether or not some dietary supplements sold in the United States contain genotoxic pyrrolizidine alkaloids
7) To determine the effect of liver carboxyesterases on DHP-derived DNA-adduct formation from rat and human liver microsomal metabolism in the presence of calf thymus DNA
8) To determine the effect of liver carboxyesterase inhibitors on DHP-derived DNA-adduct formation from rat and human liver microsomal metabolism in the presence of calf thymus DNA
9) To determine the effect of Chinese herbs, such as liquorice, and their active components, such as glycyrrhizin and glycyrrhetinic acid, on inhibition of DHP-derived DNA-adduct formation *in vivo* and *in vitro*

PI: Desai, Varsha, Ph.D.

Development of Mitochip, a Glass-Based Oligonucleotide Microarray Containing Mitochondrial and Nuclear Genes Associated with Mitochondrial Function (E0718601)

Responsible Division: Systems Toxicology

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

Collaborating FDA Center(s): ORA

Objective(s):

1) To develop a MitoChip containing genes associated with mitochondrial function such as oxidative phosphorylation, B-oxidation of free fatty acids, tricarboxylic acid cycle, apoptosis, as well as genes involved in the replication, transcription, translation of mitochondrial DNA, DNA repair, and regulation of DNA copy number
2) To validate the developed MitoChip by evaluating gene-expression profiles of AZT, an anti-HIV drug, and 3-NPA, neurotoxins known to alter mitochondrial function
3) To verify the relative expression levels of differentially expressed genes by real-time quantitative PCR

PI: Desai, Varsha, Ph.D.

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

External Funding:
Office of Women's Health

Responsible Division: Systems Toxicology

Collaborating Division(s): Genetic and Reproductive Toxicology

Objective(s):

To elucidate molecular mechanisms of mitochondrial dysfunction that will address gender-based differences in adverse effects of anti-retroviral drugs, such as AZT. This will provide critical information to the FDA for the development of guidelines to plan new treatment strategies to reduce the frequency and severity of antiretroviral-related toxic effects in women, particularly in pregnant women.

PI: Dobrovolsky, Vasily, Ph.D.

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

Responsible Division: Genetic and Reproductive Toxicology

Objective(s):

1) To produce two lines of transgenic mice expressing the tetracycline-repressor protein
2) To investigate the efficiency of *in vivo* repression of green fluorescent protein in various tissues of different lines of the double-transgenic mice
3) To determine the frequency of spontaneous and y-ray-induced TetR mutation in lymphocytes of double-transgenic mice using flow cytometry

PI: Doerge, Daniel, Ph.D.

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

External Funding:
University of Maryland (CRADA)

Responsible Division: Biochemical Toxicology

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

Objective(s):

1) To develop a physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) 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 $B_6C_3F_1$/Tk+/- mice
4) To determine toxicokinetics and compare bioavailability of acrylamide and glycidamide following exposure by intravenous, oral gavage, and dietary administration
5) To correlate the levels and kinetics of glycidamide-DNA adduct in target tissues and circulating lymphocytes with acrylamide- and glycidamide-hemoglobin adducts in rodent exposure studies for future use in monitoring human exposure through occupation, smoking, and diet
6) To determine *in vivo* mutagenesis of acrylamide and glycidamide using transgenic mice (Big Blue®)

PI: Doerge, Daniel, Ph.D.

Effect of Soy-Containing Diets on Ammonium Perchlorate-Induced Thyroid Toxicity in Sprague-Dawley Rats (E0716301)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Office of Research

Objective(s):

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

PI: Doerge, Daniel, Ph.D.

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

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Genetic and Reproductive Toxicology

Compound Nominated By: CFSAN

Objective(s):

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

PI: Doerge, Daniel, Ph.D.

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

External Funding:
University of Illinois (CRADA)

Responsible Division: Biochemical Toxicology

Objective(s):

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: Elkins, Christopher, Ph.D.

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

Responsible Division: Microbiology

Collaborating Division(s): Systems Toxicology

Objective(s):

1) To evaluate intrinsic drug resistance of *Lactobacillus* isolates from various sources
2) To functionally identify *MDR* genes from currently sequenced *Lactobacillus* genomes using genomic and proteomic approaches
3) To epidemiological profile via pulsed-field gel electrophoresis (PFGE) and microarray technology for resistance-determining factors

PI: Elkins, Christopher, Ph.D.

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

Responsible Division: Microbiology

Objective(s):

The overall objective of this project will be to determine whether probiotic administration of *Lactobacillus* can thwart *S. aureus* TSST-1

production if supplemented in women's tampons. Alternatively, if time and efforts permit during the course of this study, bacteriophage therapy may be investigated as a multifaceted approach to strengthening probiotic introduction for such conditions.

PI: Erickson, Bruce, Ph.D.

Determining the Effect of Low Levels of Antibiotic Residues on the Human Intestinal Microflora Using an *In Vitro* Continuous Culture System (E0709201)

Responsible Division: Microbiology

Objective(s):

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

PI: Erickson, Bruce, 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

Objective(s):

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, Ph.D.

Assessment of Depression Risk Associated with Accutane (13-cis-Retinoic Acid or Isotretinoin) and All-Trans-Retinoic Acid Treatment: Measurement of Behavioral and Neurochemical Alterations in Adult Sprague-Dawley and Flinders Sensitive and Insensitive Line Rats (E0714501)

Responsible Division: Neurotoxicology

Collaborating Division(s): Biochemical Toxicology, Systems Toxicology

Collaborating FDA Center(s): CBER, CDER

Objective(s):

1) To establish the necessary oral doses of 13-cis-retinoic acid and all-trans-retinoic acid in rats that produce peak plasma levels similar to those of humans prescribed 13-cis-retinoic acid
2) To measure the toxicity and pathology associated with long-term oral treatment with 13-cis-retinoic acid and all-trans-retinoic acid in rats
3) To describe the behavioral alterations associated with chronic 13-cis-retinoic acid and all-trans-retinoic acid treatment in adult male and female Sprague-Dawley rats
4) To determine if such alterations resemble those described in humans treated with 13-cis-retinoic
5) To measure sex differences in behavioral response to 13-cis-retinoic acid and all-trans-retinoic acid treatment
6) To evaluate the reversibility of the 13-cis-retinoic acid induced and/or all-trans-retinoic acid-induced alterations
7) To assess if genetic predisposition to depression determines the frequency and/or magnitude of the behavioral alterations associated with 13-cis-retinoic acid and/or all-trans-retinoic acid treatment
8) To quantitate the neurochemical alterations induced by 13-cis-retinoic acid and/or all-trans-retinoic acid treatment

PI: Ferguson, Sherry, Ph.D.

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

Responsible Division: Neurotoxicology

Objective(s):

To determine potential sex differences in substance abuse susceptibility after methylphenidate (Ritalin®) treatment during adolescence. To date, substance abuse susceptibility post-methylphenidate treatment has been determined in boys and male rodents only. If sex differences do exist, the patient information for

methylphenidate can be altered such that girls with Attention Deficit Hyperactivity Disorder (ADHD) undergoing stimulant treatment receive differential monitoring for later substance use disorders. The hypothesis is that male and female rats that have been treated with methylphenidate will exhibit different levels of drug abuse susceptibility.

PI: Fuscoe, James, Ph.D.

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

Responsible Division: Systems Toxicology

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

Objective(s):

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

PI: Fuscoe, James, Ph.D.

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

External Funding:
Office of Women's Health

Responsible Division: Systems Toxicology

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

Objective(s):

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: Fuscoe, James, Ph.D.

(OTA) (E0709401)

Responsible Division: Systems Toxicology

Collaborating Division(s): Genetic and Reproductive Toxicology

Objective(s):

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

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

Objective(s):

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.

Training in Hepatocyte Perfusion and Hepatic-Cell Isolation (P00610)

Responsible Division: Systems Toxicology

Objective(s):

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

PI: Hammons, George, Ph.D.

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

Responsible Division: Personalized Nutrition and Medicine

Objective(s):

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, Ph.D.

Mechanism of Biotin Deficiency-Induced Malformations (E0713301)

Responsible Division: Genetic and Reproductive Toxicology

Objective(s):

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

PI: Hart, Mark, Ph.D.

Development of Proteomic Approaches to Identify *Staphylococcal Aureus* Extracellular Proteins Responsible for Staphylococcal Pneumonia (E0717501)

Responsible Division: Microbiology

Collaborating Division(s): Systems Toxicology

Objective(s):

1) To develop a proteomic approach of identifying proteins by first fractionating proteins in spent media using isoelectric focusing followed by nonporous, reverse phase HPLC
2) To generate a proteomic profile for *S. aureus* RN6390 and its agr and sar isogenic mutants

PI: Khan, Saeed, Ph.D.

Development of a Microarray Chip for the Detection of Multiple Antibiotic Resistance Markers (E0715101)

Responsible Division: Microbiology

Collaborating Division(s): Systems Toxicology

Objective(s):

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

PI: Lee, Taewon, Ph.D.

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

Responsible Division: Personalized Nutrition and Medicine

Objective(s):

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: Lyn-Cook, Beverly, Ph.D.

Correlating Gene Expression with Activity of Chemotherapeutic Agents to Identify the Mechanism of Resistance to Geldanamycin Analogs (E0724101)

Responsible Division: Personalized Nutrition and Medicine

Objective(s):

1) To identify genes involved in chemoresistance and sensitivity to a group of geldanamycin analogs using pharmacogenomics approach
2) To validate the functions of candidate genes in chemoresistance to these geldanamycin compounds
3) To reveal the functional network formed by multiple factors that cooperate to mediate cellular resistance to geldanamycin
4) To identify novel inhibitors of the chemoresistance genes or therapeutic combinations to avoid the resistance mechanism
5) To identify structural features of the geldanamycin analogs that are associated with their differential relationship with candidate genes using structure-activity analysis

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

CYP1 B1 Polymorphisms in Uterine Leiomyomas: Frequency in African American Women and Response to Therapy (P00443)

Responsible Division: Personalized Nutrition and Medicine

Objective(s):

To determine the frequency of the polymorphic variant and others of cytochrome P450 1B1 in human uterine leiomyoma cases compared with the frequency in patient-matched controls.

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

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

External Funding:
Office of Women's Health

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Systems Toxicology

Objective(s):

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. This is prerequisite to elucidating the mechanisms of interindividual variability in hepatic drug transport systems
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, 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)

External Funding:
Office of Women's Health

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Neurotoxicology, Biochemical Toxicology

Objective(s):

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

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

Objective(s):

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-ethylnitrosourea (ENU) and cyclosphosphamide (CP) to investigate the potential utility of the *TCR* gene mutation assay using isolated spleen lymphocytes derived from treated Fisher 344 rats
3) 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: Morris, Suzanne, Ph.D.

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

Responsible Division: Genetic and Reproductive Toxicology

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

Objective(s):

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: Morris, Suzanne, 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(s): Personalized Nutrition and Medicine

Objective(s):

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: Ning, Baitang, Ph.D.

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

External Funding:
Office of Women's Health

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Systems Toxicology, Biochemical Toxicology

Objective(s):

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 (estrogens, progestogens, and androgens) in regulation of the expression of aspirin-metabolizing genes in human ER-positive hepatic-cell line HepG2-ER(+), using biochemical procedures including DNA-protein binding assay and reporter construct assay
3) To measure sex-hormone modulation of aspirin effect on platelet aggregation and its related biomarkers (COX-1, COX-2, PGE2, TXA2, and LTB4) using human platelet precursor cells
4) To identify sex-hormone modulation of aspirin actions in human endothelial and epithelial cell lines, by measuring prostacyclin dynamics (PGE2, TXA2 and

LTB4) and aspirin-targeting enzymes (COXs, NOSs, and LOX) expression
5) To evaluate sex-hormonal modulation of response to aspirin in apoliprotein E-deficient mice

PI: Ning, Baitang, Ph.D.

Regulatory Polymorphisms of *SULT1A1* and its Impact on the Risk of Prostate Cancer in African-Americans and Caucasians (E0715801)

Responsible Division: Personalized Nutrition and Medicine

Objective(s):
1) To determine and map polymorphisms in the promoter region of the *SULT1A1* gene
2) To identify the association study between phenotype and haplotype of *SULT1A1*
3) To conduct a case-control study to assess the high susceptible haplotype(s) of *SULT1A1* for prostate cancer
4) To functionally characterize high-risk haplotype(s) of *SULT1A1*

PI: Ning, Baitang, Ph.D.

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

Responsible Division: Personalized Nutrition and Medicine

Objective(s):
1) To determine whether induction of SULT1A by 4-OH TAM results in an increase in expressed protein and enzymatic activity toward environmental estrogens in tamoxifen-treated breast-cancer patients
2) To determine the effect of 4-OH TAM on *SULT1A1* activity in breast-cancer cell lines
3) To determine *SULT1A1* genotype in tamoxifen-treated women and genotype-phenotype correlations
4) To archive blood samples, administer the Block 98 Questionnaire, and determine the survival data for future studies

PI: Parsons, Barbara, Ph.D.

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

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Division(s): Neurotoxicology, Personalized Nutrition and Medicine

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

PI: Patterson, Tucker, Ph.D.

Analyses of the Rat Hippocampus via DNA Microarrays and a Novel Antibody Array, Coupled with LCM — Evaluation of the Effect of Aging on Gene and Protein Expression Associated with Learning (E0713901)

Responsible Division: Neurotoxicology

Objective(s):
1) To measure gene and protein expression in regions of the hippocampus to determine regional distribution
2) To determine the effect of aging on regional distribution of hippocampal proteins in three strains of rats
3) To determine if aging, behavioral performance, and alterations in gene and protein expression in the hippocampus are related
4) To correlate the differences in gene and protein expression with behavioral performance of young adult and aged rats in a learning task previously shown to be sensitive to changes in protein expression

PI: Patterson, Tucker, Ph.D.

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

Responsible Division: Neurotoxicology

Collaborating Division(s): Office of the Director

Objective(s):
To assess the neurotoxicity and neurobehavioral effects of chronic treatment with the anti-HIV agents 2',3'-

dideoxycytidine (ddC) and thalidomide in rhesus monkeys.

PI: Patterson, Tucker, Ph.D.

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

External Funding:
Boehringer Ingelheim Pharmaceuticalo, Inc. (CRADA)

Responsible Division: Neurotoxicology

Objective(s):

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, including histopathology
6) To conduct a focused neuropathological evaluation

PI: Paule, Merle, Ph.D.

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

Responsible Division: Neurotoxicology

Objective(s):

To measure aspects of learning, short-term memory and attention, motivation, time perception, and color and position discrimination using a battery of automated tests (games).

PI: Paule, Merle, Ph.D.

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

Responsible Division: Neurotoxicology

Collaborating Division(s): Office of Research

Objective(s):

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

PI: Paule, Merle, Ph.D.

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

External Funding:
National Toxicology Program (IAG)

Responsible Division: Neurotoxicology

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

Compound Nominated By: CFSAN

Objective(s):

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

PI: Paule, Merle, Ph.D.

Developmental Neurotoxicity Assessment of Acrylamide in Rats: Range-Finding Studies (E0214801)

External Funding:
National Toxicology Program (IAG)

Responsible Division: Neurotoxicology

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

Objective(s):

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

PI: Paule, Merle, Ph.D.

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

Responsible Division: Neurotoxicology

Objective(s):

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: Paule, Merle, Ph.D.

Effects of Chronic Methylphenidate (Ritalin®) Administration on 'Cognitive' Functions in the Rhesus Monkey (E0683700)

Responsible Division: Neurotoxicology

Collaborating Division(s): Office of the Director

Objective(s):

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

PI: Paule, Merle, Ph.D.

Effects of Prenatal Cocaine on Behavioral Plasticity (E0663307)

External Funding:
University of Arkansas at Little Rock (CRADA)

Responsible Division: Neurotoxicology

Collaborating Division(s): Neurotoxicology

Objective(s):

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

PI: Paule, Merle, Ph.D.

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

External Funding:
Astra Charnwood (CRADA)

Responsible Division: Neurotoxicology

Objective(s):

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

PI: Paule, Merle, 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

Objective(s):

To develop and implement a comprehensive assessment of all levels of the neuraxis in an effort to determine CNS deficits due to balance disorder and vertigo and develop and assess strategies to restore those deficits.

PI: Pogribna, Marta

Folic Acid Metabolism in Children with Down Syndrome (DS) (E0708501)

Responsible Division: Biochemical Toxicology

Objective(s):

To determine whether supplementation with the nutrients folic acid and betaine will increase plasma levels of methionine, S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), which have shown to be low in children with DS. The experiments will focus on the biochemical lesions in one-carbon metabolism stemming from trisomy 21-gene overdose and the potential to normalize metabolic imbalance

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

PI: Pogribny, Igor, Ph.D.

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

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

Responsible Division: Biochemical Toxicology

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

Objective(s):

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- 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 potentia

PI: Rafii, Fatemeh, Ph.D.

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

Responsible Division: Microbiology

Collaborating Division(s): Biochemical Toxicology, Systems Toxicology

Objective(s):

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. This information is necessary for extrapolation to humans of the data obtained from treatment of animals with phytoestrogens.

PI: Rafii, Fatemeh, Ph.D.

Elucidation of the Mechanism of Resistance Development in Anaerobic Bacteria from the Human Intestinal Tract (E0709301)

Responsible Division: Microbiology

Objective(s):

To evaluate the effects of fluoroquinolones on resistance development in bacteria from the human intestinal tract and analysis of the fluoroquinolone-resistance mechanisms in anaerobic bacteria from the human intestinal tract.

PI: Schmued, Laurence, Ph.D.

Proteomics of Toxicant-Induced Neuronal Degeneration (E0711101)

Responsible Division: Neurotoxicology

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

Objective(s):

1) To resolve the chemical identity of the endogenous protein(s) associated with neuronal cell death as identified by Fluoro-Jade B binding

2) To determine if the same proteins are expressed regardless of the mechanism of neurodegeneration
3) To resolve the chemical identity of the fluorescent component in Fluoro-Jade B responsible for the high-affinity labeling of degenerating neurons
4) To resolve the metabolic pathway by which the "degeneration protein" is generated

PI: Slikker, William, Ph.D.

Preliminary Studies for the Effects of Chronic Dexfenfluramine Administration in the Rhesus Monkey (E0702601)

Responsible Division: Neurotoxicology

Collaborating FDA Center(s): CDER

Objective(s):

1) To determine if the rhesus monkey demonstrates cardiac valve changes due to chronically administered dexfenfluramine
2) To determine if the rhesus monkey demonstrates neurobiological changes due to chronically administered dexfenfluramine

PI: Tareke, Eden, Ph.D.

The Effects of Acrylamide and PhIP on Normal Human Brain Cortical Neuronal (HCN-1), PC12, and HepG2 Cells *In Vitro*: Activation or Inactivation of Phase I and II Enzymes (E0726301)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Neurotoxicology

Objective(s):

1) To determine the effects of acrylamide and/or PhIP on cell proliferation, transformation, toxicity, apoptosis, and neurotransmitters turnover in HCN-1 and PC12 cells
2) To determine the effects of acrylamide and/or PhIP on the expression of CYP 1A1, 1A2, 1B1, 3A4, and GSTs in HepG2, PC12, and HCN-1 cells
3) To determine whether the dietary agents, I3C, and sesame seed lignans, modulate the effects of acrylamide and/or PhIP

PI: Valentine, Carrie, Ph.D.

Evaluation of the Potential of the Gene A Forward Mutational Assay of ΦX174 for Improving Sensitivity of Transgenic Mutation Assays (E0711501)

Responsible Division: Genetic and Reproductive Toxicology

Objective(s):

1) To determine the appropriate experimental conditions to identify single bursts of mutations fixed *in vivo*
2) To develop a microplate-scoring method that will identify *in vivo* bursts within numerous aliquots
3) To determine the spontaneous mutant frequency and ENU-induced mutant frequency by single-burst analysis for mouse splenic lymphocytes
4) To continue development of a frameshift assay for ΦX174 in gene *J*

PI: Wagner, Robert, Ph.D.

Characterization of Antimicrobial-Drug Resistance Genes from *Lactococcus lactis* P1-79 (E0716201)

Responsible Division: Microbiology

Objective(s):

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

PI: Wagner, Robert, Ph.D.

Probiotic Effects on Host Defense Against Enteric Pathogens (E0709701)

Responsible Division: Microbiology

Objective(s):

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

6) To observe the roles of model host flora and probiotic bacteria to modulate host-protective systems of immunodeficient and immunocompetent mice from *Salmonella typhimurium* and *Campylobacter jejuni*

PI: Wang, Cheng, Ph.D.

Assessment of Ketamine in the Developing Nonhuman Primate (E0718901)

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

Responsible Division: Neurotoxicology

Collaborating Division(s): Office of the Director, Biochemical Toxicology, Bionetics Site Management

Collaborating FDA Center(s): CDER

Objective(s):

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

PI: Wang, Cheng, Ph.D.

NMDA Antagonist/GABA Agonist-Induced Cell Death in the Developing Rat Brain (E0215501)

External Funding:
National Toxicology Program (IAG)

Responsible Division: Neurotoxicology

Collaborating Division(s): Bionetics Site Management, Office of the Director, Biochemical Toxicology

Collaborating FDA Center(s): CDER

Compound Nominated By: CDER

Objective(s):

1) To screen and evaluate pediatric anesthetic agents
2) To determine if a one-time bolus dose or prolonged exposure of the developing rat to NMDA antagonists, GABA agonists alone, or their combinations will induce long-term behavioral deficits, as well as long-lasting pathological changes
3) To determine the dose, temporal, and pathophysiological relationships between NMDA antagonist/GABA agonist-induced neurotoxicity and long-term behavioral changes
4) To determine the neurotransmitter receptor mechanisms involved in the neuron degeneration and behavioral deficits caused by these agents, particularly the role of altered NMDA-receptor function
5) To determine by *in situ* hybridization and immunoblot the relative densities of NMDA receptor NR1, NR2A, and NR2B subunits following anesthetic-drug administration
6) To identify mechanisms that could link altered NMDA-receptor function with elevation of superoxide free radicals in response to anesthetic drug-induced apoptosis; inhibitors will be added at various times to determine the contribution and temporal distribution of several elements of the proposed pathway leading to cell death

PI: Young, John, Ph.D.

Organ Growth Polynomial Curves for the Obese Human Population (E0726001)

Responsible Division: Personalized Nutrition and Medicine

Objective(s):

1) To gather autopsy organ-weight data from obese individuals and prepare body weight vs. organ weight curves
2) To gather autopsy organ-weight data from "normal" individuals and prepare body weights vs. organ weight curves to compare to the obese individuals and to compare to similar data reported in Reference Man (1975)

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

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

Responsible Division: Systems Toxicology

Objective(s):

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

3) To identify, adapt, develop, and standardize appropriate 2-D protein separation techniques
4) To integrate results of specific aims 1-3 to provide "front-end" components of a functional proteomics facility

NCTR Strategic Goal 2

Develop science-based best-practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory decision-making process

PI: Beland, Frederick, Ph.D.

Caloric Restriction and Gene Expression in Agouti Mice (E0260301)

Responsible Division: Biochemical Toxicology

Objective(s):

To learn how calories modify the development of cancer in mice and the mechanism underlying cancer development in humans.

PI: Beland, Frederick, Ph.D.

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

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Genetic and Reproductive Toxicology

Compound Nominated By: CDER

Objective(s):

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

PI: Boudreau, Mary, Ph.D.

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

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Regulatory Compliance and Risk Management

Collaborating FDA Center(s): CFSAN

Compound Nominated By: CFSAN

Objective(s):

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

PI: Boudreau, Mary, Ph.D.

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

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Compound Nominated By: CFSAN

Objective(s):

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

PI: Buzatu, Dan, Ph.D.

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

Responsible Division: Systems Toxicology

Collaborating Division(s): Personalized Nutrition and Medicine

Objective(s):

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

PI: Cerniglia, Carl, Ph.D.

Proteomic Approaches to Elucidate Biodegradative Pathways (E0711801)

Responsible Division: Microbiology

Collaborating Division(s): Z-Tech Corporation

Objective(s):

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

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(s): CBER

Compound Nominated By: CDRH

Objective(s):

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. The time period covered is the period during which the hypothalamic-pituitary-testicular axis is active in neonatal rhesus monkeys. This exposure time mimics, to the best extent possible, exposures of human infants to DEHP in neonatal intensive care units. To avoid high and variable background levels of DEHP and MEHP, deuterated DEHP will be utilized. Metabolites monitored will include DEHP, mono(2-ethylhexyl)phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl)phthalate (5OH-MEHP), mono(2-ethyl-5-oxo-hexyl)phthalate (5oxo-MEHP), and mono(2-ethyl-5-carboxypropyl)phthalate (MECPP)
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. The same animals will be utilized for the intravenous (IV) and oral doses. Oral doses will follow each IV dose by one week. The same metabolites mentioned in objective 1 will be monitored
3) To evaluate the feasibility and utility of a subchronic toxicity study of DEHP using repeated IV exposures in neonatal rhesus monkeys; results will be essential for the planning of such a study
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

Objective(s):

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

PI: Delclos, Kenneth, Ph.D.

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

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Office of Research

Objective(s):

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

PI: Doerge, Daniel, Ph.D.

Ketamine Pharmacokinetics in Children (E0726201)

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

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Neurotoxicology

Objective(s):

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. These measurements will be the basis for pharmacokinetic evaluation of ketamine and nor-ketamine enantiomers in children. This laboratory investigation at the NCTR is part of an Arkansas Children's Hospital protocol to better understand the disposition of ketamine in infants and children undergoing cardiopulmonary bypass.

PI: Ferguson, Sherry, Ph.D.

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

Responsible Division: Neurotoxicology

Objective(s):

Determine if Neonatal Intensive Care Unit morphine treatment in preterm infants is associated with long-term alterations in short-term memory and/or motivation at approximately 6 years of age.

PI: Fu, Peter, Ph.D.

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

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Office of the Director

Collaborating FDA Center(s): CDER

Objective(s):

1) To determine if the neonatal mouse bioassay can be employed to evaluate the tumorigenic potential of therapeutic drugs
2) To examine concurrently as positive controls the genotoxic carcinogens: 4-aminobiphenyl, benzo(a)pyrene, 6-nitrochrysene, and aflatoxin B1
3) To study the metabolism and DNA-adduct formation of benzodiazepine and antihistamine drugs by mouse and human liver microsomes to determine which, if any, cytochrome P450 is responsible for metabolic activation in mice and humans
4) To study the mutations and DNA binding of the subject drugs using transgenic human lymphoblastoid cell lines expressing appropriate CYP isozymes

PI: Fuscoe, James, Ph.D.

Evaluation of Performance Standards and Statistical Software for Regulatory Toxicogenomic Studies (E0716801)

Responsible Division: Systems Toxicology

Collaborating Division(s): Personalized Nutrition and Medicine

Collaborating FDA Center(s): CDER

Objective(s):

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

PI: Fuscoe, James, Ph.D.

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

Responsible Division: Systems Toxicology

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

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

Objective(s):

To prioritize sources of variability in microarray data to determine how to focus additional experimental queries, guidance development, and experimental standards. The outcome should be an enhanced capability to address standards development and accept new technologies as they arise.

PI: Hansen, Deborah, Ph.D.

Developmental Toxicity of Bitter Orange in Rats (E0214701)

External Funding: National Toxicology Program (IAG)

Responsible Division: Genetic and Reproductive Toxicology

Collaborating FDA Center(s): CFSAN

Compound Nominated By: CFSAN

Objective(s):

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

PI: Hansen, Deborah, Ph.D.

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

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Division(s): Personalized Nutrition and Medicine

Objective(s):

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

PI: Hansen, Deborah, Ph.D.

Physiological Effects of Bitter Orange in Rats (E0214901)

External Funding: National Toxicology Program (IAG)

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Division(s): Personalized Nutrition and Medicine, Toxicologic Pathology Associates, Biochemical Toxicology, Genetic and Reproductive Toxicology

Collaborating FDA Center(s): CFSAN

Compound Nominated By: CFSAN

Objective(s):

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

PI: Heflich, Robert, Ph.D.

Effect of Azathioprine on Somatic Cell and Germline *Hprt* Mutant Frequencies in the Mouse (E0709901)

Responsible Division: Genetic and Reproductive Toxicology

Objective(s):

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

PI: Howard, Paul, Ph.D.

Comparative Toxicity of Fumonisin Derivatives in Female $B_6C_3F_1$ Mice (E0212401)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Toxicologic Pathology Associates

Objective(s):

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

PI: Howard, Paul, Ph.D.

DNA-Adduct Formation by Nicotine Metabolites (E0692501)

Responsible Division: Biochemical Toxicology

Objective(s):

1) To determine the structural identity of the nicotine delta 1',2'- and delta 1',5'-iminium ion DNA adducts, and modify existing 32P-post labelling techniques to detect the adduct
2) To quantify the presence of these adducts *in vitro* and *in vivo* in mice

PI: Howard, Paul, Ph.D.

Immunogenicity of Permanent Make-Up Inks and Their Components (E0216101)

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Compound Nominated By: CFSAN

Objective(s):

To determine the immunogenicity of permanent make-up inks using a modified LNPA (lymph node proliferation assay) protocol.

PI: Howard, Paul, Ph.D.

Methodology for Safety Testing of Pigments Used for Tattooing, Including Permanent Make-Up (E0710501)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

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 make-up inks in SKH-1 hairless mice in the presence and absence of simulated-solar light
5) To determine if tattoo pigments are photococarcinogenic in the SKH-1 hairless mouse using simulated-solar light

PI: Howard, Paul, Ph.D.

Purification of Ceramide Synthase (E0705901)

Responsible Division: Biochemical Toxicology

Objective(s):

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

PI: Howard, Paul, Ph.D.

Skin Penetration, Phototoxicity, and Photocarcinogenicity of Nanoscale Oxides of Titanium and Zinc using Quantum Dot (QDOT) (E0215611)

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Compound Nominated By: CFSAN

Objective(s):

To investigate the penetration of QDOTSinto the skin of SKH-1 mice.

PI: Howard, Paul, Ph.D.

The Role of Fumonisin B1 in Fusarium sp. Tumorigenicity in Rats (E0211101)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Office of the Director, Microbiology

Collaborating FDA Center(s): CVM

Objective(s):

1) To determine the effect of fumonisin B1 on signal-transduction pathways in cultured human esophageal epithelial tissues
2) To determine if DNA damage occurs *in vivo* in F344 rats when fed in the diet cultures of Fusarium graminearum, Fusarium

subglutinans, Fusarium moniliforme or a combination of the three fungi, using 32P-postlabeling tech

3) To determine the pharmacokinetics of fumonisin B1 in $B_6C_3F_1$ mice and F344 rats under conditions similar to those used in the chronic bioassay, and in nonhuman primates

PI: Howard, Paul, Ph.D.

Tumorigenicity of Photoactive Nanoscale Titanium Dioxide in Tg.AC Transgenic Mice (E0215801)

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Compound Nominated By: CFSAN

Objective(s):

1) To determine if topical application of nanoscale TiO_2 results in penetration of the TiO_2 into the hair follicle of FVB/N mice
2) To determine the dose-response relationship of follicular penetration by TiO_2
3) To repeat the UVB doses used in the Trempus et al., 1998, study to confirm reproducibility of UVB dose that is photocarcinogenic
4) To dose mice with high dose of UVA to determine photocarcinogenicity

PI: Leakey, Julian, Ph.D.

Effect of Caloric Restriction on Rat Testicular Tumor Formation (E0260201)

Responsible Division: Office of Research

Collaborating Division(s): Personalized Nutrition and Medicine

Objective(s):

To understand the role of dietary components (i.e., caloric restriction) in influencing the ultimate susceptibility of the male reproductive tract to chemical insult.

PI: Leakey, Julian, Ph.D.

Studies of Usnic Acid and *Usnea Barbata* Herb in Fischer 344 Rats and $B_6C_3F_1$ Mice Range-Finding Only (E0215911)

External Funding:
National Toxicology Program (IAG)

Responsible Division: Office of Research

Collaborating FDA Center(s): CFSAN

Compound Nominated By: CFSAN

Objective(s):

To establish appropriate doses of usnic acid and *Usnea barbata* preparations administered in feed to male and female Fischer 344 rats and $B_6C_3F_1$ mice for use in subsequent subchronic and chronic studies.

PI: Leakey, Julian, Ph.D.

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

External Funding:
National Toxicology Program (IAG)

Responsible Division: Office of Research

Compound Nominated By: CFSAN

Objective(s):

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, 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(s): Biochemical Toxicology

Compound Nominated By: CDER

Objective(s):

To evaluate the potential toxicity and carcinogenicity of perinatal and chronic exposures to AIDS drugs, Zidovudine (AZT) and Lamivudine (3TC) in C57BL/6(N5)trp53 (+/-) haplodeficient F1 transgenic mice.

PI: Lewis, Sherry, Ph.D.

Dosing Methods for FVBp16/19(+/-) Neonatal Mice (P00694)

External Funding:
National Toxicology Program (IAG)

Responsible Division: Office of Research

Objective(s):

To optimize dosing methods for perinatal FVBp16/19(+/-) mice so that pup survival is maintained through the dosing period.

PI: Mckinzie, Page, 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

Collaborating Division(s): Personalized Nutrition and Medicine

Objective(s):

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

PI: Morris, Suzanne, Ph.D.

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

External Funding:
NIH/National Institute of Child Health and Human Development—Methylphenidate in Rhesus Monkey and Big Blue® Mice (IAG)

Responsible Division: Genetic and Reproductive Toxicology

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

Objective(s):

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, Ph.D.

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

External Funding:
NIH/National Institute of Child Health and Human Development — Methylphenidate in Rhesus Monkey and Big Blue® Mice (IAG)

Responsible Division: Genetic and Reproductive Toxicology

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

Objective(s):

1) To determine the metabolites of methylphenidate at early times after exposure in $B_6C_3F_1$ 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 $B_6C_3F_1$ mouse after 28 days of exposure
3) To determine the effect of exposure to methylphenidate on body and organ weights of the $B_6C_3F_1$ 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: Parsons, Barbara, Ph.D.

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

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Division(s): Biochemical Toxicology

Objective(s):

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: Parsons, Barbara, Ph.D.

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

External Funding:
CIIT Centers for Health Research (CRADA)

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Division(s): Neurotoxicology

Objective(s):
1) To further develop, evaluate, and disseminate a new NCTR method, Allele-specific competitive blocker-PCR (ACB-PCR)
2) To determine whether ACB-PCR measurements of specific oncogenic-base substitutions can be used to inform and improve the dose-response and mode-of-action assessments required in cancer risk assessment

PI: Paule, Merle, Ph.D.

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

External Funding:
Pfizer, Inc. (CRADA)

Responsible Division: Neurotoxicology

Objective(s):
1) To determine the acute dose-effect relationships of several psychotropic drugs on a battery of operant-behavioral tasks in rhesus monkeys
2) To characterize the relative sensitivities of the various behavioral end-points in NCTR's OTB to these agents
3) To compare the behavioral profiles of these agents to those of a variety of reference compounds with well-characterized mechanisms of action

PI: Pogribny, Igor, Ph.D.

Mechanisms and Consequences of DNA Damage and Methylation Dysregulation During Rat Hepatocarcinogenesis (E0712801)

Responsible Division: Biochemical Toxicology

Objective(s):
1) To confirm that the presence of uracil and abasic sites in preneoplastic DNA from folate/methyl deficient rats creates nonproductive high-affinity binding sites for the DNA methyltransferase that compromise normal DNA methylation at the replication fork resulting in genome-wide hypomethylation
2) To determine:
   a. whether the double-stranded loss of cytosine methylation is maintained in folate/methyl-deficient rats after nutritional repletion of methyl donors or whether the original methylation pattern and chromatin structure can be reestablished
   b. whether the increase in expression is stimulated by global loss of methyl groups and whether DNMT1 expression is decreased by methyl repletion
3) To determine the temporal relationship between the appearance of DNA lesions and site-specific methylation within the CpG (cytosine-phosphate-guanine) island of the p16 promoter region in p16 gene expression with alterations in local chromatin structure and DNA methyltransferase mRNA levels and activity
4) To use microarray slides printed with the rat cDNA library as a tool to screen for methylation-related down-regulation of candidate genes in hepatic preneoplastic foci, preneoplastic nodules, and tumor tissue from folate/methyl-deficient rats

PI: Shi, Leming, Ph.D.

The MicroArray Quality Control (MAQC) Project—An FDA-led Effort Toward Personalized Medicine (E0720701)

Responsible Division: Systems Toxicology

Collaborating Division(s): Z-Tech Corporation, Neurotoxicology, Office of the Director, Personalized Nutrition and Medicine

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

Objective(s):
1) To establish QC metrics and thresholds for objectively assessing the performance achievable by different microarray platforms and evaluating the merits and limitations of various data-analysis methods. The MAQC project will help improve the microarray technology and foster its proper applications

in discovery, development, and review of FDA-regulated products.

2) To identify multiple disease/study types and explore all training datasets
3) To develop a generic data-analysis protocol (DAP)
4) To review and approve the generic DAP
5) To apply the generic DAP to all training datasets
6) To report classifiers and performance metrics (internal validation) to Workgroup (WG) co-chairs: Many classifiers; one to be highlighted for "validation"
7) To distribute confirmatory (validation) datasets (microarray data only)
8) To report prediction results from all classifiers to WG co-chairs
9) To calculate classifier performance metrics (external validation)
10) To switch training and confirmatory datasets: repeat Steps 5-9
11) To conduct meta-analysis of the "matrix of performance metrics" to determine the relative impact of different factors on model performance
12) To develop guidance document(s)

PI: Sutherland, John, Ph.D.

Microbial Degradation of Fluoroquinolone Antimicrobial Agents (E0722701)

Responsible Division: Microbiology

Collaborating Division(s): Biochemical Toxicology

Objective(s):

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

PI: Tolleson, William, Ph.D.

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

Responsible Division: Biochemical Toxicology

Objective(s):

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.

Development of a Novel Class-Prediction Method, Decision Forest, for Analysis of Genomic and Proteomic Data (E0716901)

Responsible Division: Systems Toxicology

Collaborating Division(s): Personalized Nutrition and Medicine, Z-Tech Corporation

Objective(s):

1) To develop the two-class Decision Forest method. The method will be developed on several publicly available gene expression and SELDI-TOF datasets, and the results will be compared with others derived from traditional classification techniques
2) To develop the multiclass Decision Forest method. The method will be demonstrated on a gene-expression dataset to classify the pediatric acute lymphoblastic leukemia (ALL) subtypes

PI: Valentine, Carrie, Ph.D.

Creation of a Web-Based Database for Mutations Associated with Exon-Skipping (E0720101)

Responsible Division: Genetic and Reproductive Toxicology

Collaborating Division(s): Z-Tech Corporation

Objective(s):

To create and update a public web-based database with reported exonic mutations associated with exon loss. The database will be posted on a public website that is searchable by gene characteristics and will be monitored for use.

PI: Valentine, Carrie, Ph.D.

Identification of Target Sites for UVB Irradiation in Gene A of 0X174 Contained as a Transgene in Mouse Embryonic Cell PX-2 (E0710101)

Responsible Division: Genetic and Reproductive Toxicology

Objective(s):

1) To determine the dose-survival response of PX02 cells to UVB/UVA light to determine UV doses that optimize mutation induction and cell survival
2) To determine the induced mutant frequency in gene A of X174 by a forward mutation assay using cultures of PX2 exposed to UVB
3) To sequence the UVB/UVA-induced mutants from treated and untreated cultures to identify specific target sequences

PI: Valentine, Carrie, Ph.D.

UV-Induced Mutations in Mouse Epidermis Using Gene A of OX174: Proof of Principle (E0718701)

Responsible Division: Genetic and Reproductive Toxicology

Objective(s):

To establish that a UVB-induced dose response in mutant frequency of mouse epidermis can be detected by the forward assay for OX174 analyzed by single bursts.

PI: Wagner, Robert, Ph.D.

Maintenance of Defined Flora Associated BALB/c and TG26 Mice in Isolators for use in Future Protocols (E0727001)

Responsible Division: Microbiology

Objective(s):

To maintain a colony of defined-microbiota BALB/c and Tg26 mice in gnotobiotic isolators between approved protocols.

PI: Wang, Cheng, Ph.D.

Methods Development for High-Resolution Dedicated Positron Emission Tomography (microPET) to Rodent Neuroplasticity and Toxicity During Development (E0726401)

Responsible Division: Neurotoxicology

Collaborating Division(s): Bionetics Site Management, Office of the Director

Objective(s):

To minimize risks to children resulting from the use of anesthesia, it is necessary to understand the effects of anesthetic drugs on the developing nervous system. This study will utilize microPET to screen and evaluate *in vitro* and *in vivo* measurements from a broad range of pathophysiological or pharmacological parameters using specific tracers in the developing rat. Three different age groups of developing rats will be used: pregnant day-18 female rats, PND-7 rat pups and PND-35 rats. This study will also attempt to elucidate the relationship between apoptosis identifying ligands (specific tracers) and subsequent behavioral deficits.

NCTR Strategic Goal 3
Conduct research to strengthen our understanding of food safety and food defense

PI: Buzatu, Dan, 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

Objective(s):

To take advantage of the unique physical and electrical properties of nanotubes to develop:

a) novel technologies for the filtration of chemical and biological hazards from air, water, blood, and other media

b) technologies that protect public health or otherwise benefit the public

c) novel nanotube/monoclonal antibody-based cancer therapies

PI: Buzatu, Dan, Ph.D.

The Development of Rapid Spectral-Based Pathogen Identification Methods for Food Defense and Counter-Bioterrorism (E0714601)

Responsible Division: Systems Toxicology

Collaborating Division(s): Office of Commissioner/Office of Management/Office of Information Management

Objective(s):

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

PI: Khan, Ashraf, 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(s): ORA

Objective(s):

To characterize representative isolates of *Salmonella* and *Vibrio* spp. by molecular techniques, such as pulsed-field gel electrophoresis (PFGE), multilocus sequencing, ERIC, and REP-PCR methods. The results of this study will be used as a template for development of a diagnostic gene chip capable of simultaneous detection of multiple foodborne pathogens.

PI: Khan, Saeed, Ph.D.

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

External Funding:
USDA/FSIS/OFDER (IAG)

Responsible Division: Microbiology

Objective(s):

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 inactivation kinetics of spores of Sterne strain at different temperatures.

PI: Melchior, William, Ph.D.

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

Responsible Division: Biochemical Toxicology

Objective(s):

1) 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(s): Regulatory Compliance and Risk Management

Collaborating FDA Center(s): ORA

Objective(s):

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(s): Regulatory Compliance and Risk Management

Collaborating FDA Center(s): ORA

Objective(s):

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

PI: Nawaz, Mohamed, Ph.D.

The Fate and Degradation of Antimicrobials, Oxytetracycline (OTC), and Sulfadimethoxine-Ormetoprim (Romet-30®) from Aquaculture Environmental Samples (E0707501)

Responsible Division: Microbiology

Collaborating FDA Center(s): CVM

Objective(s):

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

PI: Nayak, Rajesh, Ph.D.

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

Responsible Division: Microbiology

Objective(s):

1) 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 fingerprinting the bacteria using the CDC's PulseNet pulsed-field gel electrophoresis (PFGE) protocol
3) To identify the genes in the multiple antibiotic region (MAR) of the Salmonella Genomic Island-class 1 integron gene cassettes in the resistant phenotypes
4) To detect antimicrobial-resistance genes in select multidrug-resistant Javiana isolates by a PCR-based and microarray biochip methodologies

PI: Nayak, Rajesh

Molecular Epidemiology and Characterization of Multiple Antibiotic-Resistant *Salmonella* Isolated from Turkey Production Environment (E0717301)

Responsible Division: Microbiology

Collaborating Division(s): Systems Toxicology

Objective(s):

1) To determine what source(s) of horizontal transmission are most closely related to the frequency and persistence of *Salmonella* detection in turkey flocks
2) To assess the genetic diversity and epidemiological profiles of *Salmonella* strains isolated in a turkey production environment
3) To evaluate the intrinsic resistances of *Salmonella* isolates to multiple antibiotics
4) To develop DNA-based and microarray assays to detect genes in *Salmonella* isolates that are involved in antibiotic resistance and pathogenicity

PI: Tolleson, William, Ph.D.

Detection of Staphylococcal Enterotoxin in Yogurt Products (P00692)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

To determine the steady-state levels of IL-2 mRNA and 18S rRNA in each cDNA sample using quantitative real-time PCR technology and compare the abundance of each RNA species using standard procedures used in other NCTR experiments.

PI: Tolleson, William, Ph.D.

Effect of Primary Yogurt Fermentation on the Cytotoxic Activity of the Bioterrorism Agents Ricin and Abrin (P00683)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

To test the applicability of ELISA and cell-based toxicity techniques developed previously to quantify residual ricin and abrin cytotoxic activity when added to yogurt fermentation cultures.

PI: Tolleson, William, Ph.D.

Thermal Stability of Ricin in Fruit Juice (P00682)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center(s): CFSAN

Objective(s):

To detect and quantify residual cytotoxic activity present in thermally treated ricin-contaminated fruit juice samples.

PI: Wagner, Robert, Ph.D.

Measurement of Antimicrobial Drug Concentrations that Inhibit Colonization Resistance (E0708601)

Responsible Division: Microbiology

Objective(s):

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

PI: Wagner, Robert, Ph.D.

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

Responsible Division: Microbiology

Objective(s):

1) 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 RT-PCR expression arrays to compare signal transduction in MALT from HMA mice treated with or without probiotic bacteria and orally challenged with *S. enterica*
3) 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 IEC-derived cytokines

PI: Wilkes, Jon, Ph.D.

Combining Metastable Atom Bombardment/Mass Spectrometry (MAB/MS) with Pattern Recognition to Subtype Bacteria for Food Safety and Food Defense (E0707901)

Responsible Division: Systems Toxicology

Collaborating Division(s): Microbiology

Collaborating FDA Center(s): CFSAN

Objective(s):

To demonstrate the validity of the combination of pyrolysis/metastable atom bombardment (MAB)/mass spectrometry (PyMAB/MS) with computerized pattern recognition (PattRec) for bacterial subtyping. The work should produce a scientifically and technologically validated

basis for commercial licensing of an NCTR-patented process: a method for assembling coherent spectral data bases for use in rapid chemotaxonomy at the strain and substrain level.

PI: Wilkes, Jon, Ph.D.

Innovative, Static, and Dynamic Chemical Sensors for Food Safety (E0719901)

External Funding:
Litmus, LLC (CRADA)

Responsible Division: Systems Toxicology

Objective(s):

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

PI: Wilkes, Jon, Ph.D.

Rapid Bacterial Identification with Subspecies-Level Specificity (E0714701)

Responsible Division: Systems Toxicology

Collaborating Division(s): Microbiology

Collaborating FDA Center(s): ORA

Objective(s):

To develop a complete instrumental/ computational system for rapid bacterial identification at the subspecies level and demonstrate its utility in simulated counterterror and food defense.

NCTR Strategic Goal 4

Modernize science management and infrastructure, and promote management expertise to effectively and efficiently support FDA/DHHS goals

PI: Tong, Weida, Ph.D.

Development of an FDA Resource for Improved Efficiency of Sex Difference-Related Pharmacogenomic Data Analysis and Review (E0727501)

External Funding:
Office of Women's Health

Responsible Division: Systems Toxicology

Objective(s):

To take advantage of the unique opportunity presented in VGDS and unique capability available from ArrayTrack™ to develop an FDA database populated with sex difference-related PGx information.

PI: Tong, Weida, Ph.D.

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

External Funding:
SAS Institutes, Inc. (CRADA)

Responsible Division: Systems Toxicology

Objective(s):

To develop modules in ArrayTrack™ that integrate the functionalities of ArrayTrack™ with SAS Scientific Discovery Solutions to provide the research community more comprehensive bioinformatics capabilities than each solution does alone.

PI: Delclos, Kenneth, Ph.D.

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

External Funding:
National Toxicology Program (IAG)

Responsible Division: Biochemical Toxicology

Collaborating Division(s): Office of Scientific Coordination

Objective(s):

1) To evaluate the effects of ethinyl estradiol, a potent synthetic estrogen widely used in prescription drugs, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats in the diet over multiple generations
2) To determine if subtle effects observed in the dose range-finding study are magnified through multiple generations
3) To evaluate the reversibility of any observed effects
4) To evaluate the chronic toxicity of ethinyl estradiol, particularly the potential induction of cancer of the reproductive organs, following exposures that will include various life stages

Results:

Ethinyl estradiol showed clear biological activity and potentially adverse effects at 10 and 50 ppb exposure concentrations in a low-phytoestrogen diet in NCTR CD (Sprague-Dawley) rats. Some statistically significant effects were also observed at 2 ppb, the lowest dose tested. Ethinyl estradiol clearly suppressed postweaning body weights in both males and females in the F_0 through F_2 generations where there was direct consumption of dosed feed until termination of the experiment. Reduced body weights were also observed in lower-dose groups (at 10 ppb for F_0 females and at 2 and 10 ppb for F_2 males). The weaning body weights were reduced in pups of both sexes in the 50 ppb dose groups of the F_1 through F_3 generations where there was continuous exposure to dosed feed from conception through weaning. Ethinyl estradiol accelerated the attainment of puberty in females under continuous exposure conditions (F_1 and F_2) and in animals where dosing was terminated at weaning (F_3) at 50 ppb. Perturbation of the estrous cycle (prolonged cycles, aberrant cycles, increased time in estrus) in young females after vaginal opening and prior to mating was observed in the 50 ppb dose groups of the F_1 and F_2 generations, with prolonged cycle time also seen at 2 and 10 ppb in the F_1 generation. In males, induction of male mammary hyperplasia and mild mineralization of renal tubules occurred at 50 ppb in the Fo through F_3 generations and F_1 and F_2 generations, respectively. In the continuously exposed F_1 and F_2 generations, increased male mammary hyperplasia was also observed in the 10 ppb (F_1 and F_2) and 2 ppb (F_1 only) dose groups. None of these effects appeared to carry over into unexposed generations. With the possible exception of a 1.5 day delay of preputial separation in the F_2 males, effects of ethinyl estradiol did not appear to be magnified across exposed generations, but rather for the most part appeared to be similar or reduced.

PI: Kodell, Ralph, Ph.D.

Dose-Response Modeling for Microbial Risk Assessment (E0704501)

Responsible Division: Personalized Nutrition and Medicine

Objective(s):

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

Results:

A study of eight mathematical dose-response models for microbial risk assessment was conducted using infectivity and illness data on a variety of microbial pathogem from published studies with human volunteers. The purpose was to evaluate variability among the models for human microbial dose-response data to determine whether two-parameter models might suffice for most microbial dose-response data or whether three-parameter models should generally be fitted. Model variability was

measured in terms of estimated ED_{01}s and ED_{10}s, with the view that these effective dose levels correspond to the lower and upper limits of the 1-10% risk range generally recommended for establishing benchmark doses in risk assessment. An investigation of the ranks of the ED_{01} and ED_{10} values among the models led to the conclusion that the two-parameter models captured at least as much uncertainty as the three-parameter models for the data examined. A further evaluation of the two-parameter models did not result in the selection of one "best" model, but it did provide some insights into the model's relative behavior. The model uncertainty analysis proposed by Kang et al. 81 using four two-parameter models was reinforced.

PI: Moon, Hojin, Ph.D.

Development of Improved Survival-Adjusted Tests for Animal Carcinogenicity/Tumorigenicity Data (E0717101)

Responsible Division: Personalized Nutrition and Medicine

Objective(s):

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

Results:

The assumption of an asymptotic normal distribution of some test statistics may be invalid in certain dose-response trend tests. For instance, the survival-adjusted Cochran-Armitage test, known as the Poly-k test, is asymptotically standard normal under the null hypothesis. However, the asymptotic normality is not valid if there is a deviation from the tumour onset distribution that is assumed in this test or if the competing-risks survival rates differ across groups. We develop an age-adjusted bootstrap-based method to assess the significance of assumed asymptotic normal tests for animal carcinogenicity data. The proposed method differs from conventional bootstrap methods in the aspect of preserving the mortality rate in each dose group under the null hypothesis of equal tumour incidence rates among the groups. We investigate an empirical distribution of the Poly-3 (P3) trend test statistic using the proposed age-adjusted bootstrap-based method and compare it with the P3 test statistic referenced to the assumed standard normal distribution. A simulation study is conducted to evaluate the robustness of these tests to various Weibull-family tumour onset distributions. The proposed method is applied to National Toxicology Program datasets to evaluate a dose-related trend of a test substance on the incidence of neoplasms.

PI: Turturro, Angelo, Ph.D.

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

Responsible Division: Personalized Nutrition and Medicine

Objective(s):

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

Results:

This project has developed a dynamic model based on both susceptibility and exposure subpopulations that tracks the course of epidemics and identifies the major subpopulation reservoirs for their continuation. Interestingly, this project has also explained some phenomena that appear

counter-intuitive. For instance, in Milwaukee, the existence of a peak of response at 30 years of age to C. parvum in the age distribution of response is not explicable based on any known model. Using this analysis, the explanation falls out quickly that this is the manifestation of the secondary transmission of infection to the age groups most likely to be caring for their children, who are especially susceptible to the parasite. Also, their exposure is likely to be massive, overwhelming any refractoriness due to prior exposure. Given the fairly low primary exposure in Milwaukee, it is impossible to generate an epidemic in adults unless some fairly drastic assumptions are made in the face of the evidence, albeit incomplete, that exists. Previous analyses have required such heroic mechanisms as the contamination of Lake Michigan by wastes that were somehow untreated or simply averring that the dose must have been underestimated by orders of magnitude. Using this analysis, it quickly becomes clear that an especially sensitive subpopulation, the children, was a major factor driving the epidemic.

There are warning flags that have arisen as a result of this analysis. One is the extreme sensitivity to infection with C. parvum given corticosteroids. Another is the unexpectedly strong effect of all forms of food restriction, something especially important for the Third World and perhaps to dieting and poor Americans. Finally there are potential effects on pregnant women, with unknown effects on their fetuses. Each of these areas should be explored in a public-health context.

The methodology used here can, with some modifications for specific transmission factors, be used across a wide variety of agents.

PI: Young, John, Ph.D.

Bio-Preg to Windows 2000 Upgrade (E0713001)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Office of Commissioner/Office of Management/Office of Information Management

Objective(s):

To upgrade Bio-Preg to a Windows-based program which will be called Win-Preg.

Results:

A physiologically based pharmacokinetic (PBPK) model and program (called PostNatal) was developed, which focuses on postnatal growth from birth through adulthood, using appropriate growth curves for each species and gender. The model also includes linkages for the simulation of pharmacodynamic (PD) effects. Postnatal growth equations for organs/tissues and total weight for male and female humans, dogs, rats, and mice are an integral part of the software and are utilized to calculate the appropriate weight and blood flow for each organ/tissue. This Windows®-based program is actually four PBPK models in one with each PBPK model acting independently or totally integrated with the others through metabolism by first order or Michaelis-Menten kinetics. Dosing may be by intravenous (IV), ingestion, dermal, intraperitoneal (IP), intramuscular (IM), subcutaneous, inhalation, or infision; 20 doses per input route are allowed. Elimination may be through the feces, urine, and/or hair. Data fitting is by a weighted least square-regression algorithm. Special features include elimination interactions, enterohepatic recirculation, and a copy link attribute.

PI: Young, John, Ph.D.

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

Responsible Division: Personalized Nutrition and Medicine

Collaborating Division(s): Systems Toxicology, Z-Tech Corporation

Objective(s):

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

Results:

Standard classification algorithms are generally designed to maximize the number of correct predictions (concordance). The criterion of maximizing the concordance may not be appropriate in certain applications. In practice, some applications may emphasize high sensitivity (e.g., clinical diagnostic tests) and others may emphasize high specificity (e.g., epidemiology screening studies). This project considers effects of the decision threshold on sensitivity, specificity, and concordance for four classification methods: logistic regression, classification

tree, Fisher's linear discriminant analysis, and a weighted k-nearest neighbor. We investigated the use of decision threshold adjustment to improve performance of either sensitivity or specificity of a classifier under specific conditions. We conducted a Monte Carlo simulation showing that as the decision threshold increases, the sensitivity decreases and the specificity increases; but, the concordance values in an interval around the maximum concordance are similar. For specified sensitivity and specificity levels, an optimal decision threshold might be determined in an interval around the maximum concordance that meets the specified requirement.

FY 2007 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 2007**.

1. Adjei, M.D., Deck, J., Heinze, T.M., Freeman, J.P., Williams, A.J., and Sutherland, J.B., (2007), Identification of metabolites produced from N-phenylpiperazine by *Mycobacterium* spp., *Journal of Industrial Microbiology and Biotechnology*, 34(3):219-224.
   Responsible Division: Microbiology
   Collaborating Division(s): Biochemical Toxicology

2. Ahn, H., Moon, H., Fazzari, M.J., Lim, N., Chen, J.J., and Kodell, R.L., (2007), Classification by ensembles from random partitions for high-dimensional data, *Computational Statistics and Data Analysis*, 51(12):6166-6179.
   Responsible Division: Personalized Nutrition and Medicine

3. Arlt, V.M., Glatt, H., Gamboa Da Costa, G., Reynisson, J., Takamura-Enya, T., and Phillips, D.H., (2007), Mutagenicity and DNA adduct formation by the urban air pollutant 2-nitrobenzanthrone, *Toxicological Sciences*, 98(2):445-457.
   Responsible Division: Biochemical Toxicology
   Collaborating Division(s): Genetic and Reproductive Toxicology

4. Bagnyukova, T.V. and Lushchak, V.I., (2007), Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23° C, *Journal of Thermal Biology*, 32(4):227-234.
   Responsible Division: Biochemical Toxicology

5. Bagnyukova, T.V., Luzhna, L.I., Pogribny, I.P., and Lushchak, V.I., (2007), Oxidative stress and antioxidant defenses in goldfish liver in response to short-term exposure to arsenite, *Environmental Molecular Mutagenesis*, 48:658-665.
   Responsible Division: Biochemical Toxicology

6. Bagnyukova, T.V., Pogribny, I.P., and Chekhun, V.F., (2006), MicroRNAs in normal and cancer cells: a new class of gene expression regulators, *Experimental Oncology*, 28(4):263-269.
   Responsible Division: Biochemical Toxicology

7. Bagnyukova, T.V., Danyliv, S.I., Zin'ko, O.S., and Lushchak, V.I., (2007), Heat shock induces oxidative stress in rotan *Perccottus glenii* tissues, *Journal of Thermal Biology*, 32(5):255-260.
   Responsible Division: Biochemical Toxicology

8. Beger, R.D., (2007), Cambridge Healthtech Institute's 7th annual identifying and validating metabolic markers for drug development and clinical studies, *Expert Review of Molecular Diagnostics*, 7(2):113-115.
   Responsible Division: Systems Toxicology

9. Bendre, S.V., Shaddock, J.G., Dobrovolsky, V.N., Albertini, R.J., and Heflich, R.H., (2007), Effect of chronic azathioprine treatment on germ-line transmission of *Hprt* mutation in mice, *Environmental and Molecular Mutagenesis*, 48(9):744-753.
   Responsible Division: Genetic and Reproductive Toxicology

10. Binienda, Z.K., Przybyla-zawislak, B.D., Robinson, B.L., Salem, N., Virmani, A., Amato, A., and Ali, S.F., (2006), Effects of L-carnitine pretreatment in methamphetamine and 3-nitropropionic acid-induced neurotoxicity, *Annals of the New York Academy of Sciences*, 1074:74-83.
Responsible Division: Neurotoxicology
Collaborating Division(s): Toxicologic Pathology Associates

11. Bowyer, J.F., Pogge, A., Delongchamp, R.R., O'Callaghan, J.P., Patel, K.M., Vrana, K.E., and Freeman, W.M., (2006), A threshold neurotoxic amphetamine exposure inhibits parietal cortex expression of synaptic plasticity-related genes, *Neuroscience*, 144(1):66-76.
Responsible Division: Neurotoxicology
Collaborating Division(s): Personalized Nutrition and Medicine

12. Boyko, A., Kathiria, P., Zemp, F.J., Yao, Y., Pogribny, I.P., and Kovalchuk, I., (2007), Transgenerational changes in the genome stability and methylation in pathogen-infected plants: (virus-induced plant genome instability), *Nucleic Acids Research*, 35:1714-1725.
Responsible Division: Biochemical Toxicology

13. Branham, W.S., Melvin, C., Han, T., Desai, V.G., Moland, C.L., Scully, A., and Fuscoe, J., (2007), Elimination of laboratory ozone leads to a dramatic improvement in the reproducibility of microarray gene expression measurements, *BMC Biotechnology*, 7(8):10.1186/1472.
Responsible Division: Systems Toxicology
Collaborating Division(s): FDA/Office of Real Property Services

14. Cederroth, C.R., Vinciguerra, M., Kuhne, F., Madani, R., Doerge, D.R., Visser, T.J., Foti, M., Rohner-Jeanrenaud, F., Vassalli, J., and Nef, S., (2007), A phytoestrogen-rich diet increases energy expenditure and decreases adiposity in mice, *Environmental Health Perspectives*, 115(10):1467-1473.
Responsible Division: Biochemical Toxicology

15. Cerniglia, C.E. and Sutherland, J.B., Fungal metabolism of polycyclic aromatic hydrocarbons In *Microbial Degradation of Aromatic Compounds, 2nd edition,* (J. Kukor and G. Zylstra), Marcel Dekker, Inc, Book Chapter.
Responsible Division: Microbiology

16. Chan, P.C., Xia, Q., and Fu, P.P., (2007), Ginkgo biloba leave extract: biological, medicinal, and toxicological effects, *Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis and Ecotoxicology Reviews*, 25(3):211-244.
Responsible Division: Biochemical Toxicology

17. Chekhun, V.F., Lukyanova, N.Y., Kovalchuk, O., Tryndyak, V.P., and Pogribny, I.P., (2007), Epigenetic profiling of multidrug-resistant human MCF-7 breast adenocarinoma cells reveals novel hyper- and hypmethylated targets, *Molecular Cancer Therapeutics*, 6:1089-1098.
Responsible Division: Biochemical Toxicology

18. Chen, C., Tsai, C., Tzeng, S., and Chen, J.J., (2007), Gene selection with multiple ordering criteria, *BMC Bioinformatics*, 8(74).
Responsible Division: Personalized Nutrition and Medicine

19. Chen, D.T., Chen, J.J., Cheng, G., Shi, L.H., and Soong, S.J, (2007), A two-stage binomial test approach of gene identification in oligonucleotide arrays, *Journal of Biopharmaceutical Statistics*, 17(5):903-918.
Responsible Division: Personalized Nutrition and Medicine
Collaborating Division(s): Systems Toxicology

20. Chen, H.C., Xia, Q., Cherng, S.H., Chen, S., Lai, C.C., Yu, H., and Fu, P.P., (2007), Synthesis and photoirradiation of isomeric ethylchrysenes by UVA light leading to lipid peroxidation, *International Journal of Environmental Research and Public Health*, 4(2):145-152.
Responsible Division: Biochemical Toxicology

21. Chen, J.J., (2007), Key aspects of analyzing microarray gene expression data, *Pharmacogenomics*, 8(5):473-482.
Responsible Division: Personalized Nutrition and Medicine

22. Chen, J.J., Lee, T., Delongchamp, R.R., Chen, T., and Tsai, C., (2007), Significance analysis of groups of genes in expression profiling studies, *Bioinformatics*, 23(16):2104-2112.
Responsible Division: Personalized Nutrition and Medicine
Collaborating Division(s): Genetic and Reproductive Toxicology

23. Chen, J.J., Moon, H., and Baek, S., (2007), Tree-based ensemble classifiers for high-dimensional data, *Journal of the Chinese Statistical Association*, 45:1-17.
Responsible Division: Personalized Nutrition and Medicine

24. Chen, J.J., Moon, H., and Kodell, R.L., (2007), A probabilistic framework for noncancer risk assessment, *Regulatory Toxicology and Pharmacology*, 48:45-50.
Responsible Division: Personalized Nutrition and Medicine

25. Chen, J.J., Statistics for environmental teratogenesis In *Encyclopedia of Quantitative Risk Assessment*, Book Chapter.
Responsible Division: Personalized Nutrition and Medicine

26. Chen, J.J., Wang, S.J., Tsai, C.A., and Lin, C.J., (2007), Selection of differentially expressed genes in microarray data analysis, *The Pharmacogenomics Journal*, 7(3):212-220.
Responsible Division: Personalized Nutrition and Medicine

27. Chilakapati, J., Korrapati, M.C., Hill, R.A., Warbritton, A, Latendresse, J.R., and Mehendale, H.M., (2007), Role of thioacetamide sulfoxide in saturation of toxicokinetics of thioacetamide, *Toxicology,* 230:105-116.
Responsible Division: Toxicological Pathology Associates

28. Chilakapati, J., Korrapati, M.C., Shankar, K., Hill, R.A., Warbritton, A., Latendresse, J.R., and Mehendale, H.M., (2007), Role of CYP2E1 and saturation kinetics in the bioactivation of thioacetamide: Effects of diet restriction and phenobarbital, *Toxicology and Applied Pharmacology,* 219(1):72-84.
Responsible Division: Toxicological Pathology Associates

29. Dearfield, K.L. and Fuscoe, J., (2007), Impact of genomic technologies on regulatory policies—presentations at the 37th annual meeting of the Environmental Mutagen Society, *Environmental Molecular Mutagenesis*, 48(5):347-348.
Responsible Division: Systems Toxicology

30. Dervishi, E., Biris, A.S., Biris, A.R., Lupu, D., Trigwell, S., Miller, D.W., Schmitt, T.C., Buzatu, D.A., and Wilkes, J.G., (2006), Transparent and electrically conductive carbon nanotube-polymer nanocomposite materials for electrostatic charge dissipation, *Proceeding of ESA/IEJ/IEEE-IAS/SFE Joint Conference on Electrostatics 2006*, 636-646.
Responsible Division: Systems Toxicology
Collaborating Division(s): Systems Toxicology, Genetic and Reproductive Toxicology

31. Desai, V.G., Lee, T., Delongchamp, R.R., Moland, C.L., Branham, W.S., Fuscoe, J.C., and Leakey, J.E., (2007), Development of mitochondria-specific mouse oligonucleotide microarray and validation of data by real-time PCR, *Mitochondrion*, 7(5):322-329.
Responsible Division: Systems Toxicology
Collaborating Division(s): Office of Research, Personalized Nutrition and Medicine

32. Divi, R.L., Doerge, D.R., Twaddle, N.C., Shockley, M.E., St.Clare, M.C., Harbaugh, J.W., Harbaugh, S.W., and Poirier, M.C., (2008), Metabolism and pharmacokinetics of the combination Zidovudine plus Lamivudine in the adult Erythrocebus patas monkey determined by liquid chromatography-tandem mass spectrometric analysis, *Toxicology and Applied Pharmacology*, 226:206-211.
Responsible Division: Biochemical Toxicology

33. Dobrovolsky, V.N. and Heflich, R.H., (2007), On the use of the T-REx tetracycline-inducible gene expression system *in vivo*, *Biotechnology and Bioengineering*, 98(3):719-723.
Responsible Division: Genetic and Reproductive Toxicology

34. Dobrovolsky, V.N., Shaddock, J.G., Mittelstaedt, R.A., Bishop, M.E., Lewis, S.M., Lee, F.W., Aidoo, A., Leakey, J.E., Dunnick, J.K., and Heflich, R.H., (2007), Frequency of *Hprt* mutant lymphocytes and micronucleated erythrocytes in p53-haplodeficient mice treated perinatally with AZT and AZT in combination with 3TC, *Environmental and Molecular Mutagenesis*, 48(3-4):270-282.
Responsible Division: Genetic and Reproductive Toxicology
Collaborating Division(s): Office of Research

35. Doerge, D.R., Twaddle, N.C., Boettcher, M.I., McDaniel, L.P., and Angerer, J., (2007), Urinary excretion of acrylamide and metabolites in Fischer 344 rats and B6C3F(1) mice administered a single dose of acrylamide, *Toxicology Letters*, 169(1):34-42.
Responsible Division: Biochemical Toxicology

36. Donthamsetty, S., Bhave, V.S., Mitra, M.S., Latendresse, J.R., and Mehendale, H.M., (2007), Nonalcoholic fatty liver sensitizes rats to carbon tetrachloride hepatotoxicity, *Hepatology,* 45(2):391-403.
Responsible Division: Toxicological Pathology Associates

37. Duffy, P.H., Lewis, S.M., Mayhugh, M.A., Trotter, R.W., Hass, B.S., Latendresse, J.R., Thorn, B.T., Tobin, G., and Feuers, R.J., (2008), Neoplastic pathology in male Sprague-Dawley rats fed the AIN-93M purified diet *ad libitum* or at restricted intakes, *Nutrition Research*, 28(1):36-42.
Responsible Division: Office of Research
Collaborating Division(s): Genetic and Reproductive Toxicology

38. Elkins, C.A. and Mullis, L.B., (2007), Substrate competition studies using whole cell accumulation assays with the major tripartite multidrug efflux pumps of *Escherichia coli*, *Antimicrobial Agents and Chemotherapy*, 51(3):923-929.
Responsible Division: Microbiology

39. Esperandi, P., Zhang, J., Rosenzweig, B.A., Vaidya, V.S., Sun, J., Schnackenberg, L., Herman, E.H., Knapton, A., Bonventre, J.V., Beger, R.D., Thompson, K.L., and Hanig, J., (2007), The utility of a rodent model in detecting pediatric drug-induced nephrotoxicity, *Toxicological Sciences*, 99(2):637-648.
Responsible Division: Systems Toxicology
Collaborating Division(s): Toxicologic Pathology Associates

40. Esperandi, P., Zhang, J., Schnackenberg, L., Miller, T.J., Knapton, A., Herman, E.H., Beger, R.D., and Hanig, J.P., (2007), Age-related differences in susceptibility to toxic effects of valproic acid in rats, *Journal of Applied Toxicology*.
Responsible Division: Systems Toxicology

41. Evans, R. and Siitonen, P.H., (2008), Determination of caffeine and sympathomimetic alkaloids in weight loss supplements by high performance liquid chromatography, *Journal of Chromatographic Science*, 46(1):61-67
Responsible Division: Biochemical Toxicology

42. Fang, H., Fan, X., Guo, L., Shi, L., Perkins, R.G., Ge, W., Dragan, Y., and Tong, W., (2007), Self-self hybridization as an alternative experiment design to dye swap for two-color microarrays, *Omics: A Journal of Integrative Biology*, 11(1):14-24.
Responsible Division: Systems Toxicology
Collaborating Division(s): Z-Tech Corporation

43. Ferguson, S.A. and Berry, K.J., (2007), Oral Accutane (13-cis-retinoic acid) has no effects on spatial learning and memory in male and female Sprague-Dawley rats, *Neurotoxicology and Teratology*, 29(2):219-227.
Responsible Division: Neurotoxicology

44. Foley, S.L., Lynne, A.M., and Nayak, R., (2008), Salmonella challenges: prevalence in swine and poultry and potential pathogenicity of such isolates, *Journal of Animal Science*, 86(14 Suppl).
Responsible Division: Microbiology

45. Fostel, J.M., Burgoon, L., Zwickl, C., Lord, P., Corton, J., Bushel, P.R., Cunningham, M., Fan, L., Edwards, S.W., Hester, S., Stevens, J., Tong, W., Waters, M., Yang, C., and Tennant, R., (2007), Towards a checklist for exchange and interpretation of data from a toxicology study, *Toxicological Sciences*, 99(1):26-34.
Responsible Division: Systems Toxicology

46. Fu, P.P., Xia, Q., Boudreau, M.D., Howard, P.C., Tolleson, W.H., and Wamer, W., (2007), Physiological role of retinyl palmitate in the skin In *Vitamin & Hormones,* 75: 223-256, Book Chapter.
Responsible Division: Biochemical Toxicology

47. Fu, P.P., Xia, Q., Yin, J.J., Cherng, S.H., Yan, J., Mei, N., Chen, T., Boudreau, M.D., Howard, P.C., and Wamer, W., (2007), Photodecomposition of vitamin A and photobiological implications for the skin, *Photochemistry and Photobiology*, 83(2):409-424.
Responsible Division: Biochemical Toxicology
Collaborating Division(s): Genetic and Reproductive Toxicology

48. Fuscoe, J., Tong, W., and Shi, L., (2007), QA/QC issues to aid regulatory acceptance of microarray gene expression data, *Environmental and Molecular Mutagenesis*, 48(5):349-353.
Responsible Division: Systems Toxicology

49. Gamboa Da Costa, G., Marques, M.M., Fu, X., Churchwell, M.I., Wang, Y., Doerge, D.R., and Beland, F.A., (2007), Effect of N,N-didesmethyltamoxifen upon DNA adduct formation by tamoxifen and alpha-hydroxytamoxifen, *Cancer Letters*, 257(2):191-198.
Responsible Division: Biochemical Toxicology

50. Gamboa Da Costa, G., Pereira, P.C., Churchwell, M.I., Beland, F.A., and Marques, M.M., (2007), DNA adduct formation in the livers of female Sprague-Dawley rats treated with toremifene or alpha-hydroxytoremifene, *Chemical Research in Toxicology*, 20(2):300-310.
Responsible Division: Biochemical Toxicology

51. Garey, J. and Paule, M.G., (2007), Effects of chronic low-dose acrylamide exposure on progressive ratio performance in adolescent rats, *Neurotoxicology*, 28(5):998-1002.
Responsible Division: Neurotoxicology

52. Gopee, N.V. and Howard, P.C., (2007), A time course study demonstrating RNA stability in postmortem skin, *Experimental and Molecular Pathology*, 83(1):4-10.
Responsible Division: Biochemical Toxicology

53. Gopee, N.V., Roberts, D.W., Webb, P., Cozart, C.R., Siitonen, P.H., Warbritton, A.R., Yu, W.W., Colvin, V.L., Walker, N.J., and Howard, P.C., (2007), Migration of intradermally injected quantum dots to sentinel organs in mice, *Toxicological Sciences*, 98(1):249-257.
Responsible Division: Biochemical Toxicology
Collaborating Division(s): Toxicologic Pathology Associates

54. Govindarajan, R., Ratnasinghe, L., Simmons, D.L., Siegel, E.R., Midathada, M.V., Kim, L., Kim, P.J., Owens, R.J., and Lang, N.P., (2007), Thiazolidinediones and the risk of lung, prostate, and colon cancer in patients with diabetes, *Journal of Clinical Oncology*, 25(12):1476-1481.
Responsible Division: Personalized Nutrition and Medicine

55. Guo, L., Mei, N., Dial, S., Fuscoe, J., and Chen, T., (2007), Comparison of gene-expression profiles altered by comfrey and riddelliine in rat liver, *BMC Bioinformatics*, 8 Suppl 7:S22.
Responsible Division: Genetic and Reproductive Toxicology
Collaborating Division(s): Systems Toxicology

56. Haefele, A.R., Word, B.R., Xiao, Y., Hammons, G.J., and Lyn-Cook, B.A., (2007), Indole-3-Carbinol (I3C) modulates expression of DNA Methyltransferases 1, 3a, and 3b in pancreatic cancer cells: effects of gender and a novel (C→T) polymorphism in the promoter region of DNMT 3b, *International Journal of Cancer Prevention*, 2(4):245-255.
Responsible Division: Personalized Nutrition and Medicine

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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
3TC	lamivudine
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care, International
*aadA*1	aminoglycoside adenyltransferase
AALAS	American Association for Laboratory Animal Science
ACB-PCR	allele competitive blocker-polymerase chain reaction
ACLAM	American College of Laboratory Animal Medicine
ACP-PCR	annealing control primer–polymerase chain reaction
ADHD	Attention Deficit Hyperactivity Disorder
AIDS	acquired immunodeficiency syndrome
ALL	acute lymphoblastic leukemia
AMPH	amphetamine
ASCP	American Society for Clinical Pathology
ATP	adenosine 5'-triphosphate
AZT	zidovudine or azidothymidine
$B_6C_3F_1$	mouse strain
CBER	Center for Biologics Evaluation and Research, FDA
CBPR	community-based participatory research
CDER	Center for Drug Evaluation and Research, FDA
cDNA	complementary DNA
CDRH	Center for Devices and Radiological Health, FDA
CFSAN	Center for Food Safety and Applied Nutrition, FDA
CMAR	Certified Managers of Animal Resources
CNS	central nervous system
CRADA	Cooperative Research and Development Agreement
CVM	Center for Veterinary Medicine, FDA
CYP	cytochrome
DBS	deep brain stimulation
ddC	zalcitabane
DEHP	di-(2-ethylhexyl)phthalate
DGRT	Division of Genetic and Reproductive Toxicology
DHHS	Department of Health and Human Services
DHP	6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine

Acronym/ Abbreviation	Name
DNMT	DNA methyltransferase
DPNM	Division of Personalized Nutrition and Medicine
DS	Down Syndrome
DVS	Division of Veterinary Services
EDTA	ethylene-diamine-tetra-acetic acid
ENU	ethylnitrosourea
EPA	Environmental Protection Agency
ERIC	enterobacterial repetitive intergenic consensus
F344	strain of rats
FDA	Food and Drug Administration
FDA Centers	Center for Biologics Evaluation and Research (CBER) Center for Devices and Radiological Health (CDRH) Center for Drug Evaluation and Research (CDER) Center for Food Safety and Applied Nutrition (CFSAN) Center for Veterinary Medicine (CVM) National Center for Toxicological Research (NCTR) Office of Regulatory Affairs (ORA)
FMN	flavin mononucleotide
FY	fiscal year
GABA	gamma-aminobutyric acid
GC-MS	gas chromatography-mass spectrometry
GD	gestational day
GGT	guanine guanine thymidine
GI	gastrointestinal
GST	glutathione S-transferase
GTT	guanine thymidine thymidine
HIV	human immunodeficiency virus
HMA	human microbiota-associated
HPLC	high-performance liquid chromatography
hprt	hypoxanthine guanine phosophoribosyl transferase
IACUC	Institutional Animal Care and Use Committee
IAG	Interagency Agreement
IM	intramuscular
in silico	modeled on a computer
in situ	in place; localized and confined to one area
in utero	in the womb
in vitro	in animal models
in vivo	in cell cultures
IP	intraperitoneal
IV	intravenous
LC/MS	liquid chromatography-mass spectrometry
LCM	laser capture microdissection
LIMS	laboratory information management system

Acronym/ Abbreviation	Name
LPNA	lymph node proliferation assay
MAB/MS	metastable atom bombardment/mass spectrometry
MAQC	MicroArray Quality Control
MALT	mucosal-associated lymphoid tissues
MIC	minimum inhibitory concentration
MLA	Mouse Lymphoma Assay
Mn	manganese
MPP+	1-methyl-4-phenylpyridinium
MPTP	1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine
MRI	magnetic resonance imaging
mRNA	messenger RNA
MRS	magnetic resonance spectroscopy
MS	mass spectrometry
MSI	Metabolomics Standards Initiative
NCFST	National Center for Food Safety and Technology
NCI	National Cancer Institute
NCTR	National Center for Toxicological Research, FDA
NICHD	National Institute of Child Health and Human Development
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIST	National Institute of Standards and Technology
NMDA	n-methyl-d-aspartate
NMR	nuclear magnetic resonance
NP	*p*-nonylphenol
NPP	N-phenylpiperazine
NTP	National Toxicology Program
ORA	Office of Regulatory Affairs, FDA
OTA	ochratoxin A
OTB	operant test battery
OTC	oxytetracycline
OWH	Office of Women's Health, FDA
PAH	polycyclic aromatic hydrocarbon
PattRec	pattern recognition
PBPK	physiologically based pharmacokinetic
PCR	polymerase chain reaction
PD	Parkinson's Disease
PET	positive emission tomography
PFGE	pulse-field gel electrophoresis
PhIP	2-amino-1-methyl-6-phynelimidazo[4,5-f]pyridine
PI	Principal Investigator
PPAR	peroxisome proliferator-activated receptors
ppb	parts per billion

Acronym/ Abbreviation	Name
QA	quality assurance
QC	quality control
RNA	ribonucleic acid
Romet-30®	sulfadimethoxine-ormetoprim (SDO)
ROS	reactive oxygen species
RT-PCR	reverse transcriptase-polymerase chain reaction
SAB	Science Advisory Board
SAGE	Serial Analysis of Gene Expression
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SDO	Romet-30® (sulfadimethoxine-ormetoprim)
SELDI	surface-enhanced laser desorption/ionization
SKH-1	species of mouse
SLE	systemic lupus erythematosus (lupus)
SNP	single nucleotide polymorphism
SSL	simulated-solar light
SULT1A1	sulfotransferase 1A1, a human gene
TiO_2	titanium dioxide
TOF	time of flight
TSST-1	toxic shock syndrome toxin-1
USDA	United States Department of Agriculture
USDA/FSIS/OFDER	United States Department of Agriculture/Food Safety and Inspection Service/Office of Food Defense and Emergency Response
UV, UVA, or UVB	ultraviolet (A or B indicates the region)
VGDS	Voluntary Genomic Data Submission

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Index of Key Terms

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