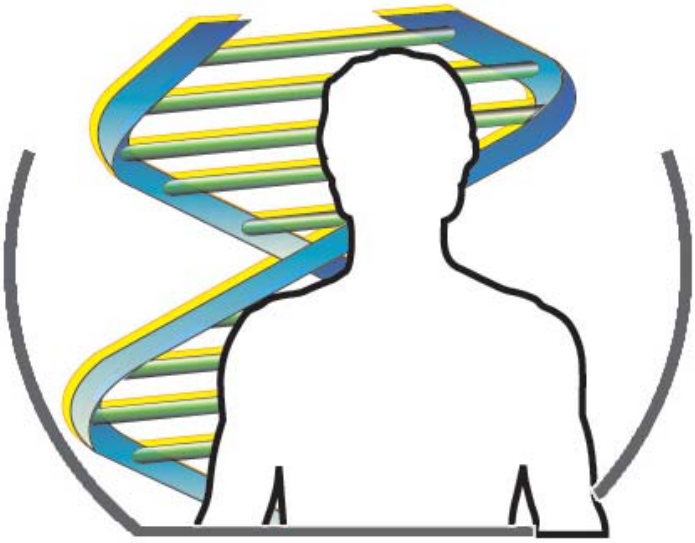
National Center for Toxicological Research



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Preface

The National Center for Toxicological Research (NCTR) is an important research component of the U.S. Food and Drug Administration (FDA) that plays a critical role in the FDA's and the Department of Health and Human Services' (DHHS) mission to promote and protect public health. 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 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 existing strengths in methods development, statistics, analytical chemistry, and spectroscopy, NCTR is developing and standardizing new technologies, such as genomics, proteomics, metabonomics, and nanotechnology to identify and characterize early biomarkers of toxicity 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. 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

NCTR: An Internationally Recognized Resource

NCTR has the staff, the facilities, and the research tools to conduct innovative, integrative research, that is used to guide and support regulatory decisions.

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.

Research Structure

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 (listed below) work together in a seamless effort to support the 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.

- 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

New Technologies and Integrative Approaches to Product Safety and Efficacy

A major focus of NCTR research involves the development of new and innovative technologies and approaches that support the FDA Regulatory Centers and, in particular, the FDA's Critical Path Initiative – Science Enhancing the Health and Well-Being of All Americans – "FDA's effort to stimulate and facilitate a national effort to modernize the sciences through which FDA-regulated products are developed, evaluated, and manufactured."[1]

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

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

NCTR scientists play an integral role in developing, standardizing, and integrating microarray and other omics data. They have developed ArrayTrack™, an integrated solution for managing, analyzing, visualizing, and interpreting microarray data currently used by FDA regulatory Center scientists as well as academics and industry scientists. ArrayTrack™ is under continuous development and is currently being enhanced to accept whole-genome, gene expression, proteomic, metabolomics, clinical, and nonclinical data and has been integrated into many commercial tools. Through this integration, these tools are freely available to FDA scientists and reviewers. NCTR scientists were essential partners in the initial Voluntary Genomic Data Submission (VGDS) Program with training for reviewers, analysis of submitted data, and formal discussions between the FDA review centers, NCTR, and pharmaceutical sponsors. NCTR staff have been involved in other aspects of standardization of microarray technology. Specifically, the MicroArray Quality Control (MAQC) project catapulted NCTR/FDA into front-line discussions and contributions toward standardization of microarray use. MAQC 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, and showed that current microarray technology provides the reliability and consistency necessary for regulatory submissions. With the completion of Phase I and the publication of the results in *Nature Biotechnology*, MAQC Phase II focuses on developing consensus on predictive signatures. The existence of a consensus-building consortium under the leadership of

[1] http://www.fda.gov/oc/initiatives/criticalpath/initiative.html

NCTR/FDA, positions FDA to play a defining role in the integration of omics data into review processes.

Research Leading to Personalized Medicine

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

NCTR research on classification algorithms provides the fundamental basis for making personalized medicine a reality by enabling the assignment of therapies to patients to maximize efficacy and minimize toxicity. These algorithms can combine diverse, high-dimensional biomarkers to achieve increased accuracy in predicting which patients will benefit most from medical products and which are most likely to experience toxicities that might outweigh treatment benefits. Research on probabilistic techniques that minimize uncertainty in risk assessment provides risk managers tools for ensuring adequate public health protection, including protection of sensitive populations. Research on physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models is providing software that enables risk assessors and managers to make scientifically sound decisions based on data from animal studies to characterize the risk to humans of FDA-regulated products. NCTR has developed a Windows-based general purpose PBPK/PD modeling software that can simulate pharmacokinetic data during postnatal growth in mice, rats, dogs, and humans.

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

Key Research for High-Priority Safety Issues

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

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

complex brain function, now known widely as the NCTR Operant Test Battery (OTB), has proven successful not only in the animal laboratory, but also in the clinical setting – primarily in children – where it has been proven to be sensitive not only in differentiating clinical syndromes, such as Attention Deficit Hyperactivity Disorder, but also in detecting the efficacy of drug treatment. Since this kind of assessment can be performed repeatedly in the same subjects, is noninvasive, is cost-effective and can be performed in laboratory animal surrogates (rodents through nonhuman primates), the utility of this approach becomes more evident daily. Ongoing studies are examining the ability of the OTB to identify specific cognitive profiles in children with anxiety, depression, exposure to morphine as a neonate, anxiety disorders, and childhood abuse. Thus, Critical Path Initiatives concerning biomarkers, assessment of depression, pediatrics, preclinical models, and human surrogates are being directly assessed in these efforts.

NCTR researchers are determining the gene-expression changes associated with long-term exposure to ketamine (a commonly used pediatric anesthetic) and remacemide (a sodium channel blocker and NMDA-receptor antagonist). In addition, gene changes associated with acute exposure to ketamine during the peak of the brain growth spurt are also being analyzed. The results from these studies will not only provide fundamental insight into normal developmental processes, but should also provide important data that can inform regulatory decisions for these and related compounds.

NCTR researchers, in collaboration with the National Institute of Child Health and Human Development (NICHD), are conducting a comprehensive study on methylphenidate (a drug commonly used to control Attention Deficit Disorder in children) to assess the possibility that this drug may cause genetic alterations in children. Studies are being conducted using nonhuman primates and rodents.

To provide further support of our research endeavors, genomic, proteomic, and bioinformatics approaches were developed or enhanced to allow for the identification of gene and protein-expression profiles associated with neurotoxic events. These data indicate that multiple pathways such as apoptosis, oxidative stress, iron binding, cellular metabolism, and signal transduction may be involved in aspects of Parkinson's disease therapies.

Research for Food Safety and Food Defense

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

In the areas of counterterrorism and food defense, NCTR scientists collaborate with scientists from other FDA Centers and several federal and state agencies to rapidly and accurately evaluate the disease-causing potential of foodborne pathogens. NCTR researchers and the FDA's Center for Food Safety and Applied Nutrition (CFSAN) have developed a biochip for high-throughput screening of virulence genes in *Salmonella* serovars to assess the threat of these bacteria in outbreak investigations. This microarray biochip has been used for threat assessment of

Salmonella serovars isolated from preharvest poultry sources and Roma tomatoes. Researchers are developing diagnostic microarray gene chips and other rapid molecular techniques for the simultaneous detection of multiple foodborne pathogens and antimicrobial resistance genes.

NCTR has continued developing methods for rapid detection of bacterial pathogens using pyrolysis and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS) technology with multivariate statistical and artificial neural-network pattern recognition (RAPID-B). NCTR has developed rapid flow cytometric techniques to detect and count fluorescent antibody-tagged target cells, specifically *Salmonella* spp. and *Listeria monocytogenes*, and is exploring other bacterial pathogens such as nonpathogenic *E. coli*, *E. coli* O157:H7, *Staphylococcus aureus*, methicillin-resistant *S. aureus,* and *Campylobacter jejuni.*

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

Summary

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

Science Advisory Board

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

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Division of Biochemical Toxicology

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Biochemical Toxicology Executive Summary

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

FY 2006 Accomplishments

A major emphasis within the Division is to conduct research on compounds nominated by FDA for evaluation by the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP). This focus reflects the 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 the FDA's needs. These studies currently serve as the benchmark by which toxicological assessments are made by federal agencies, including the FDA. In addition to providing basic information on toxicological endpoints such as cancer, these experiments form the basis for mechanistic studies to ascertain if the response detected in the experimental model is pertinent to humans.

During FY 2006, Division investigators continued chronic bioassays on acrylamide, a carcinogen found in certain baked and fried foods, in response to an NTP nomination by the Center for Food Safety and Applied Nutrition (CFSAN). The bioassays include assessing the carcinogenicity of glycidamide, a genotoxic metabolite of acrylamide. The assessment also includes administering the chemicals to newborn mice to determine if infants are particularly susceptible to the carcinogenic properties of these chemicals. 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 adducts levels associated with 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. As part of assessing the

potential toxicities associated with *Aloe vera* ingestion, the expression of cyclooxygenases, alterations in DNA methylation, and the ability of *Aloe vera* polysaccharides to alter the growth of colonic bacteria to ferment nonstarch polysaccharides were examined. Division investigators also completed and successfully defended reports on multigeneration and chronic studies on genistein, a component of soy products, including soy-based infant formula and dietary supplements. The results indicate that there are subtle multigeneration effects from this endocrine-disrupting chemical. As part of the investigations into endocrine-disrupting agents, draft NTP reports were prepared on the multigeneration and chronic studies of ethinyl estradiol. 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 diets in conducting toxicological studies for safety assessments. As part of this effort, high-performance liquid chromatography (HPLC) tandem mass spectrometry methods were developed for measuring DEHP and its metabolites in serum and urine. HPLC tandem mass spectrometry methods were also developed to detect and quantify pyrrolizidine alkaloid-derived DNA adducts. The method has the potential to detect carcinogenic pyrrolizidine alkaloids in foods, including dietary supplements. In collaboration with investigators at the Environmental Protection Agency, 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 particular concern to 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 a phototoxicity facility located within the Division. During FY 2006, evaluations were completed on the toxicity and carcinogenicity of topically applied alpha-hydroxy acids and beta-hydroxy acids in the presence of simulated terrestrial sunlight, and histopathological assessments were completed on the photocarcinogenesis studies of *Aloe vera* and retinyl palmitate. Studies were also initiated to assess the phototoxicity of nanoscale titanium dioxide, a component of certain sunscreens and other cosmetic products. The results indicated that in mice the regional lymph nodes and liver are the primary sentinel organs for the dermal penetration of topically applied nanoscale materials. They further indicated that nanoparticles did not penetrate intact skin, but did penetrate skin compromised by dermabrasion. In addition, Division investigators developed a novel neural net-based image analysis method and applied the method to identify and quantify DNA damage (*i.e.*, cyclobutane dimers) in very-large-format photomicrograph image files. A positive correlation was found between the levels of cyclobutane dimers and the extent of melanoma induced by ultraviolet (UV) treatment.

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 2006, Division investigators completed the Inlife phase of a bioassay to assess the effects of transplacental exposure of the antiretroviral drugs zidovudine and lamivudine in combination with nevirapine and nelfinavir. In addition, a bioassay was initiated to assess carcinogenic potential of the drugs administered transplacentally and neonatally. In further studies, Division investigators measured the transplacental transfer and metabolism of the anti-HIV-1 drugs in pregnant mice and evaluated the genotoxic consequences of the transplacental transfer of the drugs to the offspring. The results indicate that the treatments result in substantial damage to the genomes of the developing fetuses.

Tamoxifen is an adjunct chemotherapeutic agent for the treatment of breast cancer and a chemoprotective agent for breast cancer prevention. Despite being beneficial for the treatment and prevention of breast cancer, tamoxifen is known to increase the risk of endometrial cancer in women. During FY 2006, Division investigators compared the ability of tamoxifen and the tamoxifen analogue toremifene to form DNA adducts *in vivo*. The results demonstrated that toremifene is considerably less genotoxic than tamoxifen. In further work, Division investigators developed methodology for the simultaneous analysis of soy isoflavones, tamoxifen, and their bioactive metabolites. The technique is being applied to study diet-drug interactions between soy isoflavones and tamoxifen with respect to changes in breast cancer incidence and survival.

During FY 2006, Division investigators developed a robust cell-based functional assay for the bioterrorism agents ricin and abrin. This method can be applied to FDA-regulated foods and has been used to assess 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, *Clostridium botulinum* toxin, and other neurotoxins that 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 2006, 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 2007 Plans

In FY 2007, Division investigators will complete and defend the final NTP reports on the multigenerational reproductive and carcinogenic effects of ethinyl estradiol. The Inlife phases of chronic two-year bioassays on *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.

Investigators associated with the NCTR Center for Phototoxicology will continue to study the interaction of light with tattoo pigments. Specifically, photocarcinogenesis studies will continue on various tattoo inks using full-spectrum simulated solar-light. 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 will also continue on the characterization of transgenic mouse models for photocarcinogenesis with an emphasis on the induction of cutaneous and ocular melanoma.

In collaboration with investigators at the Environmental Protection Agency, Division staff will apply HPLC tandem mass spectrometry methods to assess the levels of stable and unstable DNA adducts in mice administered benzo[*a*]pyrene. HPLC tandem mass spectrometry methods will also be applied to investigate the pharmacokinetics of intravenously administered DEHP in

neonatal rhesus monkeys. 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 Division of Systems Toxicology, experiments will be initiated to apply omics techniques to determine biomarkers for liver toxicity.

In additional experiments, Division investigators will develop HPLC tandem mass spectrometry methods to identify and quantify melamine and melamine derivatives in tissues and biofluids.

With support from the FDA's Office of Women's Health, Division investigators, in collaboration with scientists in NCTR's Division of Personalized Nutrition and Medicine and clinical researchers at the Brody School of Medicine, East Carolina University, will evaluate the effects of a common single nucleotide polymorphism in the gene for prolactin on the efficacy of prasterone therapy for systemic lupus erythematosus. The frequency of the variant prolactin allele will be evaluated among male and female patients and matched controls and correlated with response to prasterone (dehydroepiandrosterone), an FDA-designated orphan drug.

Contribution to FDA's Strategic Goals

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 presence of potential bioterrorism agents, for example ricin and abrin, in various food products. The Division also emphasizes toxicological assessments of chemicals found in cosmetic products. These chemicals include 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 assessing potential risks associated with permanent makeup and tattoos that are being also used by an increasing proportion of the U.S. population.

Biochemical Toxicology Ongoing Research Projects

NCTR's Strategic Goal 1 — Advance the scientific approaches and tools to attain personalized nutrition and medicine for the American public

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

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

Objective(s):

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

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

Objective(s):

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

DNA Adducts of Tamoxifen (E0701101)

Objective(s):

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

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

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: Boudreau, Mary D., Ph.D.

Bioassays in the F344 Rat and the $B_6C_3F_1$ Mouse-Administered *Aloe Vera* Plant Constituents in the Drinking Water (E0214201)

Objective(s):

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 – in view of the complexities inherent in *Aloe vera* pharmacology and the inconsistencies reported in literature. 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.

PI: Chou, Ming W., Ph.D.

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

Objective(s):

1) To characterize the structures of the eight DHP-derived DNA adducts; 2) To study metabolism of retronecine-based pyrrolizidine alkaloids, heliotridine-based pyrrolizidine alkaloids, otonecine-based pyrrolizidine alkaloids, and pyrrolizidine alkaloid N-oxides by liver microsomes of F344 rats, $B_6C_3F_1$ 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; and 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: Doerge, Daniel R., Ph.D.

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

Collaborating Division(s):

Genetic and Reproductive Toxicology
Personalized Nutrition and Medicine

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; and 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.

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

Collaborating Office/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.

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

Collaborating Division(s):

Genetic and Reproductive Toxicology

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 adducts in target tissues and circulating lymphocytes with acrylamide- and glycidamide-hemoglobin adducts in rodent exposure studies for future use in monitoring human exposure through occupation, smoking, and the diet; and 6) To determine *in vivo* mutagenesis of acrylamide and glycidamide using transgenic mice (Big Blue®).

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

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: Pogribna, Marta V.

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

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 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 P., Ph.D.

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

Collaborating Office/Division(s):

Office of 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 upregulated or downregulated in target tissue during the promotion stage of carcinogenesis; and 3) To

evaluate whether or not the global and locus-specific DNA hypomethylation, along with aberrant expression of related genes and changes in chromatin conformation, is specific only to target tissues and may be used for early detection of chemicals with carcinogenic potential.

NCTR's Strategic Goal 2 — Develop science-based best practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory science process to create a seamless bench-to-bedside continuum

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

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

Collaborating Division(s):

Genetic and Reproductive Toxicology

Objective(s):

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

PI: Boudreau, Mary D., Ph.D.

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

Objective(s):

1) To determine the dose response and acute kinetics of topical exposure to *Aloe vera* plant components on the structure of SKH-1mouse skin in the absence of simulated solar-light exposure; and 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; and 5) To determine the influence of *Aloe vera* components on simulated solar-light-induced tumor formations in mice.

PI: Delclos, K. Barry, Ph.D.

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

Objective(s):

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

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

Objective(s):

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

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

Collaborating Office/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; and 3) To evaluate the reversibility of any observed effects.

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

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

Collaborating FDA Center(s):

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.

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

Collaborating Office/Division(s):

Office of Director

Personalized Nutrition and Medicine

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*]yrene, 6-nitrochrysene, and aflatoxin B_1; 3) To study the metabolism and DNA adduct formation of benzodiazepine and antihistamine drugs by mouse and human liver microsomes to determine which, if any, cytochrome P450 is responsible for metabolic activation in mice and humans; and 4) To employ transgenic human lymphoblastoid cell lines expressing appropriate CYP isozymes to study the mutations and DNA binding of the subject drugs.

PI: Heinze, Thomas M.

Analytical Methodology Development for Assessing Bioactive Herbal Ingredients in Functional Foods (E0716101)

Objective(s):

To develop qualitative and quantitative methods for determination of specific marker compounds, such as terpene trilactones (ginkgolides and biolobalide) and kava lactones in raw plant materials, dietary supplements and functional food products containing ginkgo, kava kava or their extracts. A minor objective of this proposed work is to include other minor compounds which do not meet the selection criteria but may be of safety concerns. These compounds include ginkgolic acids, ginkotoxin and urushiols in gingko products and unknown factors in kava.

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

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

Objective(s):

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

DNA Adduct Formation by Nicotine Metabolites (E0692501)

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 labeling techniques to detect the adduct; and 2) To quantify the presence of these adducts *in vitro* and *in vivo* in mice.

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

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 makeup inks in SKH-1 hairless mice in the

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

Purification of Ceramide Synthase (E0705901)

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; and 4) To use the antibodies to study tissue-specific expression of ceramide synthase.

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

Collaborating Office/Division(s):

Office of 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; and 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.

Tumorigenicity of Photoactive Nanoscale Titanium Dioxide (TiO2) in Tg.AC Transgenic Mice (E0215801)

Objective(s):

1) To determine if topical application of nanoscale TiO2 results in penetration of the TiO2 into the hair follicle of FVB/N mice; 2) To determine the dose-response relationship of follicular penetration by TiO2; 3) To repeat the UVB doses used in the Trempus *et al.*, 1998, study to confirm reproducibility of UVB dose that is photocarcinogenic; and 4) To dose mice with high dose of UVA to determine photocarcinogenicity.

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

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

Collaborating Office/Division(s):

Systems 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; and b) whether the increase in dnmt1 expression is stimulated by global loss of methyl groups and whether dmnt1 expression is decreased by methyl repletion; 3) To determine the temporal relationship between the appearance of DNA lesions and site-specific methylation within the CpG island of the p16 promoter region in *p16* gene expression with alterations in local chromatin structure and DNA methyltransferase mRNA levels and activity; and 4) To use microarray slides printed with the rat cDNA library in collaboration with the Division of Systems Toxicology as a tool to screen for methylation-related downregulation of candidate genes in hepatic preneoplastic foci, preneoplastic nodules, and tumor tissue from folate/methyl-deficient rats.

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

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

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; and 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.

NCTR's Strategic Goal 3 — Develop and apply rapid detection technologies and testing platforms to assure food safety, biosecurity, food biodefense, and to combat bioterrorism

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

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

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

Biochemical Toxicology Research Projects Completed in FY 2006

PI: Chou, Ming W., Ph.D.

A Collaborative Research Proposal to Assess Cancer Risk Posed by Intermittent Exposure to Aflatoxin B_1 in Rats (E0688801)

Objective(s):

1) To test the hypothesis that a chemically induced tumor incidence is a function of the accumulated lifetime exposure and is predictable from the average daily dose for various dosing regimens, such as continuous and intermittent dosing; and 2) To study correlations between the chemically induced tumor incidence and various biomarkers of the initiation and the promotion stage of carcinogenesis for continuous and intermittent dosing.

Results:

Fischer 344 male rats were fed 1.6 ppm of aflatoxin B_1 (AFB_1) continuously and intermittently for several weeks. At various time periods, DNA was isolated from the testes and livers and analyzed for AFB_1-DNA adducts. The ability of the testis to detoxify AFB_1 was also investigated by the glutathione S-transferase (GST) activity assay and compared with that of the liver. The levels of testicular AFB_1-DNA adducts were 2.4 to 8.1 times lower than those of the liver after 4 to 16 weeks of continuous treatment and 2.2 to 46.2 times lower after 8 to 20 weeks of intermittent treatment. The testicular DNA adducts markedly decreased over time. By 16 weeks of continuous and 20 weeks of intermittent exposure, they had decreased 37 and 91%, respectively. In contrast, hepatic AFB_1-DNA adducts increased four-fold from 4 to 16 weeks of continuous treatment but increased at a much slower rate after intermittent exposure. In both the liver and testis, significant levels of AFB_l-DNA adducts persisted for at least one month after ending the treatment, suggesting that this type of lesion was poorly repaired. In control rats, the testis showed significantly higher GST activity than the liver. In treated rats, these differences were significant during the first 12 weeks of continuous treatment but not at later times. Tissue specific differences, such as germcell depletion and increased testicular detoxification, may play an important role in the observed differential pattern of DNA-adduct formation between the testis and liver.

Effects of Dietary Restriction on the Post-Initiation Stages in Aflatoxin B_1-Induced Carcinogenesis on Male F344 Rats Fed Methyl-Deficient Diets (E0695201)

Objective(s):

To study the interactions of dietary restriction (DR) and methyl deficiency (MD) on the alterations of hepatic oxidative DNA damages, DNA methylation, cell proliferation, oncogene and tumor-suppressor gene mutation, preneoplastic foci formation and tumor incidence during the post-initiation stages of AFB_1-induced carcinogenesis in male F344 rats. The results of these studies will: i) To test the hypothesis that DR may be an antagonist to the promotional effect of MD in the AFB_1-induced carcinogenesis; and ii) To evaluate the correlations between the effects of DR and MD on the formation of AFB_1-induced preneoplastic foci and tumors and various biomarkers during the post-initiation stages of carcinogenesis.

Results:

The effects of methyl deficiency and dietary restriction (DR) on hepatic cell proliferation and telomerase activity was studied in male Fischer 344 rats pretreated with aflatoxin B1 (AFBl). Five-week-old rats were gavaged 5 days per week for 3 weeks with AFBl (25 µg/rat per day) or solvent (100 µl 75% dimethylsulfoxide). Rats were then divided into four groups. Two groups were fed a methyl-sufficient (MS) diet either *ab libitum* (AL) or with DR. The other two groups were fed a methyl-deficient (MD) diet either AL or with DR. At 15, 20, and 32 weeks of age, hepatic cell proliferation, telomerase activity, and the number of glutathione S-transferase-P positive (GST-P+) foci were determined. DR reduced hepatic cell proliferation, while the MD diet and AFBl pretreatment increased cell proliferation. Telomerase activity was decreased by DR and increased by the MD diet and AFBl pretreatment. The same trend was observed with GST-P+ foci: in AFBl-pretreated rats, methyl deficiency increased the number of foci, while DR decreased the number. These results are consistent with a role of telomerase in hepatocarcinogenesis.

PI: Delclos, K. Barry, Ph.D.

Effects of Endocrine Active Agents on Bone (E0710601)

Objective(s):

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

Results:

Genistein has been shown to prevent bone loss in ovariectomized adult rats. However, the effects of genistein on bone in developing and reproductively intact rats have not been examined. A large multigenerational experiment involved feeding 0, 5, 100, or 500 ppm genistein in the diet to intact male and female rats from conception until either weaning, postnatal day 140, or continuously for two years. Vertebrae (lumbar and caudal) were collected from these animals at necropsy at 2 years of age and subjected to dual-energy x-ray absorptiometry (DXA) scanning to measure bone mineral density (BMD), bone mineral content (BMC), and bone area. Femurs were collected, and length, crosssectional area, and cortical bone area were measured directly. Serum was collected for measurement of pyridinoline (PYD) and alkaline phosphatase (ALP). BMD was not affected by genistein in any phase of the experiment. In female rats treated continuously with genistein, BMC and bone area were reduced in the 500 ppm group compared to the 5 ppm group in the lumbar vertebrae, and in all treatment groups compared to control in the caudal vertebrae. In both males and females treated continuously, the cross-sectional area of the femur was reduced in rats treated with 500 ppm compared to those treated with 5 ppm. In female rats treated continuously, PYD was higher in the 100 and 500 ppm groups than in the 0 and 5 ppm groups. In conclusion, the effects of genistein on reproductively intact rats were not dramatic. High dose of genistein throughout the lifespan resulted in decreased bone size, which may reduce the force required to break the bone.

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

Objective(s):

1) To determine the effects of genistein, a naturally occurring isoflavone, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations; 2) To determine if subtle effects observed in the dose range-finding study are magnified through multiple generations; 3). To evaluate the reversibility of any observed effects; and 4) To evaluate the chronic toxicity of genistein, particularly potential induction of cancer of the reproductive organs, following exposures that will include various life stages (*in utero* through early adulthood, *in utero* and continuous for 2 years after birth, *in utero* and lactational only, and postweaning only).

Results:

Although genistein did show biological activity at 100 and 500 ppm dietary doses, there were few if any clear overtly toxic effects that carried over across directly exposed generations or were imprinted to carry over into unexposed descendents under the conditions of exposure in this study. Genistein clearly affected postweaning body weight gain, predominantly in females who were directly consuming dosed feed, at 500 ppm. Preweaning body weight gain in pups of both sexes was reduced in the 500 ppm dose groups of all generations. Genistein accelerated the attainment of puberty in females under continuous exposure conditions at 500 ppm and caused perturbation of the estrous cycle in younger animals. There was some evidence for reduced litter size in the 500 ppm dose group in continuously exposed generations. In males, genistein induction of male mammary hyperplasia and mild mineralization of renal tubules occurred at 100 and 500 ppm in generations (F_1 and F_2) that were continuously exposed from conception. Weaker effects on male mammary hyperplasia were found in the 500 ppm dose group F_0 generation, which was exposed to genistein from six weeks of age, and in the F_3 generation, which was exposed to genistein only until weaning. With the exception of reduced body-weight gain during the preweaning period in pups, none of these effects appeared to carry over into unexposed generations. Effects observed in the F_1 generation did not appear to be magnified in subsequent

exposed generations, but rather for the most part appeared to be similar or reduced.

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

Objective(s):

To determine whether or not, in the absence of endogenous ovarian estrogens, dietary genistein can promote or suppress the growth of neoplastic mammary tissue at various stages of the carcinogenic process.

Results:

In this study, dietary modulation of 7,12-dimethylbenz[a]anthracene (DMBA)-induced adrenal toxicity in rats was investigated. Beginning at postnatal day (PND) 21, female Sprague-Dawley rats were fed either soy-containing NIH-31 diet or soy- and alfalfa-free 5K96 diet. On the first day of diestrus when the animals were PND 50 ± 5, rats received either an oral dose of 80 mg/kg DMBA or sesame oil, the vehicle, and were sacrificed at 24, 36, or 48 hours after treatment. Apoptosis was manifested at 24 and 36 hours after DMBA treatment in the zona reticularis (ZR) and the zona fasciculata (ZF) of the adrenal cortex; this was followed by severe hemorrhagic necrosis at 48 hours. DMBA-induced apoptosis, evaluated by the TUNEL assay, immunohistochemical analysis of activated caspase 3, and the ratio of expression of pro-apoptotic Bax to anti-apoptotic Bcl2, was greater in rats fed NIH-31 diet relative to rats fed 5K96 diet at 24 hours after treatment. Four of six DMBA-treated rats fed 5K96 diet had severe adrenal necrosis by 48 hours, whereas this lesion was present in only two of six DMBA-treated rats fed NIH-31 diet. DMBA also caused a significant decrease of serum corticosterone relative to controls at 48 hours in rats fed 5K96 diet. The present study indicated that diet modulates DMBA-induced adrenal toxicity in female rats, with increased apoptosis early and reduced necrosis later in rats fed a soy-containing diet.

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

Development of Methods for Analysis and Confirmation of β-Agonists (E0694501)

Objective(s):

1) To develop determinative and confirmatory procedures using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCI/MS) for multiresidue screening β-agonists in livestock tissues; 2) To develop synthetic procedures to produce authentic β-agonist standards for use in regulatory screening. These methods will provide the flexibility to adapt to the potential for "designer drug" modifications by clandestine laboratories; and 3) To explore the use of packed column supercritical fluid chromatography (SFC) coupled to APCI/MS as a more efficient technique for chromatographic separation in the screening of large numbers of β-agonists in livestock tissues.

Results:

LC/MS methods were developed and validated for the quantification and confirmation of multiple β-agonists in several biological matrices (e.g., serum, liver, retina). The level of confirmation was determined for nine diverse β-agonists to be less than 1 ppb in liver and retina. This methodology was adopted for use by the United States Department Agriculature/Food Safety and Inspection Service (FSIS) for screening β-agonist misuse in the meat supply.

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

Effect of Topically Applied Skin Creams Containing Glycolic and Salicylic Acid on the Photocarcinogenicity of Simulated Solar-Light in SKH-1 Mice (E0213701 and E0213101)

Objective(s):

To determine if the application of creams containing α- βαand β-hydroxy acids to the skin of male and female SKH-1 hairless mice alters the tumor incidence induced by simulated solar-light in the mouse skin.

Results:

These experiments investigated the impact of topical application of a cosmetic formulation containing 4% or 10% glycolic acid (pH 3.5) or 2% or 4% salicylic acid (pH 4) on the photocarcinogenesis of filtered 6.5 kW xenon-arc simulated solar-light (SSL) in SKH-1 hairless mice. Taking into consideration the survival data, time-to-tumor data, and the pathology results: (1) glycolic acid did not affect the photocarcinogenesis of SSL; (2) salicylic acid was photoprotective, reducing the carcinogenicity of 0.3 MED SSL.

Division of Genetic and Reproductive Toxicology

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Genetic and Reproductive Toxicology Executive Summary

Introduction

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

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

Currently there are four focus areas in the Division research program – genetic toxicology, reproductive/developmental toxicology, dietary research, and omics research. Genetic toxicology research addresses the development of methods to assess the potential for chemicals to negatively impact human genetic material or the function of the genetic material. Reproductive/developmental toxicology focuses on methods to understand normal human development and how chemicals might alter normal development. Dietary research primarily focuses on understanding the physiological and genetic consequences of dietary modulation and on the potential hazards of dietary supplements. Emphasis on omics research is rapidly increasing in the Division and is coupled with more traditional approaches to improve the ability of FDA to incorporate these new and powerful technologies into regulatory decision-making.

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

FY 2006 Accomplishments

In FY 2006, DGRT scientists actively provided genetic toxicology advice to FDA regulatory Centers (CDER, Center for Veterinary Medicine (CVM), Center for Food Safety and Nutrition (CFSAN), and Center for Devices and Radiological Health (CDRH). These consultations included general advice concerning the conduct and interpretation of data from specific assays and the evaluation of data from submissions to FDA. DGRT scientists participated in the

International Workshop for Genotoxicity Tests and authored manuscripts that provide harmonized guidance for the conduct of specific assays and on 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 research areas are highlighted as follows:

1) DGRT scientists developed a series of allele competitive blocker-polymerase chain reaction (ACB-PCR) genotypic selection methods that can directly measure specific mutations in genes (oncogenes and tumor-suppressor genes) involved in tumor induction. These assays were used to measure mutations in tumors resulting from solar-light exposure and in mice treated with 4-aminobiphenyl. These studies have yielded significant new information. For instance, all the skin tumors resulting from solar exposure had relatively high frequencies of *p53* mutations, indicating that while this mutation was not the initiating event for the tumor, it was important for tumor formation. This research was conducted in collaboration with the NCTR Center for Phototoxicology. Two companion studies evaluating the presence on *p53* mutations in colon cancer from both mice and humans is nearing completion. A collaboration involving researchers in NCTR's Division of Biochemical Toxicology and investigators in the Carcinogenesis Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, is well underway. This study addresses the shape of the dose-response curve for low-dose exposure to carcinogens. NCTR research indicates ACB-PCR technology 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 new strategy that might ultimately lead to the replacement of the traditional two-year cancer bioassay. Such an approach would hasten the development, safety assessment, and approval of new drugs.

2) A study was completed and published that provided a methodology for using the *in vitro* mouse lymphoma mutation assay to assess photomutagenicity and showed that retinyl palmitate can be activated by ultraviolet light to a mutagenic form.

3) In collaboration with scientists in NCTR's Division of Personalized Nutrition and Medicine and Division of Systems Toxicology, studies were completed using gene-expression microarray data to address several issues of specific chemical activity and to assist with understanding particular assays.

4) Division scientists completed and published results from studies evaluating the genotoxicity of several additional specific chemicals (zidovudine, comfrey, and aristollochic acid) of regulatory interest to FDA. Many of these compounds were nominated by FDA for evaluation, and the research on them is supported through an Interagency Agreement (IAG) with the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP). Studies evaluating the *in vivo* mutagenicity of acrylamide, which is found in baked goods, also are ongoing, and results on the *in vivo* genotoxicity of acrylamide and its major metabolite glycidamide were recently published.

5) In collaboration with NCTR's Division of Neurotoxicology and funded by NICHD (National Institute of Child Health and Human Development), a comprehensive study for assessing methylphenidate (a drug commonly used to control Attention Deficit Disorder

in children) was initiated to assess the possibility that this drug may cause genetic alterations in children. Studies are being conducted using nonhuman primates and rodents.

FY 2007 Plans

1) In collaboration with scientists in NCTR's Division of Personalized Nutrition and Medicine, a new approach for using the quantitative analysis of *in vivo* mutation data to inform the mode-of-action assessment for carcinogens was developed in 2005 and 2006. This approach will be evaluated for its utility in a Cooperative Research and Development Agreement((CRADA) with 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 to studies involving colon cancer in humans, a skin cancer model in mice, and a colon cancer model in rats. The rat studies will involve examining mutations in cells with specific morphologies related to cancer induction; the cells will be isolated using laser capture microdissection. The collaboration with the Environmental Protection Agency to apply this technology to understanding the shape of the dose-response curve at low dose will continue. A CRADA(with CIIT Centers of Health Research is currently in preparation to evaluate if this technology can be developed as a biologically based dose-response model for carcinogens. A new emphasis will be placed on developing this technology as a biomarker to quickly and easily identify potential carcinogens and provide an alternative to the two-year cancer bioassay.

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

4) A new approach for directly analyzing mutations is under development. This assay uses fluorescent probes to detect mutation in the endogenous, X-linked *PIG-A* gene. In theory, detection of mutations in this gene does not require cell culture (as do many other *in vivo* mutation detection methods) and lends itself to both *in situ* and high-throughput analyses in humans and animal models. All of these properties make *PIG-A* an attractive reporter gene for *in vivo* mutation studies. Experiments are being conducted to determine the level of sensitivity of the analysis using cultures of cells that are wild-type and mutant for *PIG-A*.

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

and mutation. In an additional study, an NTP-sponsored experiment will evaluate the ability of *p53* haplodeficient ($p53^{+/-}$) mice to detect the tumorigenicity of AIDS drugs in a relatively short-term bioassay.

6) DGRT scientists will continue collaborating with scientists in NCTR's Division of Neurotoxicology to assess the potential for methylphenidate (a drug commonly used to control Attention Deficit Disorder in children) to induce mutagenic damage. This study is being conducted as a part of an interagency agreement with the NICHD.

7) Work will continue on several ongoing projects including: 1) a collaborative project with the University of Arkansas for Medical Sciences investigating the influence of biotin on the developing embryo; 2) the further development and characterization of the ΦX174 transgenic mouse, particularly as it applies to detecting mutation in mouse skin following exposure to solar-light; 3) NTP projects investigating the genetic toxicity of AIDS therapeutic drugs and the developmental toxicity and physiological impact of exposure to bitter orange; 4) the evaluation of flow cytometry for the high-throughput analysis of micronucleus frequency in mice, rats, monkeys, dogs, and humans; and 5) a project to investigate whether the developing embryo and/or the neonate is particularly sensitive to the induction of mutation following exposure to known carcinogens.

Contribution to FDA's Strategic Goals

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

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

Reproductive/developmental toxicology is important to FDA because one of the difficult challenges facing 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 may play a role in the development of normal offspring, and that interaction between diet and toxicants may be important in producing certain birth defects.

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

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Genetic and Toxicology Ongoing Research Projects

NCTR's Strategic Goal 1 — Advance the scientific approaches and tools to attain personalized nutrition and medicine for the American public

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)

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 B_1, a human hepatocarcinogen that requires a metabolic activation; 3) To determine the effect of exposure of neonatal and adult Big Blue® mice to 17β-estradiol, a human hormone carcinogen, on subsequent spontaneous and carcinogen-induced mutations in the *cII* gene of the target organs; and 4) To determine the types of *cII* mutations in the mutants from Obj. 1, 2, and 3.

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)

Collaborating Division(s):

Biochemical 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; and 5) To correlate gene-expression patterns with DNA adduct formation and mutation induction in treated rats.

Further Evaluation of the Types of Genetic Events Detected by the Mouse Lymphoma Assay (MLA) and the Role of the Assay in Mechanistically Based Risk Assessment (E0711701)

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; and 3) To determine what is/are the fundamental genetic mechanism(s) causing the small and large colony thymidine kinase mutant phenotypes.

PI: Dobrovolsky, Vasily N., Ph.D.

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

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 (GFP) in various tissues of different lines of the double-transgenic mice; and 3) To determine the frequency of spontaneous and y-ray-induced TetR mutation in lymphocytes of double-transgenic mice using flow cytometry.

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

Mechanism of Biotin Deficiency-Induced Malformations (E0713301)

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*; and 6) To determine if arachidonic acid is able to

prevent biotin deficiency-induced orofacial clefting and limb hypoplasia *in vivo*.

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

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

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; and 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.

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)

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

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

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

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; and 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: Valentine, Carrie R., Ph.D.

Evaluation of the Potential of the Gene *A* Forward Mutational Assay of PhiX174 for Improving Sensitivity of Transgenic Mutation Assays (E0711501)

Objective(s):

1) To determine the appropriate experimental conditions to identify single bursts of mutations fixed *in vivo*; 2) To develop a microplate-scoring method that will identify *in vivo* bursts within numerous aliquots; 3) To determine the spontaneous mutant frequency and ENU-induced mutant frequency by single burst analysis for mouse splenic lymphocytes; and 4) To continue development of a frameshift assay for FX174 in gene *J* by our collaborator, Dr. Bentley Fane.

NCTR's Strategic Goal 2 — Develop science-based best practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory science process to create a seamless bench-to-bedside continuum

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

Developmental Toxicity of Bitter Orange in Rats (E0214701)

Collaborating FDA Center(s):
CFSAN

Objective(s):
To determine potential developmental toxicity of synthetic synephrine and citrus aurantium extract in rats.

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

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 (GD) 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 downregulated 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; and 7) To use *in situ* hybridization, laser capture microdissection, and Northern techniques to determine if altered gene expression is specific for subsets of embryonic cells.

Physiological Effects of Bitter Orange in Rats (E0214901)

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

Collaborating FDA Center(s):
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 H., Ph.D.

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

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: 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)

Collaborating Division(s):
Neurotoxicology
Personalized Nutrition and Medicine

Objective(s):
1) To 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; and 2) To 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 M., Ph.D.

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

Collaborating Office/Division(s):

Office of Director
Biochemical Toxicology
Neurotoxicology
Personalized Nutrition and Medicine
The Bionetics Corporation

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; and 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.

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

Collaborating Office/Division(s):

Office of Director
Biochemical Toxicology
Neurotoxicology
Personalized Nutrition and Medicine

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 the 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 *cII* gene mutant frequency of Big Blue® mouse; and 5) To determine the pharmacokinetics of methylphenidate and its major metabolite, ritalinic acid, in $B_6C_3F_1$a mice.

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

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

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; and 4) To measure the frequency of this mutation in skin exposed to decreasing levels of SSL.

Detection of K-*ras* Mutations in Mice Treated with Benzo[*a*]pyrene at Low Exposure levels (E0723711)

Collaborating Division(s):

Biochemical Toxicology

Objective(s):

1) To establish the background levels of K-*ras* codon 12 GGT to TGT mutation in lungs of A/J mice using the Allele-specific Competitive Blocker PCR (ACB-PCR) method; 2) To determine the amount of inter-animal variability in the levels of this K-*ras* mutation, as measured by ACB-PCR; and 3) To determine the levels of the K-*ras* codon 12 mutations in the lungs of A/J mice treated with various levels of benzo[*a*]pyrene (BP) at two different times after treatment.

PI: Valentine, Carrie R., Ph.D.

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

Collaborating Division(s):

Biochemical Toxicology

Objective(s):

To create and update a public website 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.

Identification of Target Sites for UVB Irradiation in Gene *A* of ΦX174 Contained as a Transgene in Mouse Embryonic Cell PX-2 (E0710101)

Objective(s):

1) To determine the dose-survival response of PX-2 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 ΦX174 by a forward mutation assay using cultures of PX-2 exposed to UVB; and 3) To sequence the UVB/UVA-induced mutants from

treated and untreated cultures to identify specific target sequences.

UV-Induced Mutations in Mouse Epidermis Using Gene *A* of ΦX174: Proof of Principle (E0718701)

Collaborating Division(s):

Biochemical 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 ΦX174 analyzed by single bursts.

Genetic and Reproductive Toxicology Research Projects Completed in FY 2006

PI: Aidoo, Anane, Ph.D.

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

Objective(s):

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

Results:

Age-dependent accumulation of point mutations in mitochondrial DNA (mtDNA) was determined from the livers of young mice (2 months old) and old mice (22 months old) using short-extension PCR (SE-PCR). Mitochondrial DNA was chosen because it exists in a highly genotoxic environment caused by constant exposure to reactive oxygen species, somewhat deficient DNA repair, and the relatively low fedelity of polymerase gamma, indicative of mutations accumulation by several orders of magnitude compared to nuclear genome. In addition, mutations in the mitochondrial genome are associated with numerous chronic diseases. Fragments from the D-loop region of mtDNA were amplified and cloned into plasmid vectors, and plasmid DNAs from individual clones were purified and sequenced. In total, 197 plasmids containing a fragment of the D-loop region were analyzed. Samples from five young mice (a total of 110 plasmids) did not contain any mutations. However, in the colonies from older animals (87) we found nine mutated sequences containing five base substitutions, three different G:C→T:A transversions. Mutations in young mice apparently occurred at levels below the detection limits in this study (2.3x10-5), while the calculated mutant frequency for the old animals was 25.4±7.8x10-5. The difference between the two groups was statiscially significant (p=0.001). Double tandem (CC→TT) transition mutations were also observed in the mtDNA of older mice. The results suggest that at 22 months of age (which roughly corresponds to ¾ of the mouse natural life span), mtDNA molecules carry multiple point mutations. This high accumulation of mtDNA mutations could reduce the generation of cellular energy, which in turn may be responsible for some of the clinical manifestations of aging.

To demonstrate the utility of the short extension PCR assay in risk-assessment evaluations of drugs/chemicals, a total DNA preparation from splenic lymnphocytes derived from animals treated with a single dose (10 mg/kg) of the chemotherapeutic drug bleomycin were processed in the SE-PCR assay. The results indicated that spontaneous deletion mutations were typically flanked by direct repeats (78.5%), while in the bleomycin-treated group, direct repeats were found in only 46.6% of breakpoints. The ratio between deletions based on direct repeats and random sequence deletions changed from 3.7 in control group to 0.87 in bleomycin-treated animals., approximately 1.7-fold increase in the deletion mutation frequency. The dose of bleomycin used also induces nuclear gene mutation in the lymphocyte Hprt assay, thus suggesting the utility of SE-PCR method for evaluating drug-induced genotoxicity.

PI: Duffy, Peter H., Ph.D.

Effect of Diet and Different Levels of Caloric Restriction (CR) on Physiological, Metabolic, Biochemical, Immunological, Molecular, and Body Composition Variables in Rats (E0692401)

Objective(s):

1) To determine how various levels and durations of CR affect physiological function, enzymes related to intermediary and drug metabolism, hormonal regulation, blood chemistry, etc.; 2) To determine the relationship between body fat (BF), fat-free mass (FFM), total body water (TBW), and total body electrical conductivity (TOBEC) as a function of strain, age, mass, and nutritional status in rats; 3) To validate and automate the use of a new noninvasive electromagnetic scanning device to measure BF, FFM, and TBW and to compare the results to a conventional chemical fat extraction technique; 4) To determine if CR alters the relative quantity and disposition of various types of lipids such as cholesterol, phospholipids,

free fatty acid, etc. in various tissues, as well as in urine, feces, and blood serum; 5) To develop control data related to CR that can be used by CFSAN to evaluate the toxicity and efficacy of low-calorie foods, food additives, and food substitutes; 6) To determine temporal and environmental factors that modulate the effects of CR; 7) To develop experimental methods for utilizing a low level of CR to increase the survival rate and to decrease variability in the chronic bioassay; to provide the concomitant control data for comparison; and 8) To develop control data for a reference purified diet that has been formulated to conform to long-term nutrient requirements of rodent animal models typically utilized in toxicology and nutrition studies.

Results:

1) At 114 weeks of age, the survival rate was 51.7, 87.5, 85.0, and 95.0% for AL and 10, 25, and 40% DR rats, respectively. 2) No significant difference in survival was seen between 10 and 25% DR; the largest increase in survival occurred between AL and 10% DR. 3) The results of this study clearly show that a 10% reduction in calories significantly reduced the onset and progression of a wide variety of tumors and non-neoplastic diseases. 4) In many instances, the reduction in neoplastic and non-neoplastic diseases was similar for 10, 25, and 40% DR rats. 5) DR (all levels) significantly reduced the incidence and severity of nephropathy and cardiomyopathy in old rats; DR reduced the progression of foot inflammation to ulcers. 6) The incidence of benign neoplasms was significantly reduced by DR (all levels); the incidence of primary neoplasms was significantly reduced by 10 and 40% DR. 7) No malignant neoplasms were found in 10% DR rats; the incidence of skin tumors was reduced by 10 and 40% DR. 8) The number of total neoplasms and the percent of tumor-bearing animals were lower in all DR groups. 8) DR (all levels) increased the survival rate of rats by delaying the onset and/or progression of prevalent and potentially fatal tumors.

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

Mechanism(s) of Folate-Responsive Dysgenesis (E0707401)

Objective(s):

1) To determine if there is concordance between the expression of the folate receptor (FBPI) and the most proliferative cohorts of neural tube- and neural crest-cells during defined 12-hr windows on each day of gestation from GD 5 to GD 15, and to determine if the loss of these cohorts of cells during these windows of antifolate exposure gives rise to recognizable neural-tube defects and neurocristopathies in the fetus at term; 2) To characterize the basal expression of FBPI isoforms and extent and mechanism of FBPI regulation in the placenta and various fetal tissues on GD 17 among cohorts of dams fed a folate-deficient or folate-replete diet; 3) To determine if sustained quenching of placental cytotrophoblast FBPI by antisense FBPI cDNA overexpression from GD 8 to GD 16 during maternal folate deficiency has an adverse impact on cytotrophoblastic proliferation leading to small placentas and global growth retardation of fetuses; and 4) To demonstrate that neural-tube closure and neural-crest cell function in the whole mouse embryo at GD 8.5 can be perturbed by down-regulating FBPI expression in neural-tube cells through the introduction of antisense oligonucleotides to the 43-kDa trans-factor, which is required for FBPI transcription.

Results:

The purpose of this protocol was to examine regulation and expression of the folate receptor in embryonic tissues during development, focusing on the time of closure of the neural tube. We began with the hypothesis that expression and regulation of the receptor varies among tissues, and this may account, in part, for the variability in tissue sensitivity to folate deficiency. To test this hypothesis, we first examined the expression and regulation of the receptor in human placenta, since the receptor is plentiful in this tissue. We found a cytosolic *trans*-factor protein that is needed for translation of the folate receptor; this protein has been identified by several biochemical criteria as heterogeneous nuclear ribonucleoprotein E1 (hnRNP E1). Next, using antibodies to both the folate receptor and hnRNP E1, we examined the expression of these proteins in sections from mouse embryos. We observed that both proteins were expressed to varying degrees in different embryonic tissues. We also examined the expression of these proteins under conditions of dietary folate sufficiency or deficiency, and found that both proteins are upregulated under conditions of folate deficiency. Again the degree of upregulation varied among embryonic tissues. We also determined that administration of antisense oligonucleotides to either FBP1 or hnRNP E1 produced neural-tube defects in mouse embryos cultured *in vitro*, suggesting that downregulation of FBP1 altered normal development and function of neural crest and neural-tube cells.

Division of Microbiology

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Microbiology Executive Summary

Introduction

The Division of Microbiology at NCTR serves a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology. The Division of Microbiology also responds to microbial surveillance and diagnostic needs for research projects within NCTR and FDA. The Division of Microbiology has a multidisciplinary staff including 14 research scientists and 17 research support staff, postdoctoral fellows, undergraduate and graduate students, visiting scientists, and program support specialists. The Microbiology Division has the staff, the know-how, and the facilities to help address the scientific challenges encountered by FDA and other government organizations. Some examples of the research projects within the Division and collaborative research with scientists from other NCTR Divisions, FDA Centers, academic institutions, and industry are described below. Projects are based on FDA priorities and programmatic expertise. The research program is divided into five focal 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 2006 Accomplishments

Division of Microbiology scientists are engaged in research addressing a variety of critically important issues in food safety and biosecurity relevant to the missions of FDA and other regulatory agencies. Recent outbreaks of *E. coli* O157:H7 and *Salmonella* underscore the necessity to address the threat of foodborne pathogens in our food supply. For example, in collaboration with FDA's Center for Food Safety and Applied Nutrition (CFSAN) and United States Department of Agriculture's Agricultural Research Service (ARS), microarray biochips are being developed and validated for rapid and accurate identification of multiple virulence and antimicrobial resistance genes in *Salmonella* serovars. In collaboration with CFSAN, the Division has characterized *Salmonella* and *Vibrio* spp. isolated from seafood samples using the most current state-of-the-art-molecular techniques.

Scientists in the Division of Microbiology collaborate with microbiologists at the FDA's Arkansas Regional Laboratory (ARL) to survey imported fish samples for antibiotic-resistant bacteria. The genetic determinants conferring antibiotic resistance in these bacteria are also being characterized.

In collaboration with NCTR ARL Bioterrorism Mass Spectrometry (MS) programs, methods are being developed for the rapid detection of bacterial foodborne pathogens using pyrolysis and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS) technology with multivariate statistics and artificial neural-network pattern recognition. Division scientists have also developed and completed research for an interagency agreement (IAG) with the USDA and the Department of Homeland Security for a project on the survivability of *Bacillus anthracis* in foods.

FDA is increasingly being called upon to make regulatory judgments on safety questions related to the gastrointestinal microfloras of humans and animals. The Division of Microbiology research staff has the expertise and long-standing interest in assessing the risks to the gastrointestinal microflora of humans of antimicrobial compounds 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.

Microbiology scientists have developed a series of molecular techniques that can measure perturbations of the intestinal microflora population resulting from exposure of the consumer to antimicrobial residues, probiotics, and dietary supplements. Research has also been undertaken to address the role of intestinal microflora in the metabolism of xenobiotic compounds. In addition, experiments are being conducted on antimicrobial resistance mechanisms, such as efflux pumps, found in commensal organisms in the gastrointestinal tract. The Division is also evaluating the effects of fluoroquinolones on resistance development in bacteria from the human intestinal tract.

Division scientists have entered into 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. Results from the investigation will allow FDA to evaluate the long-term use of this antibiotic on the development of antimicrobial resistance and may give guidance for understanding the drug characteristics that minimize resistance development. An FDA Office of Women's Health project has been initiated to investigate the protective effect of *Lactobacillus* against *Staphylococcus aureus*-mediated toxic shock syndrome. This represents a new area of research for the Division in potential probiotic issues in women's health. Another new field for investigation in the Division of Microbiology is determining the critical role that the normal microflora of the human skin has in the metabolism of tattoo pigments and topically applied colorants. A comprehensive study has been completed on the enzymatic mechanisms of azo dye degradation by skin microflora. Microbiology scientists are developing proteomic approaches to identify in *Staphylococcus aureus* the extracellular proteins responsible for staphylococcal pneumonia.

The environmental biotechnology research program in the Division of Microbiology supports FDA in its assessment of potential environmental impacts of regulated chemicals during their lifetime in the environment. To this end, the research program is incorporating the new omics technologies to elucidate the microbial degradation of polycyclic aromatic hydrocarbons (PAHs) found in industrial, agricultural, and municipal effluents and in contaminated sediments. The use of systems-biology approaches for elucidating the metabolic pathways of PAH degradation will further help FDA understand the fate of these biohazardous compounds in the environment and in the human body. 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. This investigation has increased our understanding of the ability of microorganisms to degrade residues of antimicrobial drugs, thereby reducing the pressure for the selection of drug-resistant strains of bacteria.

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 2006, the Surveillance/Diagnostic Program prevented

pathogen introduction by quarantined animals by 1) detecting bacterial pathogens in the cage water from commercially purchased mice; 2) monitoring the health, environment, and food of the animals in the established NCTR breeder colonies; 3) working closely with the Division of Veterinary Services to monitor the success of a bacterial pathogen eradication program in the NCTR breeder mouse population; and 4) expanding the use of molecular techniques to detect the presence of difficult-to-culture pathogenic bacteria. The Division provided microbial cultures to researchers in the United States and foreign countries as well as to researchers at NCTR. Microbiology scientists provided technical support to NCTR researchers by 1) supplying and maintaining microbial cultures used for research; 2) supplying culture media and reagents; and 3) providing culture identification and antibiotic sensitivity testing.

FY 2007 Plans

Food Safety and Biosecurity, Food Biodefense, and Methods Development

1) In collaboration with CFSAN and USDA, two *Salmonella* biochips are being developed and validated for rapid and accurate identification of multiple virulence and antimicrobial resistance genes in *Salmonella* serovars. The joint effort between the FDA Centers and USDA will be useful in transferring microarray technology from the research stage at NCTR to the FDA field laboratories and law enforcement mobile laboratories.

2) In collaboration with the ORA ARL Bioterrorism Mass Spectrometry project, researchers in the Division of Microbiology are continuing to develop and validate methods for rapid detection of multiple bacterial pathogens using pyrolysis and MALDI-ToF MS technology with multivariate statistical and artificial neural-network pattern recognition.

3) *Salmonella* and *Vibrio* spp. isolated from seafood samples will be characterized by pulsed-field gel electrophoresis (PFGE), multiplex PCR (polymerase chain reaction) ribotyping, ERIC (enterobacterial repetitive intergenic consensus)-PCR, multilocus seqencing, and RAPD (randomly amplified polymorphic DNA) methods. Multidrug-resistant bacteria, including sulfonamide and ciprofloxacin-resistant *Salmonella* spp., will be characterized for integrons and plasmids. The amplified integrons will be sequenced to determine any unique characteristics.

4) The Division will continue working on a project in collaboration with USDA on the survivability of *Bacillus anthracis.*

Antimicrobial Resistance

1) The *van* operons from 17 human vancomycin-resistant enterococcal isolates were amplified and tested for differences by a PCR-RFLP (restriction fragment length polymorphism) method. These isolates had differences in the operons. The *van* operons from these isolates will be cloned and sequenced to determine differences at the sequence level.

2) *Aeromonas* spp. isolates obtained from FDA's Center for Veterinary Medicine (CVM) will be analyzed by PFGE, PCR, and other molecular biology-based typing methods.

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

4) 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. In addition, *tet*-resistant *Citrobacter*, *E. coli* and *Pseudomonas* isolates will be characterized from aquaculture samples.

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

6) Our study of efflux-mediated drug resistance will address the physiological role of multidrug efflux pumps in the *Escherichia coli* model system using microarrays to monitor changes in the transcriptome upon their knockout (deletion), inhibition, or substrate challenge. This will be done through collaboration with the NCTR Center for Functional Genomics.

7) In light of the grave potential outcome of an influenza pandemic, research efforts in FY 2006 included collaboration with St. Jude Children's Research Hospital in Memphis, Tennessee. Preliminary data resulted in the submission of a proposal titled "Interaction of influenza and *Staphylococcus aureus* in the mouse and ferret models of severe pneumonia" to the National Institutes of Health. While not funded on its initial submission, the animal models of influenza and bacterial superinfection that have been established in mice and ferrets will be used to identify virulence factors in the virus and bacteria that contribute to the synergism. Genes encoding suspect virulence factors will be altered or deleted and the effects on disease will be examined.

Gastrointestinal Microbiology and Host Interactions

1) Vancomycin-resistant enterococci (VRE) have become a major food safety issue. We recently discovered that a vancomycin-resistant *Lactococcus lactis* that we isolated from a competitive exclusion product used in poultry could transfer the *van*A gene to *Staphylococcus aureus*, which is often found in humans. 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 (GI) tract-associated bacteria.

2) *Lactobacillus* serves as an important indicator of GI and vaginal tract health and is heavily used by consumers, either intentionally as probiotic supplements or unintentionally in microbially fortified foods. Less complicated studies, such as assessing growth fitness or identifying metabolites of endogenous steroid molecules (hormones and bile acids), will be conducted in *Lactobacillus,* as will studies assessing the effects of microbicides and spermicides. In vaginal health, *Lactobacillus* microarrays will be used to address questions on efflux pump function.

3) The Division will analyze degradation products of azo dyes by skin and intestinal microorganisms, using specific enzymatic treatments, high-performance liquid chromatography (HPLC), and LC-MS/GC-MS methods. Since the three-dimensional structure of the azoreductase from *Enterococcus faecalis* has been solved, site-directed mutagenesis will be employed to study the enzyme activity center and flavin mononucleotide (FMN) binding motif and to characterize properties of the mutant

proteins. 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 will also continue to study structure and function of the azoreductases from *S. aureus* and environmental microorganisms.

4) Entering into the second year of the CRADA(with Pfizer Animal Health, we will continue our study of the metabolism of the third-generation cephalosporin ceftiofur by the bovine intestinal microflora.

5) In FY 2006, our proposal "Protective effect of vaginal *Lactobacillus* species against *Staphylococcus aureus*-mediated toxic shock syndrome" was funded by the FDA Office of Women's Health. In FY 2007, the Division will carry out this research to investigate whether *Lactobacillus*, when supplemented in tampons, has the capacity to exert probiotic benefits by decreasing either *S. aureus* proliferation and/or TSST-1 exotoxin production.

Environmental Biotechnology

1) The Division will conduct transcriptomic and proteomic analyses to understand PAH metabolism in *M. vanbaalenii* PYR-1. The results will be integrated with metabolomic and genomic data, which will give us a deeper understanding of how the bacterium responds to its environment and degrades PAHs at a systems-biology level.

2) To identify microorganisms that degrade fluoroquinolones, samples from soils and wastewater treatment plants to screen for the presence of fluoroquinolone resistance genes and for bacteria that degrade or transform *N*-phenylpiperazine and fluoroquinolones will be obtained. The Division will prepare DNA and sequence the 16S ribosomal RNA genes from any bacteria that appear to produce metabolites from either *N*-phenylpiperazine or fluoroquinolones, and will submit the 16S rRNA sequences of bacteria to the GenBank.

Microbiological Surveillance and Diagnostic Support of Research

The goal for FY 2007 is the development and implementation of new techniques for the detection and identification of pathogenic microorganisms.

Contribution to FDA's Strategic Goals

The Division of Microbiology uses an interdisciplinary approach to be responsive to FDA-regulatory needs. The Microbiology Division staff has a number of projects in conjunction with other FDA Centers to provide critical research to address FDA's strategic goals. Division of Microbiology scientists provide research, guidance, and expert advice to other FDA Centers, national regulatory agencies, and the World Health Organization for emerging public health issues. In addition, Division scientists respond to FDA-regulatory research needs in food safety, antimicrobial resistance, gastrointestinal microflora, and host interactions and environmental biotechnology.

A significant portion of the research in each focal area in the Division of Microbiology is focused on addressing specific FDA high-priority issues.

In food safety, researchers in the Division of Microbiology have developed diagnostic microarray assays and other rapid molecular techniques for high-throughput screening of virulence and antibiotic resistance genes in foodborne pathogens to assess the threat of bacteria in foodborne outbreak investigations.

The Division of Microbiology developed an interagency agreement with USDA to study the growth and inactivation of a surrogate strain of *Bacillus anthracis* in liquefied egg products at storage, permissive, and nonpermissive temperatures. The research in Food Biosecurity and Bioterrorism in the Division of Microbiology not only relates directly to the FDA's mission but also shows the adaptability of the scientists to develop liaisons with other federal agencies to deal with perceived threats of bioterrorism through the food chain.

The intestinal bacterial population plays an important role in human health acting as a barrier to infection as well as contributing to the digestion of dietary components and metabolism of drugs. Scientists have developed the methods to predict and monitor changes in this complex bacterial population. In addition, research is being conducted on the importance of human intestinal microflora in the metabolism and conversion of food additives, food supplements, and antimicrobial agents which have altered biological activities from those of the parent compounds resulting in activation or detoxification. 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. This research supports the FDA's Strategic Goal 3 (Increase access to new medical and food products) and increase access to safe and effective veterinary products and to safe and nutritious food products, including products for unmet animal and human needs).

Before a veterinary drug can be granted approval for marketing, FDA requires that the drug be subjected to environment risk assessment. 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. Drug residues that persist in the environment may select for resistance strains of bacteria. If the drugs can be metabolized to inactive products, they will no longer select for bacterial resistance. The research conducted by the Microbiology Division is increasing our understanding of the metabolism of these compounds and will further help FDA understand the fate and environment effects of antimicrobial compounds.

Microbiology Ongoing Research Projects

NCTR's Strategic Goal 1 — Advance the scientific approaches and tools to attain personalized nutrition and medicine for the American public

PI: Chen, Huizhong, Ph.D.

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

Collaborating Division(s):
Biochemical Toxicology

Objective(s):
To focus on metabolic capacity and enzyme expression in human skin microflora: 1) Biodegradation and bioconversion of pigments used for tattooing and permanent makeup 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 overexpress 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*; and 5) To elucidate the role of the microflora with potential genotoxic effects of tattoo and permanent makeup pigments.

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

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 discovering the species which are noncultivated; 3) To isolation and identify the bacterial species from probiotics used for human or animal health; and 4) To develop a microarray method for the detection of intestinal bacteria.

PI: Elkins, Christopher, Ph.D.

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

Collaborating Division(s):
Systems Toxicology

Objective(s):
1) To evaluate of intrinsic drug resistance of *Lactobacillus* isolates from various sources; 2) To achieve functional identification of *MDR* genes from currently sequenced *Lactobacillus* genomes using genomic and proteomic approaches; and 3) To achieve epidemiological profiling via pulsed-field gel electrophoresis (PFGE) and microarray technology for resistance determining factors (RDFs).

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

Objective(s):
To determine whether probiotic administration of *Lactobacillus* can thwart *S. aureus* TSST-1 production if supplemented in women's tampons. Alternatively, bacteriophage therapy may be investigated as a multifaceted approach to strengthening probiotic introduction for such conditions.

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

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

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; and 5) To compare the metabolism of ceftiofur with the human third-generation cephalosporin, ceftriaxone.

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

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

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; and 2) To generate a proteomic profile for *S. aureus* RN6390 and its *agr* and *sar* isogenic mutants.

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

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

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 FDA in making regulatory decisions that require banning and/or approving the use of certain antibiotics in poultry and farm animals.

PI: Paine, Donald D.

Microbiological Diagnostic Methods: Development, Testing, & Evaluation (E0026200)

Objective(s):

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

PI: Rafii, Fatemeh, Ph.D.

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

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: Wagner, Robert D., Ph.D.

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

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; and 4) To evaluate the potential for *L. lactis* P1-79 to transfer antimicrobial resistance genes to *Enterococcus faecium* or *Staphylococcus aureus*.

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

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.

Probiotic Effects on Host Defense Against Enteric Pathogens (E0709701)

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 the human flora-associated (HFA) mouse on immunodeficient and immunocompetent host-protective systems; and 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*.

NCTR's Strategic Goal 2 — Develop science-based best practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory science process to create a seamless bench-to-bedside continuum

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

Proteomic Approaches to Elucidate Biodegradative Pathways (E0711801)

Collaborating Division(s):

Biochemical Toxicology
Systems Toxicology

Objective(s):

1) To use a proteomic approach to isolate putative catabolic proteins that are overexpressed when microorganisms are grown in the presence of polycyclic aromatic hydrocarbons; and 2) To use omics approaches to understand the environmental fate of PAHs.

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

Microbial Degradation of Fluoroquinolone Antimicrobial Agents (E0722701)

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 and levels in the environment.

NCTR's Strategic Goal 3 — Develop and apply rapid detection technologies and testing platforms to assure food safety, biosecurity, food biodefense, and to combat bioterrorism

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

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

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 (Enterobacterial repetitive intergenic consensus), and REP-PCR (Repetitive Extragenic Palindromic) 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 A., Ph.D.

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

Objective(s):

1) To determine of the lag phase duration (LPD), growth rate (GR), and maximum population density (MPD) of *B. anthracis* Sterne strain at different temperatures used for storing and cooking liquid eggs; and 2) Inactivation kinetics of spores of Sterne strain at different temperatures.

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

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

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) To used in fish farming systems; and 2) To isolate, characterize, and identify OTC- and SDO-resistant organisms from aquaculture sediment and natural environment samples and conduct molecular characterization of the genes that regulate resistance to the drugs.

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

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

Collaborating Division(s):

Systems Toxicology

Objective(s):

1) To determine the preharvest sources and/or vectors of horizontal transmission *Salmonella* in turkey flocks: 2) To evaluate the intrinsic resistances of *Salmonella* isolates to multiple antibiotics; 3) To assess the genetic diversity and epidemiological profiles of *Salmonella* strains isolated in a turkey production environment; and 4) To develop DNA-based and microarray assays to detect genes in *Salmonella* isolates that are involved in antibiotic resistance and pathogenicity.

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

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

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.

Microbiology Research Projects Completed in FY 2006

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

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

Objective(s):

1) To evaluate individual component bacteria in a defined competitive exclusion (CE) product for exclusion of enteric pathogens from Caco-2 and CRL-2117 cell monolayers; 2) To define the antimicrobial susceptibility patterns of the component bacteria using Minimal Inhibitory concentration measurements; 3) To perform sequence analysis of 16s rRNA polymerase chain reaction (PCR) products from defined culture component bacteria and development of a database containing the sequences for use in subsequent identification of the organisms in undefined CE products; and 4) To apply the 16s rRNA sequence analysis procedure to detect and identify effective CE component bacteria in undefined CE products.

Results:

1) The *in vitro* assay measured the amount of a defined mixture of 29 human bacterial isolates (7.83 log10 CFU) and of the Preempt competitive exclusion product (4.05 log10 CFU) that significantly reduced *Salmonella* invasion of 6.41 log10 Caco-2 human intestinal-like cells and 6.89 log10 of CRL-2117 chicken intestinal cells; 2) Most of the bacteria isolated from the Preempt competitive exclusion product were susceptible to antibiotics; however, there were tetracycline-resistant *Bacteroides* spp., erythromycin-resistant enterococci, and vancomycin-resistant *Lactococus lactis* isolates that should be studied further for abilities to transfer resistance genes to other bacteria; and 3) The composition of the Preempt competitive exclusion product was confirmed for 48% of the strains reported to be present in the product. Of the 20 strains isolated, 48% were not strains reported to be present in the product. The 16S rRNA sequence comparisons with GenBank entries provided more reliable identifications of anaerobic bacteria than fatty acid methyl ester or biochemical profiling techniques.

Division of Neurotoxicology

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Neurotoxicology Executive Summary

Introduction

Brain disorders and other nervous system-related disorders account for more hospitalizations than any other major disease group in the U.S. with approximately one in four Americans suffering a brain-related disorder sometime during their lives. Recent advances in biomedical research are currently providing scientists with a variety of new tools with which to better understand the etiology of brain-related disorders and to further reduce the risks associated with exposure to neuroactive substances. It has long been known or suspected that the causes of brain-related disorders include exposure to chemicals such as therapeutic drugs, food additives, food stuffs, cosmetic ingredients, pesticides, and naturally occurring substances. Since the number of potential neurotoxicants that require FDA regulation is estimated to be in the thousands, it is critical that methods be developed to assess neurotoxic risk. It is clear that chemicals from the categories listed above are vital to the national economy and our quality of life; therefore, the challenge is to determine at what dose and under what conditions these compounds can be used while minimizing the likelihood that they will cause adverse effects on the nervous system. In addition, it will be important to determine individual characteristics, such as genetic or metabolic, that affect a person's response to neurotoxic insult.

The overall goals of the Division are to develop and validate quantitative biomarkers and identify biological pathways associated with the expression of neurotoxicity. An increased understanding of the processes associated with neurotoxic outcomes will provide opportunities for improved assessments of risk and identification of potential therapeutic approaches. The strategy for achieving these goals is to employ multidisciplinary approaches that capitalize upon the Division's expertise in neurochemistry, molecular neurobiology, neuropathology, neurophysiology, and behavior. Some of the more unique features of our 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) employ multiple species, including nonhuman primates, rodents, and occasionally humans, in the risk-assessment process to reduce the uncertainty associated with extrapolating findings across species.

FY 2006 Accomplishments

Several protocols have provided important data for the regulatory needs of FDA, including: 1) studies on the ubiquitous food contaminant, acrylamide (in collaboration with FDA's Center for Food Safety and Applied Nutrition); 2) pediatric anesthetic agents, including ketamine (in collaboration with FDA's Center for Drug Evaluation and Research (CDER) and the National Institute of Child Health and Human Development (NICHD) ; 3) the acne medication, retinoic acid (in collaboration with CDER and NICHD); and 4) a treatment for Attention Deficit Hyperactivity Disorder, methylphenidate (in collaboration with CDER and NICHD). In addition, we continued to address three main areas of fundamental research designed to examine

their involvement in the expression of neurotoxicity in both adult and developing animals: 1) monoamine neurotransmitter systems; 2) mitochondrial dysfunction and oxidative stress; and 3) the N-methyl-D-aspartic acid (NMDA) receptor complex. Work in these areas provides fundamental knowledge concerning mechanisms associated with neurotoxicity and a foundation from which to predict adverse effects of chemicals that affect these systems.

Data from our rodent lifetime-neurotoxicity studies on acrylamide suggest that few effects can be detected out to one year of age. A significant dose-related delay in vaginal opening, decreases in brain weight at three and six months of age, and disrupted behaviors associated with motivation and learning have been noted, but only at the highest dose tested (5 mg/kg/day).

Ketamine-induced neural cell death in our developing-monkey cell-culture model was shown to be both apoptotic and necrotic in nature, and a manuscript on these findings has been published. Based upon critical periods of rapid synaptogenesis, the timing of exposures becomes important when comparing rodent, nonhuman primate, and human neurotoxic outcomes. Initial *in vivo* nonhuman primate studies have indicated that, as observed in the rodent, there are ketamine-sensitive periods in the monkey. Parallel studies in both the rat and the monkey demonstrate the comparability and predictability of the two models.

The fluorescent anterograde tracer Fluoro-Ruby and a new and improved version of the neurodegenerative stain, Fluoro-Jade (C-version), were used to show that dopaminergic axons and terminals can be destroyed – not just damaged – by amphetamine. Other studies on amphetamine showed specific gene changes associated with neurotoxicity in discrete brain regions. The observation that NMDA antagonists protect the somatosensory cortex from amphetamine-induced neurodegeneration but do not protect the limbic cortex provides further evidence that the mechanisms involved in neurodegeneration – and the physiological responses to it – are different in differing brain areas.

The Division of Neurotoxicology is partnering with NCTR's Division of Genetic and Reproductive Toxicology in a unique study on the effects of chronic methylphenidate (a drug commonly used to control Attention Deficit Disorder in children) treatment in preadolescent rhesus monkeys. About eight months of complex behavioral data have been collected and will be used to examine the effects of weekday methylphenidate administration on cognitive function in a pediatric animal model.

The Division played a critical role in the FDA Microarray Quality Control Project (MAQC), 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 findings from the MAQC study have been made readily accessible to the microarray community and allow individual laboratories to more easily identify and correct procedural problems. One area that has particularly benefited from these improved methods involves the NMDA-receptor complex as a mediator of adult and developmental neurotoxicity. Genomics-based approaches have been utilized to investigate the cellular changes in rats that underlie the behavioral toxicity associated with developmental exposure to NMDA-receptor antagonists and related compounds. Prolonged exposure of juvenile rats and monkeys to these agents has been shown to cause severe and long-lasting changes in critical aspects of brain function, including learning and simple discriminations. Genomic analyses of these changes should provide valuable information concerning the fundamental biology underlying these aspects of brain function.

Studies on 3-nitropropionic acid (3NPA)-induced mitochondrial dysfunction in the rat showed a significant increase in uncoupling protein-2 (UCP-2) gene expression in the striatum. Real-time quantitative polymerase chain reaction (RT-PCR) techniques were used to demonstrate this effect. Modulation of UCP-2 expression by pretreatment with the mitochondrial enhancer, L-carnitine, was observed indicating that preconditioning with carnitine prevents mitochondrial uncoupling, aggravation of energy loss, and toxicity.

Studies on the neurochemical and behavioral alterations associated with Accutane (13-cis-retinoic acid) treatment were completed. Here, validated tests were used to monitor depression-related behaviors in laboratory rats. There was no indication that Accutane treatment enhanced depression-type behaviors.

Studies on the assessment of human brain function using the NCTR Operant Test Battery continued in children. Data collection is in progress for children exposed to morphine as neonates, those with major depression, and those with anxiety disorder.

To provide further support of our research endeavors, 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, one manuscript describes the use of real-time PCR to demonstrate that MPP^+ (1-Methyl-4-phenylpyridinium) induces a selective alteration in the gene profile of a neuronal cell line in culture. These data indicate that multiple pathways (such as apoptosis, oxidative stress, iron binding, cellular metabolism, and signal transduction) may be involved in aspects of Parkinson's disease.

FY 2007 Plans

Work for the coming year will continue to focus on FDA needs with respect to acrylamide, the pediatric anesthetics, and methylphenidate, in addition to our three primary areas of fundamental research of: 1) monoamine neurotransmitter systems, 2) mitochondrial dysfunction and oxidative stress, and 3) the NMDA-receptor complex. In addition, a large-scale pediatric monkey study on the effects of chronic pramipexole administration will be conducted. Pramipexole (a dopaminergic agonist) is being studied by the manufacturer for its potential to treat restless leg syndrome in children, and the Division is participating in animal pediatric toxicity studies with a focus on cognitive function.

Manuscripts from the MAQC study (conducted in response to the FDA's Critical Path Initiative) will be invaluable scientific resources to the microarray community and provide a foundation for developing a roadmap for the future use of microarrays. This effort directly contributes to NCTR's Strategic Goal 1 (Advance scientific approaches and tools to attain personalized nutrition and medicine). Continued collection of brain-function data from humans using the NCTR Operant Test Battery will further our interspecies comparisons and extrapolations, and provide unique data on specific clinical entities. These efforts support not only NCTR's Strategic Goal 1, but also NCTR's Strategic Goal 2 (Develop science-based best practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory science process to create a seamless bench-to-bedside continuum).

Data collection has been completed from rodent studies designed to identify changes in gene expression associated with long-term exposure to ketamine and remacemide (a sodium channel blocker and NMDA-receptor antagonist). Publication of these data is anticipated in the coming year. In addition, gene changes associated with acute exposure to ketamine during the peak of the brain growth spurt are also being analyzed. The results from these studies will not only

provide fundamental insight into normal developmental processes, but should also provide important data that can inform regulatory decisions for these and related compounds. Other studies will focus on gene expression both *in vivo* (in animal models) and *in vitro* (cell cultures) that may be affected by treatment with compounds of interest. These will include the anesthetic ketamine and related NMDA-receptor antagonists, remacemide, MPTP, MPP^+, 3-NPA, and methamphetamine.

In continued fundamental research into the consequences of mitochondrial dysfunction and oxidative stress, investigations will focus on post-transcriptional and translational regulation occurring during early responses to metabolic stress. RT-PCR, cDNA arrays, 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.

Contribution to FDA's Strategic Goals

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

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

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Neurotoxicology Ongoing Research Projects

NCTR's Strategic Goal 1 — Advance the scientific approaches and tools to attain personalized nutrition and medicine for the American public

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

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

Collaborating Office/Division(s):

Office of Director
Biochemical Toxicology

Objective(s):

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

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

Objective(s):

1) To evaluate the neurotoxicity of different size manganese nanoparticles using PC-12 cultured cells; 2) To determine if *in vitro* exposure to manganese nanoparticles selectively induces specific genomic changes in PC-12 cultured cells using oligonucleotide microarrays; 3) To determine if multiple doses of Mn-nanoparticles produce reactive oxygen species, alterations in lipid peroxidation and/or changes in antioxidant enzymes (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 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; and 7) To determine if multiple doses of Mn-nanoparticles produce morphological alterations in the brain or visceral organs of the mouse.

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

Collaborating Office/Division(s):

Office of Director
The Bionetics Corporation

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 (UAF). Data gathered in this phase will be compared with data from

controls; b) study patterns of tremor and dyskinesia after DBS treatment in a PD/MPTP animal model. Data will be compared in this phase within each animal as its own control; and 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: Binienda, Zbigniew K., Ph.D.

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

Collaborating Office/Division(s):

Office of Director
Systems Toxicology

Collaborating FDA Center(s):

CFSAN

Objective(s):

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

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

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

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; and 5) To determine, using laser capture microdissection (LCM), whether astrocytes and microglia respond differentially to the two dosing paradigms in the absence or presence of neurodegeneration.

PI: Ferguson, Sherry A., 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)

Collaborating Division(s):

Systems Toxicology

Collaborating FDA Center(s):

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 acid; 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; and 8) To quantitate the neurochemical alterations induced by 13-cis-retinoic acid and/or all-trans-retinoic acid treatment.

The Effects of Developmental/Chronic Nonylphenol Exposure over Multiple Generations on Sexually Dimorphic Behaviors and Neurochemical Measures (E0213513)

Objective(s):

To determine whether chronic exposure of rats over multiple generations to nonylphenol, a compound with estrogenic and/or androgenic properties, will alter maternal behavior, the female lordosis response, male-mating behavior, sodium solution intake, amphetamine-induced release of striatal dopamine, or serum levels of testosterone and estradiol in males.

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

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

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; and 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.

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

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

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)

Collaborating Office/Division(s):

Office of Director

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; and 5) To conduct standard postmortem toxicological investigations, including histopathology.

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

Automated Cognitive Assessment of Persons with Alzheimer's Disease (E0715301)

Objective(s):

To investigate whether performance on a variety of behavioral tests that measure timing ability, memory, and learning is different between persons with mild-to-moderate Alzheimer's Disease (AD) and persons who have no diagnosis of AD. This research will also determine which of these tasks is most sensitive to disease severity.

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

Objective(s):

To measure aspects of learning, short-term-memory and attention, motivation, time

perception, and color and position discrimination through a battery of automated tests (games) that will be given to children aged 5 to 13 years.

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

Collaborating Office/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.

Developmental Neurotoxicity Assessment of Acrylamide in Rats (E0215101)

Collaborating Office/Division(s):

Office of Director
Biochemical Toxicology

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.

Developmental Neurotoxicity Assessment of Acrylamide in Rats (E0214801)

Collaborating Office/Division(s):

Office of 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.

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

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.

Effects of Prenatal Cocaine on Behavioral Plasticity (E0663307)

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.

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

Objective(s):

1) 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; and 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.

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

Objective(s):

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

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

Proteomics of Toxicant-Induced Neuronal Degeneration (E0711101)

Collaborating Office/Division(s):

Office of 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; and 4) To resolve the metabolic pathway by which the "degeneration protein" is generated.

PI: Wang, Cheng, Ph.D.

Assessment of Ketamine in the Developing Nonhuman Primate (E0718901)

Collaborating Office/Division(s):

Office of Director
Biochemical Toxicology
The Bionetics Corporation

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; and 4) To determine, at the most sensitive stage and dose, genomic and proteomic responses to ketamine treatment.

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

Collaborating Office/Division(s):

Office of Director
Biochemical Toxicology
The Bionetics Corporation

Collaborating FDA Center(s):

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; and 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.

NCTR's Strategic Goal 2 — Develop science-based best practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory science process to create a seamless bench-to-bedside continuum

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

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

Objective(s):

To determine if Neonatal Intensive Care Unit morphine treatment in male and female preterm infants is associated with long-term alterations in short-term memory and/or motivation at 5-6 years of age.

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

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

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 Operant Test Battery (OTB) to these agents; and 3) To compare the behavioral profiles of these agents to those of a variety of reference compounds with well-characterized mechanisms of action.

Neurotoxicology Research Projects Completed in FY 2006

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

Pharmacological Countermeasures for Space Motion Sickness (E0712401)

Objective(s):

To establish effectiveness and quantify side effects for potential drug countermeasures for Space Motion Sickness (SMS).

Results:

The findings from this study clearly established the utility of using the NCTR Operant Test Battery (OTB) in assessing cognitive function in human subjects. In normal, young adult volunteers, motion sickness was induced using a rotating chair. Performance of the visual short-term memory task of the OTB was assessed before and after spinning, both in the presence and absence of drug treatment (countermeasures) for motion sickness. Spinning alone was sufficient to decrease short-term memory accuracy and response speed. In addition, it was determined that, of the four drugs tested (lorazepam, meclizine, promethazine, and scopolamine), scopolamine was the most efficacious in terms of ameliorating motion sickness while minimally affecting cognitive function. The benzodiazepine lorazepam had the most adverse effects on short-term memory and did not significantly affect motion sickness. OTB tasks are, thus, sensitive to both induced motion sickness and drug countermeasures and, since they are also useable in animal models, may serve to provide models for the preclinical assessment of drug efficacy and toxicity.

Use of the NCTR Nonhuman Primate Operant Test Battery (OTB) as a Predictor of Acute Neurobehavioral Toxicity: Pharmacological Manipulation at Specific Neurotransmitter Receptor Subtypes (E0697901)

Objective(s):

1) To further explore the extent to which the use of operant behavioral techniques in nonhuman primates can serve to reliably model the effects of compounds selected to act on specific neurotransmitter systems; 2) To determine the acute dose-effect relationships of several drugs believed to act primarily at subtypes of specific neurotransmitter receptors using rhesus monkey OTB performance; 3) To characterize the relative sensitivities of the various behavioral endpoints in the NCTR OTB to pharmacological manipulation of specific neurotransmitter systems and to add new tasks to the NCTR OTB; and 4) To more thoroughly characterize the role of specific neurotransmitter systems in the expression of complex brain functions through the pharmacological manipulation of specific receptor subtypes of some of the known major neurotransmitter systems.

Results:

This study served to elucidate the extent to which the use of the NCTR Operant Test Battery can serve to reliably model, in nonhuman primates, the cognitive effects of compounds selected to act via specific neurotransmitter systems. Where comparable human data exist, the effects of drugs on monkey behavior are predictive of drug effects in humans. The acute dose-effect relationships for a variety of psychoactive drugs believed to act via different mechansma were determined in young adult rhesus monkeys. These drugs included: delta-9-tetrahydrocannabinol; marijuana smoke; diazepam; pentobarbital; caffeine; cocaine; methylphenidate; d-amphetamine; chlorpromazine; d-fenfluramine; lysergic acid diethylamide; methylenedioxymethamphetamine; atropine; physostigmine; morphine; naloxone; phencylclidine; and dizocilpine. The profiles of the relative sensitivities of the various behavioral endpoints in the NCTR OTB were different for each and every drug, without exception. Thus, even drugs in the same pharmacological class (i.e., CNS stimulants) like cocaine, d-amphetamine and methylphenidate each affected OTB performance differently. In general, it was found that time estimation behavior was the most sensitive cognitive function of those assessed in the OTB, though others being motivation, learning, short-term memory, and color and position discrimination. The utility of the monkey OTB model is remarkable, and it should serve as a standard for future studies assessing the cognitive effects of drugs.

PI: Xu, Zengjun, Ph.D.

Adolescent Nicotine Administration Effects on CNS Serotonergic Systems (E0709801)

Objective(s):

1) To determine whether adolescent nicotine administration elicits axonal/terminal damage in 5HT systems; 2) To determine if adolescent nicotine administration alters 5HT presynaptic activity; 3) To determine 5HT receptor signaling activity and functions induced by adolescent nicotine exposure; and 4) To determine if adolescent nicotine administration produces

changes in cAMP-medicated signal transduction, 5HT metabolic enzymes, and/or 5HT receptors.

Results:

These studies showed that both fetal and adolescent nicotine exposure caused apparent damage to serotonin projection areas in the brain, with reactive increases seen in regions containing serotonin nerve cell bodies. It is thought that long-term changes in serotonin innervation and/or synaptic activity may play a role in the subsequent development of depression in the offspring of women who smoke during pregnancy or in adolescent smokers.

Division of Personalized Nutrition and Medicine

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Personalized Nutrition and Medicine Executive Summary

The Division of Personalized Nutrition and Medicine was established to leverage current expertise to address research needs supporting DHHS' and FDA's Strategic Goals that address personalized nutrition and medicine. The Division utilizes modern innovative technologies and designs new approaches to identify persons most likely to benefit from, or experience adverse reactions to, particular drugs, devices, biologics, cosmetics, and nutrients. This pharmacogenomic profiling will utilize individualized assignment of exposure on a scientifically sound predictive basis rather than on exposures based upon the expected general population response. The Division personnel are designing protocols to identify single nucleotide polymorphisms (SNPs) and developing statistical classification algorithms to accurately predict efficacy and/or toxicity.

Introduction

NCTR's Division of Personalized Nutrition and Medicine was formed in October, 2006 by combining personnel from the Division of Biometry and Risk Assessment and the Division of Pharmacogenomics and Molecular Epidemiology on the retirement from government service of the two Division Directors. The respective staffs form the Biometry Branch and the Pharmacogenomics Branch within the Division of Personalized Nutrition and Medicine.

The Biometry Branch conducts research to develop new and improved methods for assessing human-health risks associated with exposure to chemicals and biological organisms. The Pharmacogenomics Branch conducts research to identify genetic polymorphisms in various populations and *in vitro,* and to determine how these phenotypes affect not only drug metabolism and toxicity but also disease risk. All of the Division scientists conduct both individual research within the Division and collaborative research with scientists from other NCTR Divisions, other FDA Centers, other government agencies, and academic institutions.

The main functions of the Division of Personalized Nutrition and Medicine are listed below:

- provide leadership and direction in the planning and implementation of strategies for achieving annual and long-range plans for research and research support in the areas of biometry, risk assessment, nutrition, pharmacogenomics, and molecular epidemiology
- design and conduct in-depth studies in the broad areas of classification methodologies, epidemiology, pharmacogenomics, and nutrigenomics as well as studies aimed at assessing interindividual and/or intergroup variability as concerns pharmacokinetic, pharmacodynamic, pharmacogenomic, and/or metabolic parameters
- develop statistical methods and predictive systems for identifying potential health hazards associated with toxic substances and apply statistical methods to the clinical application of personalized medicine

- participate in interagency risk assessment activities to maintain state-of-the-art knowledge and promote the improvement and unification of risk-assessment practices across agencies
- conduct studies aimed at the development, validation, and clinical application of molecular biomarkers of exposure and susceptibility, and conduct epidemiological studies for postmarket surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices.

The main functions of the Pharmacogenomics Branch are listed below:

- provide leadership in the development and conduct of scientific research in the areas of nutrition, pharmacogenomics, and molecular epidemiology
- extrapolate results of animal bioassays and mechanistic studies to humans
- develop and validate alternatives to the standard rodent bioassay for carcinogenicity
- quantitate measures of human exposure to chemical carcinogens, along with an estimate of dose to the carcinogen target tissue
- identify genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy
- assess individual differences in human susceptibility to diseases by a chemical or class of chemicals
- develop studies aimed at the discovery of SNPs, haplotype reconstruction, epigenetic regulation and its application to human diagnostics, and personalized medicine.

The main functions of the Biometry Branch are listed below:

- develop biometrical methods for estimating risks associated with toxic substances and for setting safe exposure levels that correctly reflect underlying uncertainties
- assist FDA centers in conducting risk assessments for the regulation of specific products and in investigating generic risk-assessment issues
- develop mathematical models and computer systems for analyzing pharmacokinetic and pharmacodynamic components of toxic mechanisms
- develop classification algorithms for biomedical decision-making, including identifying food hazards and assigning patients to drug therapies
- develop statistical methods for analyzing genomic, proteomic, metabolomic, and toxicoinformatic data
- apply statistical methods to evaluate toxicological, pharmacological, and nutritional concerns
- provide expertise to NCTR scientists on the design, conduct, and analysis of research studies to evaluate the toxicity of regulated products.

FY 2006 Accomplishments

During FY 2006, Division scientists engaged in research that addressed a variety of problems relevant to science-based regulations:

- developed classification algorithms to facilitate the use of high-dimensional genomic biomarkers for confident clinical assignment of therapies on an individual-patient basis
- identified and discussed key issues in selection of differentially expressed genes for microarray data analysis
- developed improved survival-adjusted statistical methods for animal carcinogenicity/ tumorigenicity data
- identified elevated levels of NAD(P)H:quinone oxidoreductase 1 (NQO1) as a potential early biomarker in pancreatic cancer
- demonstrated that dietary components found in cruciferous vegetables modulate DNA methyltransferase enzymes (which are involved in methylation or epigenetic regulation) in pancreatic cancer
- functionally and epidemiologically characterized a novel SNP in the *ERCC6* gene that is associated with susceptibilities of lung cancer and age-related macular degeneration
- developed Windows-based general purpose PBPK/PD (physiologically based pharmacokinetic/pharmacodynamic) modeling software that can simulate pharmacokinetic data during postnatal growth in mice, rats, dogs, and humans
- developed and validated a PBPK/PD model for acrylamide and its metabolites in rats and mice and then utilized to predict potential DNA adduct levels in humans
- developed a LC/MS-MS (liquid chromatography-mass spectrometry) method for simultaneous analysis of dopamine, dopac, 3MT, HVA, serotonin, 5HIAA, epinephrine, and norepinephrine and applied the method to brain samples from rats exposed to acrylamide
- developed statistical methods to evaluate treatment effects on gene expression within biological pathways; these methods substantially improve the accuracy of p-values by adjusting for correlations among genes
- delineated the statistical models and benchmark approach for quantitative risk assessment of developmental effects
- developed classification algorithms to balance sensitivity and specificity when class sizes are unbalanced
- developed hierarchical models for probabilistic dose-response assessment.

FY 2007 Plans

During FY 2007, Division scientists will continue research that addresses a variety of problems relevant to science-based regulations:

- develop computational algorithms that will efficiently compute adjusted p-values for the large numbers of subsets defined through gene ontology
- develop decision models for clinical assignments of patients based on the patient's genomic features and disease phenotypes
- develop methods to identify genomic biomarkers to improve class-predictive algorithms
- develop new statistical methods for estimating elapsed time between onset of, and death from, an occult tumor when cause of death is not available
- develop an optimal tree-based ensemble statistical method for class prediction

- develop methods for integrating the associations between the genomic predictor variables (such as biomarkers) and phenotype class variables (such as tumor types)
- assess interindividual variability in expression of DNA methyltransferases in liver samples selected according to smoking status, gender, and age
- screen chemicals found in natural products for potential as anticancer agents against pancreatic cancer
- develop an *in vitro* model to determine gender differences of differently expressed proteins in response to gemcitabine pancreatic cancer treatment alone and in combination with indole-3-carbnol
- determine the effects of nicotine and other cigarette components on exocrine and endocrine human pancreatic cells *in vitro*
- identify gender differences in gene expression of chemical transporters known to be involved in transport of chemotherapeutic drugs and with hepatic expression in human liver tissues
- examine the role of interindividual variability in response to exogenous agents as it may relate to breast cancer risk in African-American women
- determine the effects of 4-OH TAM on SULT1A1 (sulfotransferase 1A1) activity in breast cancer cell lines and in tamoxifen-treated breast cancer patients
- determine the impact of selected genomic markers on colorectal cancer case risk, prognosis, and efficacy of treatment
- determine and map polymorphisms in the promoter region of the *SULT1A1* gene and determine its impact on the risk of prostate cancer in African-Americans and Caucasians
- evaluate the variability in hormone metabolism and susceptibility to carcinogen exposure as measured by phenotypic and genotypic variability in carcinogen metabolism
- develop software for a PBPK/PD model, which includes postnatal development
- collect autopsy organ weight data from obese and normal-weight individuals for inclusion in the PBPK/PD software program
- develop predictive models and computational methods for quantitative assessment of benefit/risk models for regulatory decisions in personalized medicine
- identify genes involved in chemoresistance and sensitivity to a group of geldanamycin analogs using pharmacogenomic approaches
- determine the effects of acrylamide and/or PhIP (2-amino-1-methyl-6-phynelimidazo[4,5-f]pyridine) on normal human brain cortical neuronal cells *in vitro*.

Contribution to FDA's Strategic Goals

FDA's Strategic Goal 3 — Increase access to new medical and food products

NCTR's Strategic Goal 1 — Advance the scientific approaches and tools to attain personalized nutrition and medicine for the American public.

The investigators in the Division of Personalized Nutrition and Medicine are developing new technologies to improve human health on an individual basis. The identification and verification of genetic polymorphisms of enzymes involved in the metabolism of chemotherapy drugs for

breast, prostate, pancreatic, and colorectal cancers are ongoing studies within the Division and with collaborations with researchers at the University of Arkansas for Medical Sciences and the adjacent Central Arkansas Veterans Health System. Statistical research on delineating classification algorithms enables assignment of therapies to individual patients to maximize efficacy and minimize adverse events. The development of methods to analyze and interpret data from genomic experiments helps to identify high-dimensional genomic biomarkers of risk, disease, and treatment effects.

Personalized Nutrition and Medicine Ongoing Research Projects

NCTR's Strategic Goal 1 — Advance the scientific approaches and tools to attain personalized nutrition and medicine for the American public

PI: Chen, James, Ph.D.

Network Algorithms to Analyze Gene-Expression Data (E0715901)

Collaborating Division(s):
Systems Toxicology

Collaborating FDA Center(s):
CDER

Objective(s):
1) To develop statistical models and computational methods for class prediction, class discovery, and genetic regulatory networks; and 2) To develop statistical methods for identifying differentially expressed genes, cluster analysis and classification methods, cross validation methods for class prediction, and regulatory networks.

PI: Delongchamp, Robert, Ph.D.

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

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; and 3) To implement computer algorithms that will efficiently compute adjusted p-values for the large numbers of subsets defined through a gene ontology.

PI: Hammons, George J., Ph.D.

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

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; and 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: Lyn-Cook, Beverly A., Ph.D.

Mechanistic Actions of Chemopreventive Agents in Pancreatic Cancer (E0707601)

Collaborating Division(s):
Systems Toxicology

Objective(s):
To screen a number of agents found in natural products and establish mechanistic data on their potential as anticancer agents against pancreatic cancer.

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

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; 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 evaluate the effects of sex hormones on hepatic transporter gene expression in human cancer cell lines and sandwich-cultured hepatocytes; 4) 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; 5) 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; and 6) To develop guidelines and recommendations for clinical-trial design and analysis of sex differences in new drug applications.

The Effects of Nicotine and Other Cigarette Components on Normal and Neoplastic Human Pancreatic Cells: The Role of Low Zinc Levels on *Ras*, *mdr1* Genes Activation, and Metabolizing Enzyme Activities as a Possible Risk Factor for Pancreatic Cancer (E0701701)

Collaborating Division(s):

Systems Toxicology

Objective(s):

1) To determine the effects of nicotine and other cigarette components on exocrine and endocrine human pancreatic cells *in vitro*. 2) To examine *ras*, *mdr1*, CYP1A1, and CYP1A2 expression in normal and neoplastic human pancreatic tissue obtained from a human tissue bank grouped according to race and sex.

PI: Moon, Hojin, Ph.D.

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

Objective(s):

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

Estimation of Lag Time Between Onset of and Death from an Occult Tumor via Attribution of Tumor Lethality (E0717201)

Objective(s):

To develop new statistical methods for estimating the elapsed time between onset of and death from an occult tumor when cause of death (COD) for each animal or context of observation for each tumor is not available.

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

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; and 2) To use Monte Carlo simulation techniques to compare the performance of the proposed decision forest classifiers to the performance of a single optimal decision tree.

PI: Ning, Baitang, Ph.D.

Novel Recruitment Techniques for a Study of Culture-Specific Diet, Metabolic Variability, and Breast Cancer in African-American Women (E0701501)

Objective(s):

1) To examine the role of interindividual variability in response to exogenous agents as it may relate to breast cancer risk in African-American women; 2) To elucidate the effects of substances regulated by FDA on breast cancer risk in African-American women by evaluating risk associated with exposure to oral contraceptives, hormone replacement therapy, and modification of that risk by genetic variability in their metabolism; and 3) To evaluate a model to increase African-American participation in research studies, which might greatly assist in future study design related to FDA-regulated substances in African-American populations.

Pharmacogenomics of Colorectal Cancer Treatment and Outcome (E0714301)

Objective(s):

To determine the impact of selected genomic markers on colorectal cancer case risk, prognosis, and efficacy of treatment using both a case-control study and a case-series follow-up study.

Prostate Cancer: Exposure, Susceptibility, and DNA Adducts (E0702101)

Objective(s):

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

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

Objective(s):

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

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

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; and 4) To archive blood samples, to administrate the Block 98 Questionnaire, and to determine survival data for future studies.

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

Bio-Preg to Windows 2000 Upgrade (E0713001)

Collaborating FDA Center(s):

Office of Chief Information Office/Office of Information Technology

Objective(s):

To upgrade Bio-Preg to a Windows-based program, which will be called PreNatal and to develop a parallel program that covers postnatal development, which will be called PostNatal.

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

Collaborating Division(s):

Systems Toxicology
Z-Tech Corporation

Collaborating FDA Center(s):

CFSAN

Objective(s):

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

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Personalized Nutrition and Medicine Research Projects Completed in FY 2006

PI: Chen, James, Ph.D.

Experimental Design and Analysis of GeneArray Expression Data (E0711201)

Objective(s):

To develop statistical and computational procedures for the design, analysis, and interpretation of gene-expression data from microarray experiments.

Results:

Variability in microarray data is expected and unavoidable. The identification and estimation of different sources of variation are fundamental to the design of cost-efficient microarray experiments. Procedures to estimate various sources of variation and their relative contributions to the overall variation in microarray studies were proposed. In an analysis, a large week-to-week (run-to-run) variation was observed. Development of rigid SOPs, as well as limiting the number of experimental runs, would be expected to have a big impact on reducing this run-to-run variability and, therefore, the overall variability. It is critical that each treatment is represented in each experimental run to avoid confounding and biases that would be introduced due to the different runs. Replication of biological samples is generally the most effective way of reducing this variability with a fixed number of runs and increasing the power for identifying differentially expressed genes.

Different studies have different objectives; different objectives require different experimental design and analysis strategy. Sample size planning should be based on the study objectives, which can be measured by sensitivity, true discovery (i.e., positive predictivity), or accuracy rates. Once a proper statistical model and analysis method are determined, the number of arrays needed (sample size) can be computed by a simulation method for a range of parameter values and correlation structures of interest.

Standard classification algorithms are generally designed to maximize the number of correct predictions (concordance). When the class sizes are not equal, many classification rules will favor the majority class (unfavor the minority class) and result in a high-prediction accuracy on the majority class and a low-prediction accuracy on the minority class. In many applications, such as clinical diagnostic test of rare diseases, the interest is toward predicting the minority class samples with an acceptable accuracy. Two general approaches for classifying unbalanced data sets were developed: 1) decision-threshold adjustment and 2) ensemble classification. Both procedures can improve the balance of prediction accuracies between two classes while maintaining the overall accuracy.

Division of Systems Toxicology

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Systems Toxicology Executive Summary

Introduction

The Division of Systems Toxicology supports the development of new technologies and works to facilitate integration of data from multiple technology platforms for application to questions associated with the FDA's Critical Path Initiative that are in direct support of the FDA mission. Six Centers of Excellence comprise the Division of Systems Toxicology, including the Centers for Functional Genomics, Proteomics, Metabolomics, Hepatotoxicity, Toxicoinformatics, and Chemistry. The emphasis of this Division is to provide technical expertise and guidance for the inclusion of omics and *in silico* data into the review process.

Our research is focused on novel technology development for FDA's Critical Path Research that can lead to personalized medicine. The move toward personalized medicine requires the more global analysis of the phenotypes of individuals on the health-disease continuum that can be ascertained through an integrated systems-biology approach using information from microarray analysis, metabolomics, and proteomics. Because these new technologies generate a great deal of data, a strong informatics underpinning is required. The systems-biology approach to toxicity testing can provide FDA with data that are more easily extrapolated to humans, making data interpretation more facile and relevant.

An important function of FDA is to use risk management to provide the most health promotion and protection at the least cost to the public. Presently, this is accomplished by safety assessment of FDA-regulated products in surrogate organisms and, to a lesser extent, in humans.

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

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

FY 2006 Accomplishments

Center for Metabolomics

The Center for Metabolomics has been established to aid in the assessment of preclinical and clinical safety issues and as part of an FDA-wide biomarkers-development effort. This Center has instigated 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. In FY 2006 the Center for Metabolomics accomplished the following:

- participated in all five subtopic areas of the Metabolomics Standards Initiative (MSI)

- used the UPLC and LCT-Premiere MS to analyze samples from several studies that include acute renal failure during cardiac bypass surgery in children and adults, acute and chronic acetaminophen COMET samples, gentamicin and cisplatin age-related toxicity studies in rats, and cisplatin renal toxicity studies in mice.

Center of Proteomics

The Proteomics group continues to develop methods to help clarify the biological mechanisms associated with toxicity using mass spectrometry-based proteomics. The development of mechanisms to integrate proteomic data with that of other omics data is underway. This work complements the development of software for large-scale protein identification studies and is being extended to assist in quantitative comparisons. This work is necessary to permit a higher sample number to be examined to permit both dose and time studies in support of toxicology studies. Initial work focused on improvements in throughput and sensitivity of mass spectrometry-based proteomic experiments, as well as the development of bioinformatics to handle the large volume of data that are generated. In FY 2006 the Center for Proteomics:

- developed reliable automated nano LC/MS/MS (liquid chromatography/mass spectrometry/mass spectrometry) system that runs twenty-four hours a day, which enabled remote access to monitor and adjust the instrument during nonstaff hours
- ongoing development of ProteinTrack, software that allows confidence criteria to be applied to proteomics results and comparisons made between different proteomics experiments
- identified novel proteins produced by mycobacterium involved in the catabolism of polyaromatic hydrocarbons and identified virulence factors in antibiotic-resistant bacteria
- identified serum biomarkers specific to liver cancer
- developed a workflow for identifying proteins that change in abundance in large-scale proteomics experiments
- developed targeted mass spectrometry experiments for quantitative proteomics by measuring protein fold change between treatment sets for low-abundance proteins
- completed first review of proteomic data submitted to FDA via Voluntary Genomic Data Submission (VGDS).

Center for Hepatotoxicology

The Center for Hepatotoxicology 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. Biomarkers of liver injury and disease in mouse, rat, and human samples are developed through an integrated omics analysis of biofluids and tissues from liver injury and disease. The integration of signaling pathway for cell proliferation, apoptosis, and differentiation provides the framework to assess these biomarkers. Development of new methods for identification 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. In FY 2006 the Center for Hepatotoxicology:

- conducted a mechanism analysis of hepatic effects of antidiabetic drugs and hypolipidemic drugs

- identified a mechanism-of-action of nongenotoxic and genotoxic rodent carcinogens
- determined changes in gene-expression profiles of drug metabolizing genes caused by PPAR (Proliferators Activated Receptor) alpha agonists and other compounds.

Center for Functional Genomics

Microarray data show great promise in drug safety evaluation, and FDA is actively encouraging this new technology. A major effort was continued to identify sources of technical variability in microarray experiments and develop quality assurance/quality control (QA/QC) procedures to help ensure that microarray data submitted to FDA are of sound quality. This effort included colleagues in NCTR's Division of Personalized Nutrition and Medicine and Center for Toxicoinformatics, and collaborations with FDA regulatory Centers. In standardizing toxicogenomics experiments, understanding the potential sources of biological variability so that drug and nutrient effects will not be confounded with normal biological variation is important. Therefore, the impact of age and sex on gene expression in the hearts and livers of rats was determined.

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

FDA is actively encouraging the new omics technology, including DNA gene-expression microarrays as part of the FDA's Critical Path Initiative. In FY 2006 the Center for Functional Genomics:

- developed a method for reducing ozone effects on gene-expression experiments and documenting the dramatic improvement in reproducibility of the results
- optimized hybridization and washing conditions to improve accuracy of gene-expression microarray data
- collaborated in the MicroArray Quality Control project to 1) develop RNA samples for microarray proficiency standards, 2) determine that one-color vs. two-color microarray formats provide equivalent results, and 3) determine that current microarray technology provides the reliability and consistency necessary for regulatory submissions
- collaborated with the External RNA Controls Consortium and proposed methods for the selection and testing of RNA microarray controls
- conducted a systems-biology study to determine sex differences in the hearts of rats
- validated in-house developed mouse MitoChip, the mitochondria-specific oligonucleotide microarray, using real-time PCR (polymerase chain reaction)
- conducted animal experiment in mice to understand the molecular mechanisms underlying gender-associated differences in the adverse reactions to the antiretroviral agent, Zidovudine (AZT), in relation to mitochondrial function
- participated in the development of the *Pharmacogenomic Data Submissions Guidance for Industry* document

Toxicoinformatics Center of Excellence

The Toxicoinformatics group 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, transcriptomic, proteomic, and metabolomic) datasets. In FY 2006 the Center for Toxicoinformatics:

- expanded the functionality of ArrayTrack™ to warehouse, visualize, analyze, and interpret data from proteomics, metabonomics, and clinical and nonclinical data
- provided ArrayTrack™ training to FDA reviewers and scientists; additional training courses have also been offered at NCTR and the University of Arkansas for Medical Sciences
- integrated ArrayTrack™ into the VGDS Program with training for reviewers, analysis of submitted data, and formal discussions between NCTR, FDA's Center for Drug Evaluation and Research (CDER), and pharmaceutical sponsors
- integrated ArrayTrack™ with many commercial tools, such as GeneGo, API, GeneLogic Genesis, DrugMatrix, KeyMolnet, and SpotFire, making, these tools freely available to FDA scientists and reviewers
- developed ProteinTrack to support the NCTR proteomics research
- participated in the development of the *Pharmacogenomic Data Submissions Guidance for Industry* document
- provided general support to the NCTR in omics data management and analysis
- developed several novel consensus methods, including Decision Forest, for diagnostic classifier and omics signature identification
- hosted the informatics infrastructure – the VGDS and Janus servers are funded by CDER – at NCTR to support VGDS
- directly involved in data analysis of VGDS submission
- hosted training courses for several commercial tools at NCTR
- completed the first phase of the MicroArray Quality Control (MAQC-I) Project (results are summarized in six publications of *Nature Biotechnology)* and began MAQC phase II with the focus on developing consensus on predictive signature.

Center for Chemistry

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

Flow cytometric methods were developed for ultrarapid detection (two minutes) and quantification (single cells) of foodborne bacterial pathogens. These methods differentiate bacteria with specificity inherent in fluorescence-tagged antibodies and avoid false-positive assays attributable to nonspecific antibody binding or interfering fluorescent signals arising from food matrices.

Nanotechnology is an emerging research area within the Division of Systems Toxicology. In collaboration with University of Arkansas at Little Rock, members of the Chemistry staff

patented two nanotechnology-based cancer therapies and several large-scale nanoparticle production patents. This work complements ongoing sensor technology work for food quality assessment.

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

The Chemistry Group was granted two utility patents: "Food Quality Indicator Device" and "Drift Compensation Method for Fingerprint Spectra" and an application license: "Drift Compensation Method for Fingerprint Spectra".

FY 2007 Plans

In FY 2007, the Division of Systems Toxicology will use the systems-biology approach to take an integrative and iterative approach to test questions following perturbation of biological systems. To accomplish its mission, the Division of Systems Toxicology will:

- develop an integrated, state-of-the-art omics platform (consisting of microarray, NMR (nuclear magnetic resonance)- and MS (mass spectrometry)- based metabolomic, 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
- utilize a series of liver, renal, and cardiovascular toxins to demonstrate the utility of integrated omics analyses
- develop a systems toxicology approach to the integrative analysis of omics data with conventional toxicology assessments
- develop computational models of biological activity, toxicity, and biomarker pattern identification
- continue to develop nanotechnology and sensor technology efforts.

Contribution to FDA's Strategic Goals

The Division of Systems Toxicology contributes to the FDA's Strategic Goal 3 (Increase access to new medical and food products) and Goal 4 (Improve the quality and safety of manufactured products and the supply chain) and availability through better manufacturing and production oversight) on several levels. First, the Division developed a cadre of scientists with expertise in microarray performance and analysis, proteomic tool development and application, and metabolomics. Each expertise has been applied to problems in preclinical development and is being assessed for use in a clinical setting. The microarray, metabolomics, proteomics, and bioinformatics groups have been extensively involved in standards development to aid the acceptance of omics data in a regulatory setting. This work is forming the basis for future best practices. The existence of an internal omics expert panel at NCTR poises NCTR to play a defining role in the integration of omics data into the review process. As a prelude to this, the Division of Systems Toxicology is essential in training regulatory reviewers to assess omics data.

The Toxicoinformatics Group has developed ArrayTrack™ as a reviewer tool for microarray data and at least 100 reviewers have been trained. This tool is under continuous development to

accommodate FDA needs and is currently being enhanced to accept both proteomic and metabolomic data. Division scientists have been essential in the VGDS data analyses and are an integral part of the FDA Interdisciplinary Pharmacogenomics Review Group (IPRG). The Division of Systems Toxicology has also been involved in other aspects of standardization of microarray technology. Specifically, the MAQC program catapulted NCTR into the front-line discussions and contributions to standardization of microarray use. The results of this effort, involving over 50 institutions (including all FDA Centers), were published in six manuscripts that showed that current microarray technology provides the reliability and consistency necessary for regulatory submissions. In addition, two well-characterized reference RNA samples were developed and made available for proficiency/validation testing. The Division is in the process of developing a Cooperative Research and Development Agreement (CRADA) (with a company to jointly develop preclinical biomarkers of liver toxicity using pairs of compounds within a pharmacological (and preferably structural) class to determine omics biomarker signatures of compounds with a known clinical-toxicity profile. Each of the activities of the Division of Systems Toxicology is in support of both the FDA's Critical Path Initiative and the move toward personalized medicine – goals of utmost importance to the mission of FDA.

The Center for Metabolomics contributes to the FDA's Strategic Goal 2 (Improve patient and consumer safety). The Metabolomics group has been at the forefront in discussions of the Metabolomics Standards Initiative (MSI) in this rapidly evolving field. They are working to develop metabolic preclinical biomarkers of hepatic toxicity, renal toxicity, and cardiotoxicity that might translate from preclinical to clinical tests. Metabolomics techniques have been used to determine clinical biomarkers of acute kidney injury during cardiac bypass surgery.

The discovery of novel biomarkers of disease state, drug response, and toxicity is now given highest priority in the FDA's Critical Path Initiative. As such, the development of new proteomic techniques and establishment of proteomic platforms within FDA for clinical biomarker discovery is of utmost importance. It is anticipated that these techniques may be required for future regulatory approval of new drug submissions. The Center for Proteomics is working on the development of automated high sensitivity nano LC/MS/MS (liquid chromatography/mass spectrometry/mass spectrometry) systems for proteomics, managing sample complexity and dynamic range during proteome analyses, and developing clinical proteomic biomarkers of toxicity and disease that center around these goals, and are aligned with the FDA's Strategic Goal 3 (Increase access to new medical and food products).

The Division of Systems Toxicology is utilizing omics technologies, in conjunction with traditional toxicological studies, to determine toxicity and develop biomarker signatures. For example, dyslipidemia is a major health problem in the U.S. and is often manifested by an elevation of the total cholesterol. Fibrates are a class of compounds used to treat dyslipidemias. However, they induce liver tumor in rodents. The Division of Systems Toxicology is studying this class of compounds to improve the safety of these drugs. This work is aimed at fulfilling FDA's Strategic Goal 3 (Increase access to new medical and food products).

The Center for Functional Genomics contributes to FDA protocols directed at:

- developing standards and understanding the strengths and weaknesses of the innovative microarray technology, which has enormous promise in safety assessment of new drugs and medical products and the knowledge gained by the studies will help prepare FDA for acceptance and proper interpretation of data received as parts of new medical products applications

- using a combined omics approach to understand sex differences in the heart and in adverse events associated with antiretroviral therapy. The combination of gene-expression measurements with protein and metabolomic profiles is designed to test the integration of these new innovative technologies for the development of biomarkers and key mechanistic information
- using a newly developed microarray device, the MitoChip, that allows the detailed analysis of mitochondria function and may allow early assessment of safety problems with new drugs and medical devices
- using the microarray technology to better define a standard animal model system widely used in safety testing. By understanding the genes that are active at the various life stages, the animal model system can be used to better predict safety issues in humans.

To help FDA meet the strategic goal of preventing harm from regulated products, the Chemistry staff has developed a rugged, rational, and flexible mass spectrometry-based method capable of differentiating strains. This provides an option to molecular methods for the rapid chemotaxonomy not only of novel strains but also of other sample types that cannot be presently distinguished using molecular technology. Such a capability is useful not only in relation to clinical needs and epidemiological studies, but also to the FDA's food safety assurance mandate. The system would be useful for compliance and monitoring by FDA staff. It would also be useful for the same QA/QC purpose by the food industry itself, a feature consistent with the purpose of Hazard Analysis and Critical Control Point (HACCP) and the FDA's Food Safety Initiative, programs that have been guiding the FDA's fundamental approach to its regulatory mission. If FDA can provide a mechanism by which industry can assure that food safety and quality are maintained, enforcement of compliance can become a second line rather than the first line of defense.

To meet such a need, a quick screening method for species-level identification was developed by the Chemistry staff and is currently being commercialized to the food industry. Sensitivity of the analysis is to the single cell in the volume of rinsate or concentrate assayed: 100 to 200 μL. Each assay takes from 1.5 to 2.5 minutes. The methods have been refined so that there are no false-positives or false-negatives. The detection technology has been demonstrated in food matrices as diversified (and relevant to public health) as raw or cooked chicken products, peanut butter, ice cream, and raw spinach. Litmus, LLC, an Arkansas-based company, is commercializing the Chemistry staff's rapid bacterial identification methods, food quality indicators, and the Spectrometric Data Activity Relationship (SDAR) methods (a series of techniques that can drastically reduce the time and cost of pharmaceutical development and enable rapid toxicological screening).

To address FDA's Strategic Goal 4 (Improve the quality and safety of manufactured products and the supply chain), the Chemistry staff is developing advanced pattern recognition-based algorithms that can use data from *in vivo* magnetic resonance spectroscopy (MRS) exams (an advanced form of MRI) to provide noninvasive, accurate information about brain lesions, breast tumors, and other cancers. The project will lead to improved tumor diagnostic techniques and has the potential to enable early noninvasive tissue screening.

Systems Toxicology Ongoing Research Projects

NCTR's Strategic Goal 1 — Advance the scientific approaches and tools to attain personalized nutrition and medicine for the American public

PI: Beger, Richard, Ph.D.

Biomarkers of Liver Disease and Toxicity (E0718801)

Collaborating Division(s):
Personalized Nutrition and Medicine

Objective(s):
To develop biomarker profiles for normal individuals and those with liver diseases or toxicity.

ADDEND: Metabonomic Analyses after Acute Drug Administration – Cognitive Assessments of Several Psychotropic Compounds using the NCTR Operant Test Battery (OTB) (E0721111)

Objective(s):
To determine the dose-dependent relationship between administered drug and metabonomics profiles in urine and serum.

Preclinical Metabonomic Biomarkers of Toxicity and Disease (E0720401)

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

Collaborating FDA Center(s):
CDER
CDRH

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 are related to toxicity and disease state.

PI: Desai, Varsha G., Ph.D.

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

Objective(s):
1) To develop a MitoChip containing genes associated with mitochondrial function such as oxidative phosphorylation, β-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, a neurotoxin known to alter mitochondrial function; and 3) To verify the relative expression levels of differentially expressed genes by real-time quantitative PCR.

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

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

Objective(s):
To elucidate molecular mechanisms of mitochondrial dysfunction that will address gender-based differences in adverse effects of antiretroviral drugs, such as AZT. This will provide critical information to 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: Edmondson, Rick D., Ph.D.

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

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; and 4) To integrate results objectives 1-3 to provide "front-end" components of a functional proteomics facility.

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

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

Collaborating Division(s):

Genetic and Reproductive Toxicology
Personalized Nutrition and Medicine

Objective(s):

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

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

Collaborating Division(s):

Genetic and Reproductive Toxicology
Personalized Nutrition and Medicine

Objective(s):

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

Toxicological Effects of Ochratoxin A (OTA) (E0709401)

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 liver and kidney as a function of OTA treatment; and 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)

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; and 5) To determine whether the expression of candidate target genes is PPAR-α dependent.

NCTR's Strategic Goal 2 — Develop science-based best practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory science process to create a seamless bench-to-bedside continuum

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

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

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: Fuscoe, James C., Ph.D.

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

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 FDA on sponsor-selected platforms. The experimental results and conclusions from this inter-center project will be shared with other consortial microarray standardization efforts and made publicly available through publication.

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

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

Collaborating FDA Center(s):
CBER
CDER
CDRH

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: Shi, Leming, Ph.D.

The MAQC Project: Microarray Quality Control (E0720701)

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

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

Objective(s):
To establish, through the Microarray Quality Control (MAQC) project, 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 on the identification of differentially expressed genes and the prediction of patient outcomes. The MAQC project will help improve the microarray technology and foster its proper applications in discovery, development, and review of FDA-regulated products.

PI: Tong, Weida, Ph.D.

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

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 (Surface Enhanced Laser Desorption/Ionisation Time of Flight) data sets, and the results will be compared with others derived from traditional classification techniques; and 2) To develop the multiclass Decision Forest method in this protocol. The method will be demonstrated on a gene-expression data set to classify the pediatric acute lymphoblastic leukemia (ALL) subtypes.

NCTR's Strategic Goal 3 — Develop and apply rapid detection technologies and testing platforms to assure food safety, biosecurity, food biodefense, and to combat bioterrorism

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

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

Collaborating Division(s):
Biochemical Toxicology

Objective(s):
To take advantage of the unique physical and electrical properties of nanotubes to develop: 1) novel technologies for the filtration of chemical and biological hazards from air, water, blood, and other media; 2) technologies that protect public health or otherwise benefit the public; and 3) novel nanotube/monoclonal antibody-based cancer therapies.

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

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: Wilkes, Jon G., Ph.D.

Combining MAB/MS with Pattern Recognition to Subtype Bacteria for Food Safety and Food Defense (E0707901)

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 databases for use in rapid chemotaxonomy at the strain and substrain level.

Evaluation of Pyrolysis MAB/Tof MS and Matrix-Assisted Laser Desorption (MALDI)/Tof MS for Food Safety and Food Defense (E0714701)

Collaborating Division(s):
Microbiology

Collaborating FDA Center(s):
ORA

Objective(s):
To evaluate the suitability of mass spectral data obtained from both pyrolysis-metastable atom bombardment MS and matrix-assisted laser desorption/ionization time-of-flight MS techniques for the purpose of rapidly characterizing presumptive bioterror agent samples. This includes analysis of the salient spectral features necessary for identifying microorganisms from

contaminated samples and differentiating tainted samples from hoax sample materials collected from the environment, as well as evaluating the effects of both instrumental- and pattern-definition techniques on the ability to use these features for rapid identification.

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

Objective(s):

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

NCTR's Strategic Goal 4 — Modernize science management and infrastructres to effectively and efficiently support NCTR/FDA goals

PI: Tong, Weida, Ph.D.

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

Objective(s):

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

Systems Toxicology Research Projects Completed in FY 2006

PI: Beger, Richard, Ph.D.

Methods for Predicting Toxicological Properties of Molecules from Their NMR Chemical Shifts Through-Bond and Through-Space Distance Connectivity Patterns (E0712601)

Objective(s):

To produce models that use NMR data and infuse three-dimensional atom-to-atom through-bond connectivity and atom-to-atom through-space intramolecular distance information into a three-dimensional pattern that can be used by pattern recognition software to build a model of a biological or toxicological endpoint. The results of the 3D-QSDAR models will be compared to the results of QSDAR and QSAR models from protocols E0706801, E707701 and E0708301.

Results:

Three-dimensional quantitative spectrometric data-activity relationship (3D-QSDAR) modeling technique, which uses NMR spectral and structural information that is combined in a 3D-connectivity matrix, was developed. The 3D-connectivity matrix is built by displaying all possible assigned carbon NMR chemical shifts carbon-to-carbon connections and distances between the carbons. 2D ^{13}C-^{13}C COSY and a theoretical 2D ^{13}C-^{13}C distance connectivity spectral slices are formed from the 3D-connectivity matrix and used to produce a relationship among the 2D spectral patterns for molecules and biological activities. We called this technique Comparative Structural Connectivity Spectra Analysis (CoSCoSA) modeling. Five manuscripts, one book chapter, one review article, and one patent resulted from research in this protocol. The manuscripts were written on steroids binding to the corticosteroid binding globulin, steroids binding to the aromatase enzyme, molecules binding to the estrogen receptor, models of Log P, and polychlorinated dibenzodioxins, dibenzofurans, and biphenyls binding to the aryl hydrocarbon receptor. The book chapter showed how to use both 13C and 15N NMR data in a 3D- heteronuclear connectivity matrix, and this concept was used to form models of antibiotic cephalosporins efficacy. The models of cephalosporins showed the inside backbone part of molecule was responsible for efficacy and not the side chain attachments. The book chapter also introduced the concept of using molecular dynamics of compounds to produce a 4D connectivity matrix and using the 4D connectivity matrix to estimate configurational entropy in biological processes. The 3D-QSDAR concepts were put into a patent application that has been exclusively licensed by Litmus.

PI: Harris, Angela, Ph.D.

Development and Characterization of Conditionally Immortalized Human Primary Hepatocyte Cell Lines from Female and Male Donors (E0714101)

Objective(s):

To develop *in vitro* model systems for the study of mechanisms of toxicity in humans from different genders and/or ethnic populations.

Results:

Gender differences in gene expression were estimated in liver samples from nine males and nine females. The study tested 31,110 genes for a gender difference using a design that adjusted for sources of variation associated with cDNA arrays, normalization, hybridizations and processing conditions. The genes were split into 2,800 that were clearly expressed (expressed genes) and 28,310 that had expression levels in the background range (not expressed genes). The distribution of p-values from the 'not expressed' group was consistent with no gender differences. The distribution of p-values from the 'expressed' group suggested that 8 % of these genes differed by gender, but the estimated fold-changes (expression in males/expression in females) were small. The largest observed fold-change was 1.55. The 95% confidence bounds on the estimated fold-changes were less than 1.4 fold for 79.3 %, and few (1.1%) exceed 2-fold. Observed gender differences in gene expression were small. When selecting genes with gender differences based upon their p-values, false discovery rates exceed 80 % for any set of genes, essentially making it impossible to identify any specific genes with a gender difference.

Division of Veterinary Services

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Veterinary Services Executive Summary

Introduction

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

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

DVS oversees the operation of 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.

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

The Diet Preparation Facility is a well-equipped, large-scale formulation services unit containing a specific pathogen-free barrier work environment. The majority of dosed diets, water, 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. Specific capabilities include:

- laser capture microdissection (LMD)
- tissue microarrays
- real-time PCR
- genetic monitoring of rodent strains
- proliferation assays including BrdU
- immunohistochemistry (IHC), Ki-67 (MIB-5) IHC, PCNA IHC
- *in situ* hybridization for histone mRNA
- apoptosis assays including TUNEL, ISOL, Caspase-3 IHC, ssDNA monoclonal antibody IHC
- immunoenzyme and immunofluorescent IHC on frozen sections
- immunoenzyme and immunofluorescent IHC on paraffin-embedded sections
- immunoenzyme and immunofluorescent IHC on cultured cells
- *in situ* hybridization – nonradioactive ISH with RNA probes
- nonradioactive ISH with oligonucleotide probes
- PCR solution PCR
- solution RT-PCR
- *in situ* PCR
- *in situ* RT-PCR
- light and fluorescent microscopy
- microphotography/digital and film
- digital macrophotography using necropsy gross system or stereo microscope
- glycol methacrylate and methyl methacrylate plastic embedding and sectioning capability
- clinical pathology (hematology and sperm analysis (rat/mouse) using Hamilton Thorn IVOS Sperm Analyzer).

FY 2006 Accomplishments

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 the National Institutes of Health (NIH) guidelines relative to NCTR's Animal Care and Use Program and participated in semiannual program reviews, facility inspections, and experimental protocol reviews as part of the NCTR Institutional Animal Care and Use Committee proceedings. All animal resource needs were managed for all research projects including ordering, quarantine, housing, and care of animals. Division personnel served as government project officers for the 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 Interagency Agreement (IAG) and Cooperative Research and Development Agreement (CRADA) (initiation, development, and completion. A collaborative effort between NCTR and FDA's Center for Veterinary Medicine (CVM) provided correlative information supporting conclusions concerning pathology reports/results from a swine study at DVM. Division personnel were instrumental in the solicitation, competition, and award of a new Animal Care/Diet Preparation contract in FY 2006, ensuring the continued, uninterrupted provision of high-quality services.

The Veterinary Care Program was administered through this office and, in addition to providing veterinary care to NCTR's research animals, included oversight of policies and procedures for animal procurement and transportation, preventive medicine, health and genetic monitoring, environmental enrichment, surgical protocols, anesthesia of laboratory animals, pain management, and euthanasia. To ensure state-of-the-art housing environments for research

animals, members of this Division played an integral role in planning animal facility renovation projects conducted in 2006. A comprehensive plan to relocate the rodent production colony to a more energy-efficient building was developed, presented to upper management, and accepted. State-of-the-art ventilated caging systems were ordered for the rodent production colony and for rodent quarantine.

Division personnel also provided specialized laboratory support, such as protein sample processing and organellar preparations, along with providing input for protocol development to NCTR principal investigators (PIs) as needed.

Animal Care/Diet Preparation Services

During FY 2005, contract personnel supported an average daily census of thirty-one experiments. These experiments entailed, at a minimum, the daily husbandry services for an average 6847 rodents and 110 rhesus monkeys. A variety of technical procedures were performed on many experiments, including tattooing, tumor palpations, biological sample collections, injections (SC, IM, and/or IV), oral gavage (including neonatal mice), behavior assessments on rats and rhesus monkeys, application of topical-dosed creams, rodent breeding operations, quarantine of rodents and rhesus monkeys, physical and pregnancy examinations of rhesus monkeys, microchip implantations, anesthesia of animals, and humane euthanasia. An ongoing AALAS (American Association for Laboratory Animal Science) training program ensured the maintenance of a high percentage of certified staff. Currently 85 percent of animal care and diet preparation staffs are AALAS-certified, and eight members of the animal care management group are Certified Managers of Animal Resources (CMAR). In addition to processing standard rodent chow (autoclaving, packaging, and delivery), dosed diets, dosed water, and topical creams were prepared in a barrier facility to exacting specifications for National Toxicology Program experiments. A higher percentage of irradiated rodent diet was used in FY 2006, 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 standard operating procedures (275 for animal care; 76 for diet preparation). Veterinary care for all animals was provided by two veterinarians who are board certified in laboratory animal medicine (American College of Laboratory Animal Medicine). An on-site barrier rodent production operation supplied animals for the majority of experiments. Extensive environmental and health monitoring activities were performed in cooperation with NCTR's microbiological surveillance and chemistry support groups to ensure pathogen exclusion from animal colonies, bedding, and feed. Veterinary surgical services were provided for rodent and nonhuman primate studies, and veterinarians served as Principal Investigators or Co-Investigators on several protocols. A significant achievement in FY 2006 was the eradication of *Helicobacter hepaticus* from the Center's rodent breeding colony. Other important endeavors included the expansion of the rodent enrichment program and enhancement of our disaster plan for the animal facilities.

Pathology and Pathology-Related Services

During FY 2006, the Molecular Pathology group developed the following immunohistochemical (IHC) protocols:

- Sox-9 (marker for Sertoli cells), cytochrome p450 side-chain cleavage enzyme (marker for Leydig cells), 3β-hydroxysteroid dehydrogenase (marker for Leydig cells), Pan-Ras,

TRP-1, rapid IHC protocols (~6 minutes) that allow preservation of high-quality RNA in frozen tissue sections (for laser capture microdissection)

- tissue handling and laser capture microdissection protocols for DNA and proteome analysis developed for frozen and formalin-fixed paraffin-embedded tissues
- technique for RNA isolation from microdissected samples using Qiagen RNeasy MicroKit
- specific settings of Positive Pixel Count Algorithm and Nuclear Count Algorithm (Aperio Technologies, Inc) were developed for evaluation of staining intensity and counting the numbers of cells stained with Sox-9, Ki-67, and Tunel

Besides routine pathology services, other accomplishments for FY 2006 include:

- conducted NTP (National Toxicology Program) quality assessment and peer review of pathology data for *Aloe vera* phototoxicity study
- conducted necropsy and tissue collection from minipigs without metal contamination serving as methods development
- provided nonroutine services such as digital macrophotography, laser capture microdissection, and image archiving using digital storage of microscopic images at diagnostic resolution.

FY 2007 Plans

During FY 2007, the Division of Veterinary Services will:

- prepare Animal Care and Use Program Description for AAALAC site visit in summer of 2007
- renew AAALAC certification after site visit in summer of 2007
- continue to support the research mission of NCTR through excellence in animal care, veterinary care, diet preparation, and pathology services
- continue supplying methods development and support, both technical and professional, needed to accomplish the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP) IAG work at NCTR
- continue a quality laboratory animal care and use program consistent with state and federal laws, regulations, and guidelines
- eradicate *Helicobacter hepaticus* from all NCTR research animals
- expand and improve the environmental enrichment program for rodents
- relocate the rodent production colony to Building 62A and implement ventilated caging system housing for this population
- develop and implement a housing plan for swine at NCTR
- continue active participation on research protocols as Principal Investigators and Co-Investigators
- replace chemistry analyzer in Clinical Pathology
- conduct Quality Assessment and Pathology Working Group (PWG) for retinyl palmitate, including demonstration of electronic pathology working group evaluation
- add methodologies in pathology to support research.

Contribution to FDA's Strategic Goals

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

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 or Ph.D.s whose specialties in comparative medicine, veterinary pathology, and biochemistry complement the research teams in all other Divisions. Each research Division contributes to the FDA's Strategic Goals in its own unique way through the individual and collective talents of its personnel as described in this document. DVS, through its support services, functions, and research participation, is part of each Division's contribution to these goals.

Veterinary Services Ongoing Research Projects

NCTR's Strategic Goal 2 — Develop science-based best practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory science process to create a seamless bench-to-bedside continuum

PI: Feuers, Ritchie J., Ph.D.

Evaluation of Calorically Restricted Human Surgical Samples (E0699801)

Collaborating Division(s):
Office of Research Immediate Office
Genetic and Reproductive Toxicology
Personalized Nutrition and Medicine

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

NCTR's Strategic Goal 3 — Develop and apply rapid detection technologies and testing platforms to assure food safety, biosecurity, food biodefense, and to combat bioterrorism

PI: Melvin, Cathy, Ph.D.

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

Collaborating Staff/Division(s):
Environmental Health and Program Assurance Staff

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.

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

Collaborating Staff/Division(s):
Environmental Health and Program Assurance Staff

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.

NCTR FY 2006 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 2006**.

The NCTR 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

The FDA Centers include:

CBER	Center for Biological Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDER	Center for Drug Evaluation and Research
CFSAN	Center for Food Safety and Applied Nutrition
CVM	Center for Veterinary Medicine
ORA	Office of Regulatory Affairs

Adjei, M.D., Deck, J., Heinze, T.M., Freeman, J.P., Williams, A.J. and Sutherland, J.B., Identification of metabolites produced from N-phenylpiperazine by *Mycobacterium spp*., *Journal of Industrial Microbiology and Biotechnology,* 34:219-224 (2007), Accepted: 9/25/2006.

Responsible Division: Microbiology
Collaborating Division(s): Biochemical Toxicology

Adjei, M.D., Heinze, T.M., Deck, J., Freeman, J.P., Williams, A.J. and Sutherland, J.B., Acetylation and nitrosation of ciprofloxacin by environmental strains of mycobacteria, *Canada Journal of Microbiology,* Accepted: 9/21/2006.

Responsible Division: Microbiology
Collaborating Division(s): Biochemical Toxicology

Adjei, M.D., Heinze, T.M., Deck, J.D., Freeman, J.P., Williams, A.J. and Sutherland, J.B., Transformation of the antibacterial agent norfloxacin by environmental mycobacteria, *Applied and Environmental Microbiology,* 72:5790-5793 (2006), Accepted: 6/18/2006.

Responsible Division: Microbiology
Collaborating Division(s): Biochemical Toxicology

Ahn, H., Moon, H., Fazzari, M.J., Lim, N. and Kodell, R.L., Classification by ensembles from random partitions, *Proceedings on US-Korea Conference on Science, Technology and Entrepreneurship Symposium,* Accepted: 8/14/2006.

Responsible Division: Personalized Nutrition and Medicine
Collaborating FDA Center(s): CBER

Ahn, J., Nowell, S.A., Mccann, S.E., Yu, J., Carter, L., Lang, N.P., Kadlubar, F.F., Ratnasinghe, L. and Ambrosone, C.B., Associations between catalase phenotype and genotype: modification by epidemiologic factors, *Cancer Epidemiology, Biomarkers and Prevention,* 15(6):1217-1222 (2006), Accepted: 4/26/2006.
Responsible Division: Personalized Nutrition and Medicine

Baker, S.C., Bauer, S.R., Beyer, R.P., Brenton, J.D., Bromley, B., Burrill, J.D., Causton, H., Conley, M.P., Elespuru, R.K., Fero, M., Foy, C., Fuscoe, J., Gao, X., Gerhold, D.L., Gilles, P., Goodsaid, F.M., Guo, X., Hackett, J., Hockett, R.D., Shi, L., *et al*. The External RNA Controls Consortium: a progress report, *Nature Methods,* 2(10):731-734. Accepted: 10/10/2005 (2005).
Responsible Division: Systems Toxicology
Collaborating FDA Center(s): CFSAN, CVM

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Responsible Division: Systems Toxicology

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

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Responsible Division: Neurotoxicology
Collaborating Division(s): Z-Tech Corporation, Systems Toxicology
Collaborating FDA Center(s): CFSAN, CVM

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Responsible Division: Neurotoxicology

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Responsible Division: Neurotoxicology

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Responsible Division: Biochemical Toxicology
Collaborating Division(s): Office of Director/Immediate Office

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Responsible Division: Biochemical Toxicology
Collaborating Division(s): Toxicologic Pathology Associates

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Responsible Division: Systems Toxicology

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Responsible Division: Microbiology

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Responsible Division: Systems Toxicology

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Collaborating Division(s): Systems Toxicology

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Responsible Division: Systems Toxicology

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Responsible Division: Systems Toxicology

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Responsible Division: Systems Toxicology
Collaborating Division(s): Genetic and Reproductive Toxicology

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Responsible Division: Genetic and Reproductive Toxicology

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Collaborating Division(s): Office of Director/Immediate Office, Personalized Nutrition and Medicine, Neurotoxicology, Genetic and Reproductive Toxicology, Z-Tech, Neurotoxicology
Collaborating FDA Center(s): CFSAN, CVM

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Responsible Division: Systems Toxicology
Collaborating Division(s): Genetic and Reproductive Toxicology, Z-Tech Corporation

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

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Responsible Division: Systems Toxicology
Collaborating FDA Center(s): CDER

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

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Collaborating Division(s): Z-Tech Corporation

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Collaborating Division(s): Toxicologic Pathology Associates

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Collaborating Division(s): Toxicologic Pathology Associates

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Responsible Division: Personalized Nutrition and Medicine
Collaborating FDA Center(s): CDER

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Responsible Division: Genetic and Reproductive Toxicology

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Collaborating Division(s): Genetic and Reproductive Toxicology

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Responsible Division: Neurotoxicology
Collaborating Division(s): Office of Director/Immediate Office, Biochemical Toxicology, Toxicologic Pathology Associates

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Responsible Division: Neurotoxicology
Collaborating Division(s): Office of Director/Immediate Office, Biochemical Toxicology, Toxicologic Pathology Associates, The Bionetics Corporation
Collaborating FDA Center(s): CDER

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Responsible Division: Genetic and Reproductive Toxicology

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Responsible Division: Biochemical Toxicology

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Responsible Division: Systems Toxicology

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Responsible Division: Systems Toxicology
Collaborating Division(s): Biochemical Toxicology, Microbiology, Z-Tech
Collaborating FDA Center(s): ORA

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Responsible Division: Systems Toxicology
Collaborating Division(s): Microbiology

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Responsible Division: Biochemical Toxicology

Wintz, H., Yoo, L.J., Loguinov, A., Wu, Y., Stevens, J.A., Holland, R.D., Beger, R., Perkins, E.J., Hughes, O. and Vulpe, C.D., Gene-expression profiles in fathead minnow exposed to 2,4-DNT: Correlation with toxicity in mammals, *Toxicological Sciences,* 94(1):71-82 (2006), Accepted: 7/24/2006.
Responsible Division: Systems Toxicology

Xia, Q., Chou, M.W., Yin, J., Howard, P., Yu, H. and Fu, P.P., Photoirradiation of representative polycyclic aromatic hydrocarbons and twelve isomeric methylbenz[a]anthracene with UVA light – formation of lipid peroxidation, *Toxicology and Industrial Health,* 22:147-156 (2006), Accepted: 3/8/2006.
Responsible Division: Biochemical Toxicology

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Responsible Division: Biochemical Toxicology
Collaborating FDA Center(s): CFSAN

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Responsible Division: Biochemical Toxicology
Collaborating FDA Center(s): CFSAN

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Responsible Division: Biochemical Toxicology
Collaborating Division(s): Toxicologic Pathology Associates
Collaborating FDA Center(s): CFSAN

Yu, H., Xia, Q., Yan, J., Herreno-Saenz, D., Wu, Y. and Fu, P.P., Photoirradiation of polycyclic aromatic hydrocarbons with UVA light – A pathway leading to generation of reactive oxygen species, lipid peroxidation, and DNA damage, *International Journal of Environmental Research and Public Health,* 3(4):348-354 (2006), Accepted: 8/1/2006.

Responsible Division: Biochemical Toxicology

NCTR Collaborative Activities

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

Interagency Agreements

Interagency Agreements (IAGs) are formal financial partnerships with other government agencies.

Environmental Protection Agency (EPA)

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)

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; and 7) To use these data in pathogen virulence and host susceptibility in a model for the transmission dynamics of Cryptosporidium parvum in human outbreaks.

Environmental Protection Agency/National Health and Environmental Effects Research Laboratory (EPA/NHEERL)

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

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

Objective(s):

To define dose-response curves for benzo[*a*]pyrene DNA adducts in the A/J mouse lung – utilizing the application of high-performance liquid chromatography (HPLC)-ES-MS/MS methodologies developed at NCTR.

National Cancer Institute (NCI)

PI: Pogribny, Igor P, Ph.D.

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

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 upregulated or downregulated in target tissue during the promotion stage of carcinogenesis; and 3) To evaluate whether or not the global and locus-specific DNA hypomethylation, along with aberrant expression of related genes and changes in chromatin conformation, is specific only to target tissues and may be used for early detection of chemicals with carcinogenic potential.

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

PI: Wang, Cheng, Ph.D.

Assessment of Ketamine in the Developing Nonhuman Primate (E0718901)

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; and 4) To determine, at the most sensitive stage and dose, genomic and proteomic responses to ketamine treatment.

National Institute on Drug Abuse (NIDA)

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

Effects of Prenatal Cocaine on Behavioral Plasticity (E0663307)

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.

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

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

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

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 the 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 *cII* gene mutant frequency of Big Blue® mouse; and 5) To determine the pharmacokinetics of methylphenidate and its major metabolite, ritalinic acid, in $B_6C_3F_1$a mice.

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

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; and 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.

U.S. Department of Agriculture/Food Safety and Inspection Service/Office of Food Defense and Emergency Response (USDA/FSIS/OFDER)

PI: Khan, Saeed, Ph.D.

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

Objective(s):

1) To determine of the lag phase duration (LPD), growth rate (GR), and maximum population density (MPD) of *B. anthracis* Sterne strain at different temperatures used for storing and cooking liquid eggs; and 2) To inactivate kinetics of spores of Sterne strain at different temperatures.

National Institute of Environmental Health Sciences/National Toxicology Program Interagency Agreement

William T. Allaben, Ph.D., Associate Director of Scientific Coordination; NIEHS/FDA National Toxicology Program Liaison
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In 1992 the Food and Drug Administration (FDA) entered into an Interagency Agreement (IAG) with the National Institute of Environmental Health Sciences (NIEHS). The design for this agreement concentrates on FDA's priority National Toxicology Program (NTP) nominations of chemicals and agents to be analyzed and utilizes the unique resources and facilities at NCTR. The research conducted under the IAG provides FDA the ability to better assess the safety of FDA-regulated products.

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

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

The NCTR projects listed in this section were supported by NIEHS/NTP in FY 2006 or have been approved for support in FY 2007.

National Toxicology Program

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

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

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.

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

Objective(s):

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

PI: Boudreau, Mary D., Ph.D.

Bioassays in the F344 Rat and the $B_6C_3F_1$ Mouse Administered *Aloe Vera* Plant Constituents in the Drinking Water (E0214201)

Objective(s):

To conduct – in view of the complexities inherent in *Aloe vera* pharmacology and the inconsistencies reported in literature – the bioassays in rats and mice using standardized preparations of *Aloe vera* to explore the limits of safety of *Aloe vera* leaf constituents present in commercial products. 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.

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

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

PI: Delclos, K. Barry, Ph.D.

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

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 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 and will be essential for the planning of such a study; and 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.

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

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; and 3) To evaluate the reversibility of any observed effects.

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

Objective(s):

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

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

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

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 adducts in target tissues and circulating lymphocytes with acrylamide- and glycidamide-hemoglobin adducts in rodent exposure studies for future use in monitoring human exposure through occupation, smoking, and the diet; and 6) To determine *in vivo* mutagenesis of acrylamide and glycidamide using transgenic mice (Big Blue®).

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

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

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: Hansen, Deborah K., Ph.D.

Developmental Toxicity of Bitter Orange in Rats (E0214701)

Objective(s):

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

Physiological Effects of Bitter Orange in Rats (E0214901)

Objective(s):

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

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

Skin Penetration, Phototoxicity, and Photocarcinogenicity of Nanoscale Oxides of Titanium and Zinc. QUANTUM DOT (E0215611)

Objective(s):

To investigate the skin penetration of Ti02 and Zn9 and replace those studies with an investigation of the penetration of QDOTS into the skin of SKH-1 mice.

Skin Penetration, Phototoxicity, and Photocarcinogenicity of Nanoscale Oxides of Titanium and Zinc (E0215621)

Objective(s):

To examine the dermal penetration of nanoscale titanium dioxide (TiO2) in mice following topical application. The original protocol focused on the dermal penetration of TiO2 in mice; however, due to experimental difficulties with quantifying TiO2 in the skin, the NCTR and NTP staff decided to amend the protocol and use CdSe-based quantum dots (QDots) as the focus nanoparticle, and determine the dermal penetration of these materials in mice. Sufficient experiments have been completed, providing an identification of sentinel organs following dermal penetration and biodistribution of QDots following intradermal administration and skin penetration. The current addendum outlines experiments to focus again on the dermal penetration of TiO2 and biological consequences of any penetration.

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

Objective(s):

1) To determine if topical application of nanoscale TiO2 results in penetration of the TiO2 into the hair follicle of FVB/N mice; 2) To determine the dose-response relationship of follicular penetration by TiO2; 3) To repeat the UVB doses used in the Trempus *et al.*, 1998, study to confirm reproducibility of UVB dose that is photocarcinogenic; and 4) To dose mice with high dose of UVA to determine photocarcinogenicity.

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

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

Objective(s):

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

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

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 non-insulin dependent diabetes in obese diabetic rats.

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

Objective(s):

To evaluate the potential toxicity and carcinogenicity of perinatal and chronic exposures to AIDS drugs, Zidovudine (AZT) and Lamivudine (3TC) in C57BL/6(N5)tr*p53* (+/-) haplodeficient F1 transgenic mice.

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

Developmental Neurotoxicity Assessment of Acrylamide in Rats (E0215101)

Objective(s):

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

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

Objective(s):

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

PI: Wang, Cheng, Ph.D.

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

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

Collaborative Research and Development Agreements

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

Astra Charnwood

PI: Paule, Merle G., 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)

Objective(s):

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; and 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.

BG Medicine, Inc.

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

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

Objective(s):

To establish liver toxicity biomarkers and associated algorithms for use in preclinical drug development that will predict the probability of occurrence of hepatocullular 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.

Litmus, LLC

PI: Wilkes, Jon G., Ph.D.

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

Objective(s):

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

Pfizer, Inc.

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

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

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 Operant Test Battery (OTB) to these agents; and 3) To compare the behavioral profiles of these agents to those of a variety of reference compounds with well-characterized mechanisms of action.

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

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

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; and 5) To

compare the metabolism of ceftiofur with the human third-generation cephalosporin, ceftriaxone.

SAS Institutes, Inc.

PI: Tong, Weida, Ph.D.

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

Objective(s):

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

University of Arkansas at Little Rock

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

Effects of Prenatal Cocaine on Behavioral Plasticity (E0663307)

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.

University of Arkansas at Fayetteville

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

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

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 a 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) To 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 self control; and 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.

University of Arkansas for Medical Sciences

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

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

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.

University of Illinois

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

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

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.

Wright Patterson AFB

PI: Flammang, Thomas J., Ph.D.

670 Aeronautical Systems Squadron/Wright-Patterson AFB IAG (S00689)

Objective(s):

To develop an analytical procedure(s) to detect and quantify analytes selected by the Client (670 Aeronautical Systems Squadron/Battlefield Airman: Wright-Pattterson AFB, Dayton, Ohio.

Office of Women's Health Funded Projects

The Office of Women's Health (OWH) funds research to provide a foundation for the development of sound policies and regulations to enhance women's health. OWH has provided support for women's health research studies on topics ranging from cancer, HIV, and osteoporosis to dietary supplements, dioxins, and statistical approaches to gender analysis. The NCTR projects listed in this section were supported by OWH in FY 2006 or have been approved for support in FY 2007.

PI: Desai, Varsha G., Ph.D.

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

Objective(s):

To elucidate molecular mechanisms of mitochondrial dysfunction that will address gender-based differences in adverse effects of antiretroviral drugs, such as AZT. This will provide critical information to 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: Elkins, Christopher, Ph.D.

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

Objective(s):

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: Fuscoe, James C., Ph.D.

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

Objective(s):

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

PI: Gopee, Neera, 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 and Resistance to Lymphocyte Anergy (E0727401)

Objective(s):

To provide the basis for genetic screening to identify SLE patients who could benefit most from prasterone therapy, coupled with enhanced pharmacologic understanding of the mechanism for the therapeutic benefit of prasterone in SLE patients. This will be accomplished by evaluating the sex bias of SLE from a personalized medicine perspective. A functional polymorphism in the PRL gene (-1149G) associated with SLE may influence individual responsiveness to prasterone.

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

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

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. Characterize the relationships between transporter gene expression and uptake or excretion of chemotherapeutic drugs defined with the sandwich model and transporter-transfected cell lines; 3) To evaluate the effects of sex hormones on hepatic transporter gene expression in human cancer cell lines and sandwich-cultured

hepatocytes; 4) 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; 5) 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; and 6) To develop guidelines and recommendations for clinical-trial design and analysis of sex differences in new drug applications.

PI: Ning, Baitang, Ph.D.

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

Objective(s):

To provide insights into the mechanisms underlying gender-specific effects and adverse drug reactions associated with aspirin and NSAID use. NSAIDs are important drugs with analgestic, antipyretic, anti-inflammatory and cancer-preventive properties; and most NSAIDs are regulated by FDA. Results from this study should contribute scientific data to aid FDA in its continuing efforts to develop methods for evaluating drug efficacy and toxicity.

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 Key Word Index, located at the end of this publication as a quick reference to locate other occurrences of a specific term.

3-NPA	3-nitropropionic acid or methamphetamine
3TC	lamivudine
4-ABP	4-aminobiphenyl
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care, International
AALAS	American Association for Laboratory Animal Science
ACB	allele-specific competitive blocker
ACP-PCR	annealing control primer–polymerase chain reaction
ACB-PCR	allele competitive blocker-polymerase chain reaction
AD	Alzheimer's Disease
ADHD	attention deficit hyperactivity disorder
AIDS	acquired immunodeficiency syndrome
AMPH	amphetamine
APCI/MS	atomospheric pressure chemical ionization/mass spectrometry
AZT	zidovudine
BMC	bone mineral content
BMD	bone mineral density
cAMP	cyclic adenosine monophosphate
CBER	Center for Biologics Evaluation and Research, FDA
CD	Sprague-Dawley (strain of rats)
CDER	Center for Drug Evaluation and Research, FDA
cDNA	complementary DNA
CDRH	Center for Devices and Radiological Health, FDA
CE	competitive exclusion
CFSAN	Center for Food Safety and Applied Nutrition, FDA
CNS	central nervous system
CpG	cytosine-phosphate-guanine
CR	caloric restriction
CRADA	Cooperative Research and Development Agreement(
CVM	Center for Veterinary Medicine, FDA
CYP	physiologically based pharmacokinetic
DBS	deep brain stimulation
DdC	2',3'-dideoxycytidine
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

DMBA	7,12-dimethylbenz(a)anthracene
DNA	deoxyribonucleic acid
DR	dietary restriction
DS	Down Syndrome
EE2	ethinyl estradiol
ENU	ethyl nitrosourea
EPA	Environmental Protection Agency
ERIC	enterobacterial repetitive intergenic consensus
F344	strain of rats
FDA	Food and Drug Administration
FFM	fat-free mass
FMN	flavin mononucleotide
FY	fiscal year
GABA	gamma-aminobutyric acid
GAT	guanine adenine thymidine
GC-MS	Gas chromatography-mass spectrometry
GD	gestational day
GGT	guanine guanine thymidine
GSA	genotypic selection assay
GST	glutathione S-transferase
GTT	guanine thymidine thymidine
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HPLC-MS/MS	high-performance liquid chromatography/tandem mass spectrometry
HPLC-ES-MS/MS	high-performance liquid chromatography/electrospray/tandem mass spectrometry
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
Inlife	NCTR-developed computer system for collecting animal data during study
IV	intravenous
LC	liquid chromatography
LC/MS	liquid chromatography-mass spectrometry
LC-ES/MS/MS	liquid chromatography electrospray tandem mass spectrometry
LCM	laser capture microdissection
MAB/MS	metastable atom bombardment/mass spectrometry
MALDI	matrix-assisted laser desorption
MALDI/ToF MS	matrix-assisted laser desorption ionization time-of-flight mass spectrometry
MAQC	MicroArray Quality Control
MD	methyl deficiency

MPP+	1-methyl-4-phenylpyridinium
MPTP	1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine
MR	magnetic resonance
mRNA	messenger RNA
MRS	brain magnetic resonance spectra
MS	mass spectrometry
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
NMDA	n-methyl-d-aspartate
NMR	nuclear magnetic resonance
NTP	National Toxicology Program
ORA	Office of Regulatory Affairs, FDA
OTB	operant test battery
OTC	oxytetracycline
OWH	Office of Women's Health, FDA
PAH	polycyclic aromatic hydrocarbon
PattRec	pattern recognition
PBPK	physiologically based pharmacokinetic
PBPK/PD	physiologically based pharmacokinetic/pharmacodynamic
PCR	polymerase chain reaction
PD	postnatal day
PD	Parkinson's Disease
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
QA	quality assurance
QC	quality control
QSAR	quantitative spectrometric-activity relationship
QSDAR	quantitative spectrometric data-activity relationship
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
Romet-30	sulfadimethoxine-ormetoprim (SDO)
ROS	reactive oxygen species
RT-PCR	real-time quantitative polymerase chain reaction
RT-PCR	reverse transcriptase-polymerase chain reaction
SAB	Science Advisory Board
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SAR	structure activity relationship

SD	Sprague-Dawley (strain of rats)
SDAR	spectral data activity relationships
SDO	Romet-30®
SE-PCR	short-extension PCR
SFC	supercritical fluid chromatography
SKH-1	species of mouse
SSL	simulated solar light
SNP	single nucleotide polymorphism
SULT1A1	sulfotransferase 1A1
TBW	total body water
TiO2	titanium dioxide
TOF	time of flight
UDP	up-and-down procedure
USDA	United States Department of Agriculture
USDA/FSIS	United States Department of Agriculture/Food Safety and Inspection Service
UV	ultraviolet
UVA or UVB	ultraviolet (A or B indicates the region)
VGDS	Voluntary Genomic Data Submission
WHO	World Health Organization

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