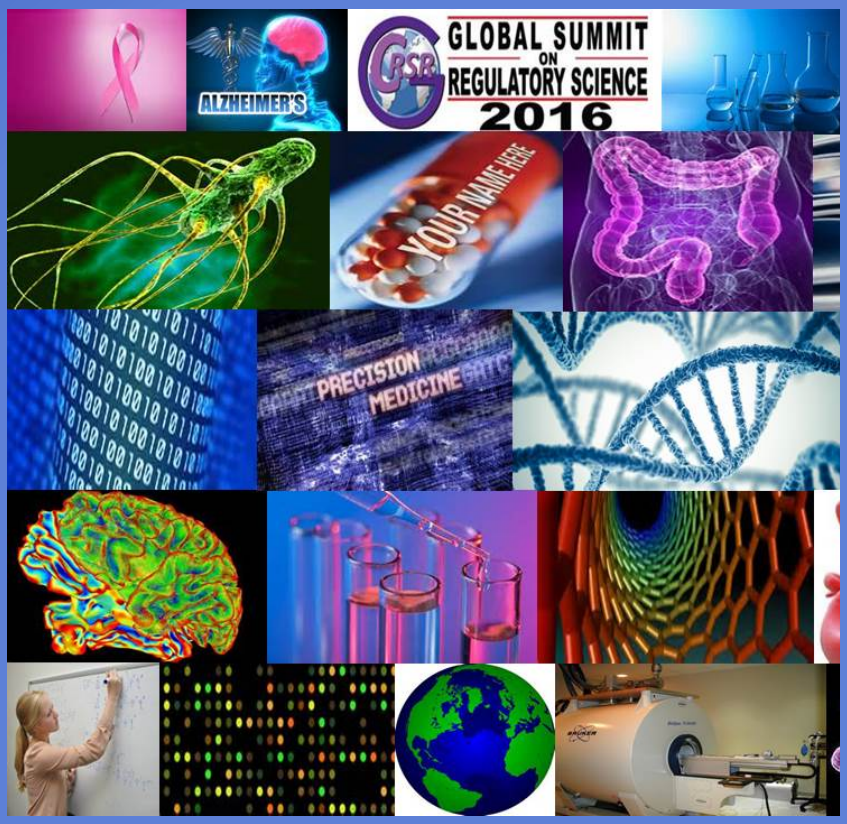

National Center for Toxicological Research

Annual Report
Research Accomplishments and Plans
FY 2015 – FY 2016

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Preface – William Slikker, Jr., Ph.D.

The National Center for Toxicological Research (NCTR) is the U.S. Food and Drug Administration's (FDA) premier laboratory research center focused on all FDA-regulated products. NCTR's primary goal is to support FDA, a critical component of the Department of Health and Human Services (HHS) in its efforts to promote and protect the health of the American public.

NCTR's goals are identified and developed based on goals outlined in FDA's Strategic Priorities document (*Enhance Oversight of FDA-Regulated Products, Improve and Safeguard Access to FDA-Regulated Products to Benefit Health, and Strengthen Organizational Excellence and Accountability*). In support of these Strategic Goals, NCTR scientists work closely with FDA Regulatory Centers in selecting, developing, and evaluating research programs that are needed to address the most pressing regulatory issues.

Once goals are identified, NCTR scientists develop innovative tools and strategies, and customize safety assessments of chemicals and materials in support of the regulatory process. Comprehensive study designs provide the data required for FDA to make science-based safety decisions. These projects involve coordinating expertise from several disciplines (toxicology, biochemistry, bioinformatics, biostatistics, chemistry, molecular biology, neurotoxicology, microbiology, genomics, nanotoxicology, molecular toxicology, scientific computing, systems biology, and others) to ensure the studies:

- maximize the ability to detect adverse outcomes
- provide information on the mechanisms underlying toxicity
- maximize the ability to translate laboratory findings to the improvement and protection of human health.

The ongoing work in multidisciplinary, multi-institutional studies evaluating bisphenol A (BPA), nanosilver, and pediatric general anesthetics exemplify this approach.

Although studies like those being conducted for BPA are designed to identify and resolve specific data gaps required for regulatory decisions, studies are also designed to be forward-thinking by evaluating the general biology and physiological principles governing associated biological responses. In studying

these more general relationships, including pharmacokinetic and pharmacodynamics modeling, NCTR projects provide strategies for evaluating similar toxicants and identifying potential biomarkers of toxicity and safety that will underpin regulatory decisions in the future.

Emerging technologies are deployed in parallel with standardized and traditional approaches to provide real-time comparisons of new tactics with accepted procedures. This also maximizes identification of potential biomarkers of toxicity to be used in further translational research and/or in supporting clinical evaluations of safety and treatment. This synergistic strategy is used in pursuit of the goal to shorten the time to biomarker discovery and qualification, and expedite acceptance of new science for evaluating regulated products in pre- and post-market evaluations.

NCTR strategies include investing in several emerging technologies that both increase the accuracy of safety evaluations and decrease the amount of time required to arrive at sound scientific decisions. A sampling of the newer techniques that are being integrated with established safety-assessment approaches include:

- Minimally invasive imaging approaches using Magnetic Resonance Imaging, Magnetic Resonance Spectroscopy, Positron Emission Tomography, and Computed Tomography for monitoring anatomical and biochemical function in live animals.
- Array and next-generation sequencing-based genomic technologies, along with proteomics and metabolomics capable of measuring hundreds—if not thousands—of simultaneous changes in gene, protein, and metabolite expression; *in silico* approaches.
- Development and standardization of bioinformatic sciences to collect, integrate, and evaluate data.

NCTR enhances FDA's capacity to collect, analyze, and interpret the unprecedented amount of biological data deriving from the omics technologies, high-throughput screening methodologies, the "big data" revolution, and public-data sharing. NCTR spearheads consortia that reach consensus on standards and approaches for routine use within the regulatory decision process. The MicroArray Quality Control (MAQC) consortium is a primary example of these efforts. MAQC was initiated to establish standards to gather and analyze microarray data, and has now completed its third phase [MAQC III, also known as Sequencing Quality Control (SEQC)]. A series of manuscripts have been published in a special issue of *Nature Biotechnology* to establish procedures and

best practice for use of RNA-sequencing technology. In addition, NCTR scientists have established the R2R (review-to-research and return) bioinformatics framework for an enhanced interaction between NCTR and FDA Product Centers.

To meet FDA's growing need for approaches to evaluate nanomaterials, NCTR developed and staffed a specialized core laboratory, in partnership with the Office of Regulatory Affairs, to characterize the nanoscale materials used in safety evaluations and to characterize these materials for use in preclinical safety-assessment studies. This facility has supported many projects at NCTR and other FDA Centers leading to publications on the toxicity and disposition of nanomaterials in cell-based and animal studies. One example is the use of sequential sectioning and imaging using a scanning electron microscope to provide 3-dimensional images of cells at extremely high resolution. A Memorandum of Understanding (MOU) between FDA and the State of Arkansas was established to advance regulatory-science research and build synergy between the five major research universities in the state and NCTR. Supported by the MOU, researchers from the state universities and NCTR investigate new approaches for evaluating the entire lifecycle of nanomaterials and have established a new Arkansas Bioinformatics Consortium to develop new tools to support the application of precision medicine and to enhance regulatory science.

In addition to newer requirements, NCTR continues to develop rapid technologies for characterizing microbial pathogens, to investigate mechanisms of antimicrobial resistance, and to investigate the virulence of microbes that may enter the food and drug supplies. Microbiome and immune responses, as an aspect of FDA product safety, are being evaluated. This research supports the FDA's emphasis on innovative emerging technologies for improving product assessment, as well as modernizes toxicology.

NCTR's scientists have also established collaborative research projects with the new FDA Center for Tobacco Products (CTP) to address priority research questions that will inform FDA's tobacco-product regulatory activities. In support of the research priorities outlined by CTP, NCTR established an inhalation core facility to study the toxicity of tobacco-smoke constituents and a core facility to evaluate the addiction properties of tobacco-product constituents. In another intra-agency collaboration, NCTR and CDER scientist are working together to complete documents concerning sunscreen ingredients and other non-prescription FDA-regulated chemicals.

The global distribution of products coming under FDA scrutiny requires

partnerships in regulatory research and training. FDA is addressing this issue via several programs including NCTR's continued long history of mentoring. NCTR has trained hundreds of scientists from over 45 countries and has expanded its efforts in communications to engage scientists from emerging economies. NCTR fosters national and international research collaborations and communications to promote rapid exchange of theories and emerging science with the promise of improving the quality and effectiveness of regulatory decisions. NCTR also supports the national and international training of scientists in the practices of modern toxicology to propagate the principles of regulatory science which support product-safety evaluation and efficacy worldwide.

NCTR formed the annual Global Summit on Regulatory Science (now in its sixth year) as a forum in which to discuss emerging science and safety evaluations, to exchange perspectives on regulatory programs, and to provide opportunity for scientific exchange and training. Cooperating and communicating, as well as advancing the principles of regulatory science through the formation of the multinational Global Coalition for Regulatory Science Research, are key factors that continue to enhance domestic and global health.

All of these areas and more are highlighted in this annual report. The robust NCTR research portfolio and strategies provide insights into how incorporating regulatory-science research enhances FDA's holistic understanding of toxicological sciences to improve public health.

/s/

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

NCTR Vision

The U.S. Food and Drug Administration's National Center for Toxicological Research is a global resource for collaboration—providing consultation, training, and innovative scientific solutions in support of FDA's mission to improve public health.

NCTR Mission

NCTR conducts scientific research to develop and support innovative tools and evaluation of approaches that FDA uses to protect and promote individual and public health.

NCTR Strategic Plan

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

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

Goal 1 identifies specific objectives that align with the priorities outlined in FDA's Advancing Regulatory Science Plan. This goal illustrates the importance of maintaining a strong basic-science core; one that provides NCTR the flexibility to address ever-changing research needs.

Goal 2: Promote global interactions in regulatory science research.

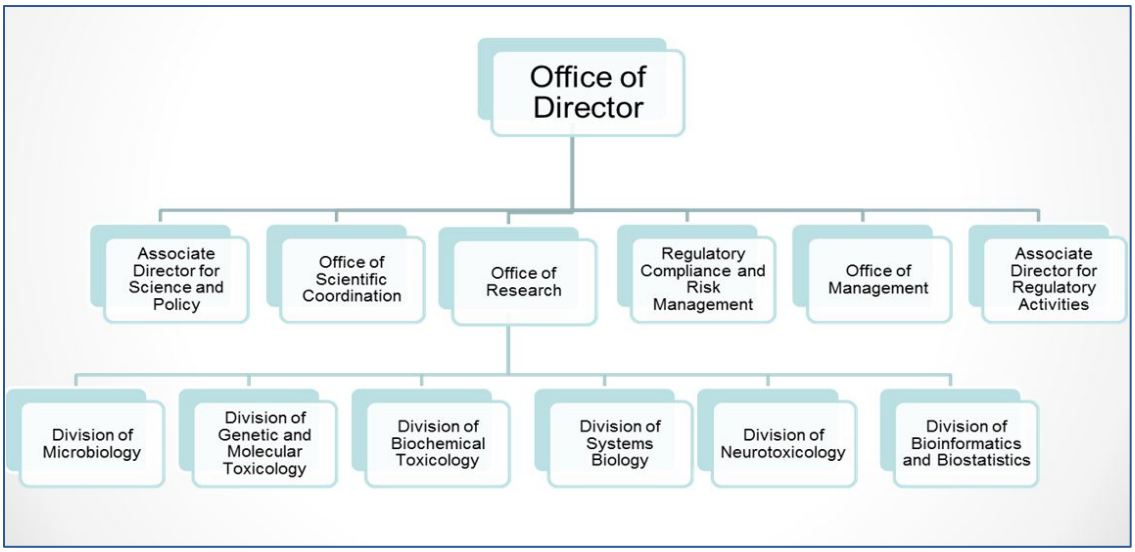
Goal 2 defines initiatives that promote NCTR's global activities dedicated to building and strengthening the product safety net around the world.

Goal 3: Improve administrative management and develop new communication materials and methods to support HHS/FDA science goals.

Goal 3 focuses on recruiting and retaining highly qualified scientists and staff, improving business processes, and extending the reach of NCTR's internal and external communications.

The NCTR Strategic Plan can be found on the FDA website at: www.fda.gov/NCTRStrategicPlan.

NCTR Organizational Structure



NCTR Location and Facilities

NCTR is co-located with the Office of Regulatory Affairs' Arkansas Regional Laboratory in Jefferson, Arkansas, about 40 minutes from Little Rock. The campus sits on 496 acres in the midst of a beautiful pine forest in central Arkansas and occupies 30 buildings spanning 1,000,000 square feet of floor space.

Some of NCTR's facilities include 132 general or special-purpose research labs and on-site housing units that are available for visiting scientists with eight two-person units and a commons building.

The NCTR Research Library holds some 12,000 monographs and 250 current titles covering a plethora of science topics. A sampling of the many services the scientific and technical library provides includes:

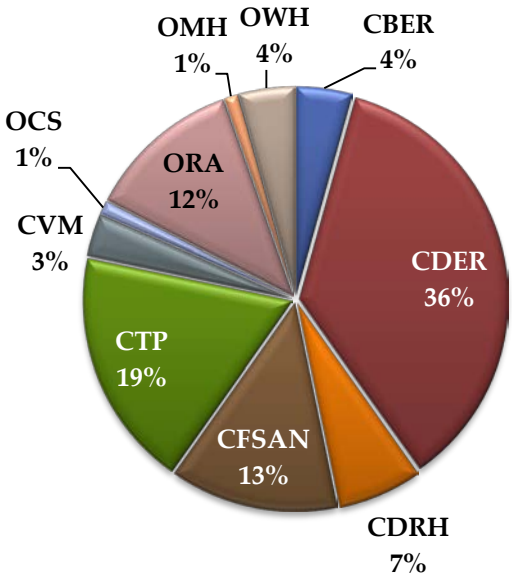
- reference information and reviews of the scientific literature
- database searches
- information-sharing services to keep the science staff informed of ongoing research worldwide
- interlibrary loans using worldwide networks of technical and academic libraries.

NCTR Advances Research Through Outreach and Collaboration

NCTR has actively sought and participated in collaborative, cooperative partnerships with other scientific and regulatory organizations in government, academia, and industry. These partnerships augment the mission of NCTR and FDA through the use of NCTR's unique resources. These opportunities to leverage resources, both public and private, enable NCTR to address questions of common concern to both FDA and the collaborating entity. 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.

NCTR has been fortunate in establishing Interagency Agreements (IAG) with several government agencies to conduct research on problems of common interest to FDA and the collaborating agency. The most significant, in terms of size, is the IAG between FDA/NCTR and the National Institute of Environmental Health Sciences/National Toxicology Program. In addition, NCTR is working with nine academic institutions and also works actively with each of the FDA Product Centers on various research efforts. Of the 166 total projects with planned or actual FY 2016 costs (as of February, 2016), NCTR is collaborating with at least one other FDA Center/Office on 46% of those projects. Shown below is the percentage of projects per FDA Center/Office of the 77 collaborative projects in place as of February 2016.

Collaborative Projects with FDA Centers/Offices



CBER – Center for Biologics Evaluation and Research
CDER – Center for Drug Evaluation and Research
CDRH – Center for Devices and Radiological Health
CFSAN – Center for Food Safety and Nutrition
CTP – Center for Tobacco Products
CVM – Center for Veterinary Medicine
OCS – Office of Chief Scientist
OMH – Office of Minority Health
ORA – Office of Regulatory Affairs
OWH – Office of Women's Health

NCTR Global Outreach and Training Activities

Research student

NCTR provides opportunities for undergraduate and graduate students, postgraduate scientists, scientists from other countries, college/university faculty members, and others to obtain hands-on laboratory experience by working with experienced regulatory science researchers. NCTR's exemplary reputation in the research community means the Center is often sought as a collaborator with and advisor for scientists from government, academia, and industry. Each year NCTR trains approximately 100 Oak Ridge Institute for Science and Education participants and visiting scientists. NCTR scientists share research knowledge, technical advice, and research training through global collaborations like the Global Summits on Regulatory Science.

Global Summit on Regulatory Science

Because of the importance for international regulators, policy makers, and scientists to exchange views on how to develop, apply, and implement innovative methodologies into regulatory assessments, NCTR established an annual Global Summit on Regulatory Science.

The Global Summit is an international conference for discussion of innovative technologies and partnerships to enhance translation of basic science into regulatory applications within the global context. The conference provides an opportunity for scientists from government, industry, and academic-research communities to objectively assess the utility of emerging technologies (such as nanotechnology, imaging, and omics for translational science, precision medicine, medical product safety, and food safety) for addressing regulatory-research questions and to discuss the best way to translate these technologies into real-world applications. The conference provides a platform

where regulators, policy makers, and bench scientists from various countries can exchange views on how

to develop, apply, and implement innovative methodologies into regulatory assessments in their respective countries, as well as harmonizing strategy via global collaboration. To engage the global community to address regulatory-science research and training needs, the Global Summit is held in different countries on an annual basis

Now in its sixth year, the Global Summit's goal is to engage the global community and harmonize research strategies via collaborations that aim to build knowledge of and promote regulatory science, define research needs, and seek to strengthen product safety worldwide by training regulatory scientists. The Global Summit is led by the Global Coalition for Regulatory Science Research which is comprised of regulatory science leaders from around the world. NCTR's Director serves as the FDA scientific representative and co-chair of the Coalition's executive committee and works with the Coalition to promote global interaction.

The Global Summit on Regulatory Science (GSRS15) was hosted by the European Food Safety Authority in Parma, Italy and included over 100 international participants representing 25 countries with a focus on "Regulatory Bioinformatics." Speakers from government and academia across the globe covered topics in four sessions: Global Trends, Initiatives, and Opportunities; Health Applications of Bioinformatics; Food Applications of Bioinformatics; and Bioinformatics Regulatory Challenges – Where Do We Go from Here and How to Overcome Barriers? A summary of the GSRS15 meeting has been written and submitted to *Regulatory Toxicology and Pharmacology* for publication. In addition, in FY 2015, the GSRS14 was summarized in a paper titled "Genomics in the Land of Regulatory Science" was published in FY 2015 in *Regulatory Toxicology and Pharmacology*[1] that summarized the 2014 Global Summit.

The 2016 Global Summit[2] will be held September 7-9, 2016, at the Natcher Auditorium, National Institutes of Health in Bethesda, Maryland, USA. The

[1] For more information: http://www.sciencedirect.com/science/article/pii/S0273230015000550

theme of this year's Global Summit is "Nanotechnology Standards and Applications."

Training Activities

The activities listed below are not comprehensive.

- **2015 Summer Student Research Program**

  During this 10-week summer program, undergraduate, and graduate students were paired with NCTR scientists and worked one-on-one with their mentors to gain hands-on research experience. At the end of the program, students gave oral presentations on their summer research; and undergraduates participated in the Central Arkansas Undergraduate Summer Research Symposium hosted by the University of Arkansas for Medical Sciences. NCTR's participants received three of the seven awards for best poster presentations. The goal of the program is to encourage students towards careers in science and to promote career possibilities in toxicology and regulatory science. This year's program was sponsored by NCTR, FDA's Office of Minority Health, and the Society of Toxicology Education Committee and hosted 23 students from colleges and universities representing 12 different states.

- **Intra-Agency Training**

  Two examples of intra-agency knowledge-sharing by NCTR staff include:

  - Annual training offered by NCTR's Division of Bioinformatics and Biostatistics to other FDA scientists on how to use NCTR-developed ArrayTrack™ (the foundation of NCTR's bioinformatics infrastructure) functionality in research and product reviews.

  - Annual hands-on training provided by the NanoCore to train laboratory and review scientists in proper methods for characterization of nanomaterials that might be incorporated into FDA-regulated products.

- **Dr. Orish Orisakwe, Visiting Scientist from Nigeria**

  NCTR welcomed Dr. Orish Ebere Orisakwe from Nigeria to conduct a three-month study stopover at FDA's premier regulatory science research Center.

His visit was coordinated through the Oak Ridge Institute for Science and Education (ORISE) Visiting Scholar program. During his visit, he rotated through various research divisions and offices at NCTR, attended seminars, presentations, and workshops. Dr. Orisakwe shared his Nigerian research experience and reviewed divisional research on endocrine disruptors and epigenetics. He expressed interest in a possible collaboration with the Division of Biochemical Toxicology, citing NCTR's state-of-the-art facilities and the tie-in to the mechanistic approach studies now being introduced at his University of Port Harcourt in Rivers State, Nigeria.

The bioinformatics research field is in the emerging stage in Nigeria regulatory science. Dr. Orisakwe expressed that certain aspects of research being conducted in NCTR's Division of Bioinformatics and Biostatistics could help boost ongoing research and graduate toxicology studies for which he has responsibility. He believes that bioinformatics, and the models it utilizes, could prove a strong research tool for, "a lean nation like Nigeria in the development of predictive toxicology," which would boost both preventive medicine and public health in the country.

NCTR Scientists – Leaders in the Research

- **NCTR Center Director Presented with 2015 Josef Warkany Lecture Award**
  William Slikker, Jr., Ph.D., NCTR Director, was presented with the 2015 Josef Warkany Lecture Award at the 55th Annual Meeting of The Teratology Society. The award recognizes a scientist who has significantly contributed to the field of teratology over their career. Dr. Slikker's multiple contributions include his work on placental transfer of compounds, fetal metabolism of compounds and the fate of those metabolites, and the long-term effects of perinatal exposures. For additional information visit *Birth Defects Research Connection, The Teratology Society*.

- **Genetic Toxicology Article Selected as Editor's Choice**
  Robert Heflich, Ph.D., Director, Division of Genetic and Molecular Toxicology. FDA/NCTR coauthored a research article titled "Derivation of Point of Departure (PoD) Estimates in Genetic Toxicology Studies and Their Potential Applications in Risk Assessment." The article was selected as the Editor's Choice and will be highlighted in an upcoming issue of *Environmental*

and Molecular Mutagenesis. The article reports the results of a study conducted by the HESI/ILSI Genetic Toxicology Technical Committee (GTTC) Quantitative Analysis Workgroup (QAW). The study's goal was to establish guidelines for the use of quantitative analyses of genetic toxicology dose-response data and point-of-departure metrics to improve risk assessments of chemical exposures. NCTR scientists are members of the GTTC QAW.

- **Bioinformatics Meetings**

  The Arkansas Bioinformatics Consortium (AR-BIC) and the MidSouth Computational Biology and Bioinformatics Society (MCBIOS) held back-to-back conferences in March 2015, in Little Rock, Arkansas. AR-BIC was formed to foster an Arkansas collaborative community in bioinformatics research and education among federal and academic institutions. The AR-BIC 2015 was organized by the Arkansas Research Alliance and NCTR. The topics this year focused on precision medicine and regulatory sciences applications. The MCBIOS is the largest such regional bioinformatics society in the United States and was co-founded by NCTR leadership about 12 years ago. MCBIOS 2015, the 12th annual meeting of the society, was supported by grants from the National Science Foundation and FDA and was attended by 200 participants. This event included preconference workshops, six plenary talks, 60 platform presentations and more than 80 posters in twelve technical sessions. MCBIOS members come from Arkansas, as well as universities from five adjoining states including Louisiana, Mississippi, Oklahoma, Tennessee, and Texas. For additional information, please contact Weida Tong, Ph.D., Director, Division of Bioinformatics and Biostatistics, FDA/NCTR.

- **Nanotechnology Symposium**

  Anil Patri, Ph.D., NCTR Director, NCTR-ORA Nanotechnology Core Facility co-chaired the Cancer Nanotechnology Symposium. The symposium is held annually at TechConnect World Meeting in Washington D.C., and features invited speakers working on cancer research using nanomaterials.

- **Councils, Committees, and Workgroup with FDA/NCTR Representatives at the Department of Health and Human Services-Level, National-Level, and International-Level** *(list below is not comprehensive)*

  - Asian Conference on Environmental Mutagens, Hangzhou, China
  - ASTM International; E56 Subcommittee on Nanotechnology
  - BioNanoMed Conference, Austria, Scientific Advisory Committee

- Critical Assessment of Massive Data Analysis (CAMDA)
- Data Quality Task Group (DQTG)
- Environmental Mutagenesis and Genomics Society Counselor
- Environmental Mutagenesis and Genomics Society Publication Policy Committee
- Environmental Mutagenesis and Genomics Society Publication Relations and Communication Committee
- EPA's FIFRA Science Advisory Panel
- Global Summit in Regulatory Science (GSRS): Nanotechnology Workshop
- Halifax Project Task Force
- HESI Cardiac Safety Committee Cardiac Biomarkers Working Group
- HESI DART Technical Committee for Nonclinical Neonatal Pediatric Models
- HESI Framework Steering Committee
- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
- International Association of Food Protection
- International Conference on Biomarkers & Clinical Research
- International Conference on Environmental OMICS, Seoul, Korea
- International Conference on Neuroprotective Agents
- International Life Sciences Institute/Health and Environmental
- International Workshop for Comet Assay (ICAW)
- International Workshop for Genotoxicity Testing (IWGT)
- Metabolomics and Epidemiology Working Group
- Molecular Pharmaceutics, Editorial Advisory Board
- Nanomedicine Nanotechnology, Biology, and Medicine
- Nanotechnology for Healthcare Conference
- National Advisory Environmental Health Services Council (NAEHS)
- National Toxicology Program (NTP) Review of Projects
- National Nanotechnology Initiative: Nanoscale Science, Engineering, Technology (NSET) Subcommittee
- National Nanotechnology Initiative: Nanotechnology Environment Health Implications (NEHI) Working group

- National Toxicology Program (NTP) Systematic Review Project
- NIEHS, National Toxicology Program Board of Scientific Counselors (BSC)
- NIEHS, National Toxicology Program (NTP) Executive Committee
- Office of Economic Cooperation and Development (OECD)
- PGX Focus Group Meeting
- Sciences Institute (ILSI/HESI)
- Sequencing Quality Control (SEQC)
- Shiga toxin *Escherichia coli* Federal Partners Summit conference
- Society of Toxicology (SOT) (multiple)
- SOT Scientific Program Committee
- TechConnect World Innovation Conference; Cancer Nanotechnology Symposium
- Toxicology Forum
- Tox21
- US-EU Communities of Research on Nanomaterial Characterization
- Wiley Journal WIREs Nanomedicine and Nanobiotechnology
- World Health Organization (multiple)

Science Advisory Board

Function

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

FY 2015 Accomplishments

The NCTR SAB held a meeting on November 6-7, 2014 to provide feedback on the scientific achievements and future plans of NCTR. The NCTR Director welcomed the SAB members, other FDA Center representatives, and NCTR participants and presented a "State of the Center" address. Each of the six research divisions at NCTR presented some of the work being done and elicited feedback from the SAB. The Director of the Division of Microbiology responded to the Subcommittee Site Visit Report. Additional speakers included the FDA's Chief Scientific Officer, representatives from FDA's Center for Drug Evaluation and Research (CDER), Center for Device and Radiological Health (CDRH), Center for Biologics and Evaluation and Research (CBER), Center for Tobacco Products (CTP), Center for Veterinary Medicine (CVM), and Office of Regulatory Affairs (ORA), as well as an envoy from the National Toxicology Program. A special session was held on "Epigenetics and Biomarkers of Organ Toxicity" and featured NCTR investigators working in these areas.

SAB meeting materials can be found here:
http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/ToxicologicalResearch/ucm379827.htm

SAB Membership Roster

CHAIR:

Martin A. Philbert, Ph.D.
Term: 06/05/13 – 06/30/16
Expertise: Neurotoxicology
Dean and Professor of Toxicology
School of Public Health
The University of Michigan
1415 Washington Heights
Ann Arbor, MI 48109-2029

DESIGNATED FEDERAL OFFICER:

Donna L. Mendrick, Ph.D.
National Center for Toxicological Research
10903 New Hampshire Avenue
Building 32, Room 2208
Silver Spring, MD 20993-0002
Tel: 301-796-8892

MEMBERS:

Susan P. Felter, Ph.D.
Term: 10/7/14 – 06/30/18
Expertise: Cancer Risk Assessment
Principal Toxicologist
Global Product Stewardship
Proctor & Gamble
8700 Mason Montgomery Road
Mason, OH 45040

Jay Gandy, Ph.D.
Term: 07/01/12 – 6/30/16
Expertise: Risk Assessment/Regulatory Science
Professor & Chair
Department of Environmental & Occupational Health
Fay W. Boozman College of Public Health
University of Arkansas for Medical Sciences
4301 W. Markham St., #820-11
Little Rock, AR 72205

Diwaker Jain, MD FACC FRCP FASNC
Term: 10/1/14 – 6/30/18
Expertise:
Imaging/Cardiotoxicity Pharmacology
Professor of Medicine (Cardiology)
Director of Nuclear Cardiology
Westchester Medical Center
Valhalla, NY 10595

Pamela J. Lein, Ph.D.
Term: 10/10/14 – 6/30/18
Expertise: Developmental Neurotoxicology
Vice Chair, Department of Molecular Biosciences
Professor of Neurotoxicology
UC Davis School of Veterinary Medicine
1089 Veterinary Medicine Dr.
2009 VM3B
Davis, CA 95616

Suresh D. Pillai, Ph.D.
Term: 01/28/14 – 6/30/17
Expertise: Microbiology & Immunology
Professor of Microbiology
Director, National Center for Electron Beam Research Room 418B, Kleberg Center
Texas A&M University
College Station, TX 77843

David Warheit, Ph.D.
Term: 07/01/12 – 06/30/16
Expertise: Inhalation Toxicology/Nanotoxicology
Senior Research Toxicologist
Acute and Developmental Toxicology Division
E.I. du Pont de Nemours & Co., Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
1090 Elkton Road
P.O. Box 50
Newark, DE 19714

Katrina M. Waters, Ph.D.
Term: 06/25/13 – 6/30/17
Expertise:
Bioinformatics/Systems Toxicology
Staff Scientist
Computational Biology & Bioinformatics
Pacific Northwest National Laboratory
902 Battelle Boulevard
P.O. Box 999, MSIN J4-33
Richland, WA 99352

Office of Scientific Coordination (OSC)

Paul C. Howard, Ph.D., Director
870-543-7672
Paul.Howard@fda.hhs.gov

Introduction

The Office of Scientific Coordination (OSC) has as its purpose "… to enable the research mission of NCTR and FDA by providing the support necessary for conducting toxicology studies." This includes support of animal-based toxicology studies, pathology, nanotechnology characterization and detection, inhalation toxicology, and management of the Interagency Agreement (IAG) with the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences (NIEHS). To accomplish this goal, many of the key support staff and operations are located in OSC.

The office oversees the IAG between NTP and NCTR, and has the responsibility of managing the IAG, communicating study progress to NTP and FDA, coordinating the generation of final reports, and generating background documents for many of the FDA-nominated chemical substances to NTP. The OSC Director serves as the liaison between FDA and NTP.
The following components of OSC are described elsewhere in this report:

- National Institute of Environmental Health Sciences/National Toxicology Program Veterinary Services Staff
- NCTR/ORA Nanotechnology Core Facility
- CTP/NCTR Inhalation Toxicology Core Facility

Pathology Services Contract

NCTR maintains an on-site pathology contract for veterinary pathology and histopathology services, and the Contract Officer Representative resides in OSC. This service is a critical component of conducting toxicological studies. The contractor maintains a staff of five board-certified veterinary pathologists and a highly trained staff that provide NCTR with services including: necropsy, clinical pathology, histopathology slide preparation, rigorous pathology examination,

and complete histopathology and pathology reports for each study. The conduct of the pathology services follows the guidelines suggested by veterinary pathology organizations, including the Specifications for the Conduct of Studies to Evaluate the Toxic and Carcinogenic Potential of Chemical, Biological, and Physical Agents in Laboratory Animals for the National Toxicology Program.

The pathology services additionally include translational and applied research that must be performed with rigid adherence to standard operating procedures, including the conduct of operations that meet the requirements of Good Laboratory Practice (21CFR58) and international test guidelines (Organisation for Economic Co-operation and Development).

The Pathology Services Contractor provides specialized analytical services which include:

- **Immunohistochemistry** for cell-type markers, tumor markers, hormones and hormone receptors, oncoproteins, oncosupressor proteins, lymphoid markers, growth factors and their receptors, virus proteins, etc.
- **Proliferation Assays**
  - BrdU immunohistochemistry
  - Ki-67 immunohistochemistry
  - Proliferating cell nuclear antigen (PCNA) immunohistochemistry
  - *In situ* hybridization for histone mRNA
- **Apoptosis Assays**
  - TUNEL
  - Caspase-3 immunohistochemistry
- **Non-Radioactive *In situ* Hybridization**
- ***In situ* PCR**
- ***In situ* RT-PCR**
- **Laser-Capture Microdissection**
  - Microdissection of cells and structures from tissues
  - Isolation of DNA or RNA from microdissected material
- **Virtual Microscopy/Pathology System (ScanScope)** Available for digital storage of microscope slides at diagnostic resolution for local and remote diagnostic collaboration and image analysis.

The Pathology Services Contractor in FY15 supported 37 NCTR research

protocols. This resulted in the trimming, processing, and embedding of 33,175 histopathology samples, generating 37,777 slides for histological examination. In support of the research studies 2,253 immunohistochemistry slides were prepared. Additionally, clinical chemistry (15,732 samples) and special pathology-related analyses were conducted as requested in research protocols.

Equipment Maintenance and Repair Contract

NCTR maintains a contract for equipment maintenance and repair, and the Contract Officer Representative resides in OSC. This contract supports research in three specific areas. The first is the routine preventative maintenance and calibration of equipment essential for the conduct of research. In FY15 this included preventative maintenance on 1,051 instruments on the campus, including preventative maintenance and calibration of 310 balances to ensure they provide accurate measurements for research. The second is the repair of equipment-supporting research that is not on a service agreement with the manufacturer. Minor equipment, such as balances, centrifuges, vacuum system, spectrometers, and chromatography systems, are repaired by the contractor to maximize utilization of aging equipment. The third is the manufacture of minor equipment to support customized research needs. This may involve the manufacture of a component of an old system when parts are no longer available, or the synthesis of new devices customized for specific applications, such as behavioral-testing devices.

Experimental Support

Two groups in OSC support animal-based research at NCTR. The Experimental Support Liaison Group specialists review the research protocols, develop an animal-use plan, review this plan with the study scientist, and interact with the computer system that collects animal data, where they input the study design into the system. This critical component of protocol execution communicates through the computer system and directs the animal-care contractor daily operations that involve animals, animal care, and animal dosing.

The Document Support Group specialists accomplish three specific tasks in support of NCTR research: 1) validation and documentation regarding animal data systems; 2) documentation of standard operating procedures for all operations of the animal data systems and computer-based systems; and 3) development of qualification and validation documents for hardware and software involved in the animal data system.

FY 2016 Plans

In FY 2016, OSC will continue to support the research at NCTR in each of the respective areas including support of the NTP interagency agreement, veterinary services, Nanotechnology Core Facility, experimental support, pathology contract support, and equipment maintenance and repair.

IntraAgency Agreement with National Institute of Environmental Health Sciences/National Toxicology Program *(NIEHS/NTP)*

Paul C. Howard, Ph.D., Director
870-543-7672
Paul.Howard@fda.hhs.gov

Introduction

The National Toxicology Program (NTP) is an interagency program established in 1978 to coordinate toxicology research and testing across the Department of Health and Human Services. The program was created to strengthen the science base in toxicology, to develop and validate improved testing methods, and to provide information about potentially toxic chemicals to health regulatory and research agencies, scientific and medical communities, and the public. NTP consists of three core agencies that provide support for NTP activities:

- National Institute of Environmental Health Sciences (NIEHS/NTP)
- U.S. Food and Drug Administration's National Center for Toxicological Research (FDA/NCTR)
- Centers for Disease Control and Prevention's National Institute for Occupational Safety and Health (CDC/NIOSH)

In support of the NTP mission, NIEHS/NTP and FDA/NCTR established an Interagency Agreement (IAG) in 1992, facilitating the conduct of toxicology studies on chemicals or substances nominated to NTP, that may be under the regulatory purview of FDA, to be studied using the unique resources and facilities at NCTR. The IAG-supported toxicology program provides FDA regulatory scientists with the opportunity for input into the toxicology-study design and dose selection to maximize the utility of the data for risk assessment. The program also provides FDA with the toxicology data that is needed for safety assessment of some FDA-regulated products.

The success of this IAG has led to 23 years of collaborative toxicity testing on compounds of interest to FDA and NTP. The IAG program has led to the investigation of toxicity assessment and mechanism-of-action studies of compounds in many classes of chemicals including:

- food contaminants
- cosmetics
- endocrine-disruptor compounds
- food cooking by-products
- dietary supplements
- drugs
- anesthetics

There is a significant probability that humans exposed to the above-listed classes of compounds will also be exposed to sunlight. To test the effect of sunlight on the toxicological risk of chemicals, the IAG supported the development of a facility (NIEHS/FDA Phototoxicology Research and Testing Laboratory) capable of conducting animal toxicology studies in the presence of simulated solar light or portions of the sunlight spectrum, such as UVB and UVA. These studies either test the acute effects of sunlight on chemical toxicity (phototoxicity) or the effects following long-term exposure of sunlight and chemical (photocarcinogenesis and photococarcinogenesis) studies.

Nanotechnology is the manipulation of matter at the near atomic scale, and materials developed with this technology have unique properties not found in bulk or larger particles. These materials require unique methods for proper characterization for toxicology studies. NCTR, together with NIEHS/NTP and FDA's Office of Regulatory Affairs (ORA) have developed a core facility in nanotechnology (NCTR/ORA Nanotechnology Core Facility), where nanoscale materials are characterized for toxicology studies. The equipment and procedures are in place to detect nanoscale materials *in vitro* and *in vivo* in support of toxicological studies.

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

The IAG fulfills one of NCTR's strategic goals (Strategic Goal 1, Advance Scientific Approaches and Tools Required to Support Public Health) through the conduct of toxicology studies that will provide FDA with appropriate data for

quantitative risk assessment of compounds. In addition, the studies are accompanied with mechanism-of-action and biomarker studies. These allow scientific understanding of the toxicology process and provide information for translation of the safety assessment to humans.

The NCTR Office of Scientific Coordination (OSC) is responsible for managing the IAG, communicating study progress to NTP and FDA, coordinating the generation of final reports, and generating background documents for many of the FDA-nominated chemical substances to NTP. These nomination documents are complete literature reviews of the use, pharmacokinetics, human exposure, and toxicity of the nominated substance. OSC will continue to produce these review documents to support FDA's need for assistance from NTP in understanding the risk of chemical substances to human health.

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

- Acrylamide (CFSAN)
- α- and β-hydroxy acids (CFSAN)
- AIDS therapeutics (Zidovudine, Nelfinavir, Nevirapine, Lamivudine)
- *Aloe vera* (CFSAN)
- Arsenic (CFSAN)
- Bisphenol A (CFSAN)
- Bitter orange, Citrus aurantium (CFSAN)
- Cellular telephone radiation (CDRH)
- Chloral hydrate (CFSAN)
- Di-(2-ethylhexyl)phthalate (CBER, CDRH)
- Ethinyl estradiol (CDER)
- Fumonisin B1 (CFSAN)
- Furan (CFSAN)
- Genistein (CFSAN)
- Glucosamine/Chondroitin (CFSAN)
- Goldenseal, berberine (CFSAN)
- Ketamine (CDER)
- Malachite green (CVM)
- Melamine with cyanuric acid (CVM)
- Microbiome
- Nanoscale silver (FDA)
- Nonylphenol (CDER)
- Oxybenzone (CDER)
- Permanent makeup pigments (CFSAN)
- Retinyl palmitate (CFSAN)
- Riddelliine (CFSAN)
- Triclosan (CDER)
- Urethane/Ethanol (CFSAN)
- Usnic acid, Usnea lichen (CFSAN)

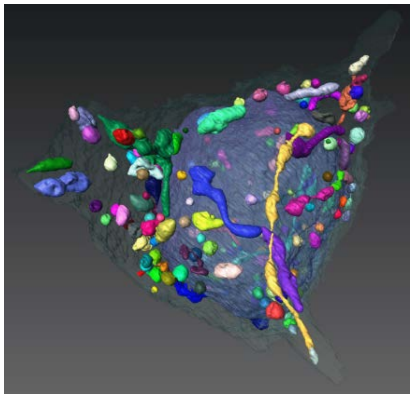
NCTR/ORA Nanotechnology Core Facility

Anil K. Patri, Ph.D., NCTR Director for NCTR/ORA Nanotechnology Core Facility
870-543-7508
Anil.Patri@fda.hhs.gov

Introduction

The NCTR/ORA Nanotechnology Core Facility (NanoCore) at Jefferson Laboratories is a joint effort by NCTR and the Office of Regulatory Affairs' (ORA) Arkansas Regional Laboratory (ARL) with a mission to:

"Provide the nanotechnology technical expertise and capability to support nanotechnology-based regulatory research and surveillance needs of NCTR, ORA, FDA, and government agency partners."



This NanoCore image shows a 3D reconstruction of a rat neuron. This is an example of the two- and three-dimensional electron microscopy techniques to quantify mitochondria defects following exposure to an FDA-regulated product.

Nanotechnology is a multidisciplinary field, drawing from applied and device physics, material science, supramolecular and polymer chemistry, interface and colloidal science, and engineering (chemical, mechanical, biological, and electrical). This field involves the manipulation of matter at the atomic level to create new materials called nanomaterials. Nanomaterials are typically defined as those with one size domain (length-width-height) between 1 and 100 nm (0.001 to 0.1 micrometer), where the size results in the presence of properties that are not found in the same material in larger sizes; however, the upper size limit (i.e. 100 nm) is not well-established in regulations. FDA has not defined the term "nanotechnology" or related terms, given the wide diversity FDA has seen with

these products. FDA has, however, published general guidance on products involving the use of nanotechnology[3]. According to this guidance, when considering whether an FDA-regulated product involves the application of nanotechnology, FDA will ask:

1. Whether a material or end product is engineered to have at least one external dimension, or an internal or surface structure, in the nanoscale range (approximately 1 nm to 100 nm), and
2. Whether a material or end product is engineered to exhibit properties or phenomena, including physical or chemical properties or biological effects that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer (1,000 nm).

The advanced and thorough characterization of nanomaterials and their detection in complex biological matrices requires specialized equipment and procedures that are not routine in either toxicology or analytical chemistry laboratories.

The NanoCore was developed to fill a need that arose within the toxicology research community, that is, a need to properly determine the physical and chemical characteristics of nanomaterials to be tested, to understand the behavior of these particles in the dosing solutions for *in vivo* studies and media for *in vitro* studies, and the ability to detect these materials or their break-down products in cells and tissues. As a result, the NanoCore has acquired the equipment and technical expertise to adapt and develop procedures to address the following:

1. Characterization of the nanomaterials used in toxicology and other studies to include:

   - Size and size distribution
   - Shape
   - Agglomeration and aggregation (i.e. particles collecting into bigger particles)
   - Concentration in solutions (mass, particle number, and surface area)
   - Overall composition (i.e. elements and crystal state)
   - Purity—elemental and organic (e.g. endotoxin contamination)
   - Surface area

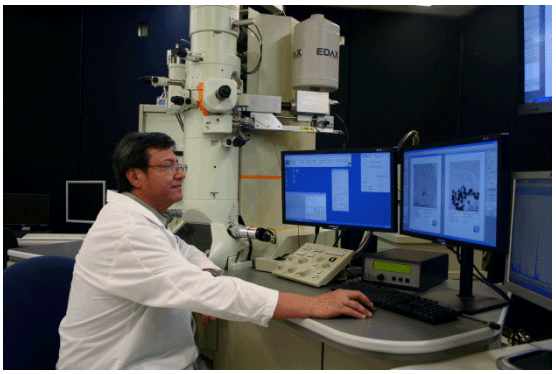
[3] For more information visit: http://www.fda.gov/regulatoryinformation/guidances/ucm257698

- Surface charge (zeta potential)
- Stability (isolated nanoparticle and when in solution)
- Dosimetrics (i.e. measurements of the dose delivered to the cell)
- Detection of nanomaterials in biological and physical matrices.

2. Detection of the nanomaterials in *in vitro* or *in vivo* toxicology assays. This requires knowledge of unique features of the test nanomaterial and developing/adapting existing methodologies to provide sensitive assays for detection. One critical starting point to any study on nanomaterials is characterization of the test article and determination of its behavior in the test environment (e.g., suspension in solution, in food matrix, in water). There is strong agreement that test articles should be characterized for many properties, including average particle size, agglomeration, shape, chemical composition, purity, crystallinity, stability, sterility, endotoxin presence, surface area, surface coating density, surface chemistry, and surface charge. The NanoCore supports investigators by providing the appropriate equipment, standard operating procedures, standards, and personnel to either conduct these characterizations or train laboratory personnel on how to conduct the analyses.

FY 2015 Accomplishments

In FY 2015, the NCTR staff of the NanoCore continued to support the core mission to characterize nanomaterial through collaborations with different FDA Product Centers and different divisions within NCTR, (e.g., nanoscale: silver, gold, iron oxide, titanium dioxide, zinc oxide, silica, core-shell particles, liposomes) used in biodistribution and toxicology studies.



NanoCore scientist conducting electron microscopy.

The newly acquired Zeiss Merlin scanning electron microscopy (SEM) for high-resolution imaging of the nanomaterials is equipped with x-ray energy dispersion spectral detectors for the analysis of the elemental composition of the sample being imaged. This instrument is used for several projects for the detection of nanomaterials and biological specimens on devices and supplements. This Zeiss Merlin SEM is

equipped with a serial block-face sectioning device (Gatan) that allows sequential sectioning and imaging of a sample and has been extensively used to interrogate biological samples to obtain high resolution 3-dimensional information. This allows the instrument to section and image in the Z-plane through a sample, creating a stack of images resulting in a 3-dimensional image of the sample. This capability allows for the imaging of the ultrastructure of samples and the 3-dimensional location of nanomaterials in biological tissues. Various biological specimens resulting from *in vitro* and *in vivo* studies using nanomaterials have been imaged this year. The past year's work on mitochondrial integrity upon ketamine treatment of brain tissue is followed by several internal research projects both from within NCTR and from CDRH. Various studies demonstrated the power of 3-dimensional stacked imaging of tissues at electron microscopic resolution, in resolving ultrastructural biology questions. While novel methods and optimization of sample preparation for these studies are underway, and we have had successes in expanding the imaging area to up to half a millimeter, the main bottleneck seems to be the storage of the significant amount of data generated from this instrument and the subsequent data analysis, segmentation, and 3D-rendering that requires collaborative development of new software. Discussions are underway with scientists from other agencies and academia to collectively solve this common problem.

These Zeiss SEMs are complemented by a Jeol SEM and two Jeol transmission electron microscopes (120 kV and 200 kV). This led to the processing and imaging of over 1,000 samples in 2015 in support of research projects at NCTR and other FDA centers.

The NanoCore additionally has a Particle Evaluation and Analytical Spectroscopy (Nano-PEAS) team with the responsibility to conduct the analyses of particle size, concentration, purity, and detection in biological matrices using a variety of techniques. The Nano-PEAS team continued to develop techniques to support multiple research projects from NCTR and FDA investigators and allow researchers to:

- Quantify the particle size of nanomaterials in solution (e.g., dynamic light scattering, particle-tracking analysis, atomic force microscopy)
- Quantitatively analyze nanoparticles in biological samples [e.g., inductively coupled plasma mass spectroscopy (ICP-MS), darkfield microscopy with hyperspectral imaging, confocal Raman spectroscopy]
- Detect unique carbon-containing nanomaterials in biological samples (e.g., Raman spectroscopy).

Research Activities:
Completed CORES-funded project on whether the accumulation of gold, silver, and silica nanoparticles within the mononuclear phagocytic system increases the susceptibility of mice to a *Listeria monocytogenes* bacteria challenge. This is a significant project involving multiple NCTR divisions and Product Centers with extensive characterization of nanomaterials through NanoCore, electron microscopy image analysis of hundreds of tissue specimen, and elemental analysis resulting from the *in vivo* study. Conclusions from this study are being drafted into a manuscript for submission.

Another significant CORES-funded project this year that is close to completion in the NanoCore is the physiologically based pharmacokinetic analysis of various liposomal doxorubicin formulations to ascertain the effects of size, polydispersity, and stability of liposomes on the pharmacokinetics of doxorubicin. Several *in vivo* studies in tumor-bearing animals have been completed and the blood analyzed through LC-MS for total and free doxorubicin. Further analysis on the drug disposition is underway and will likely result in manuscripts in early 2016.

Titanium oxide (TiO_2) is used extensively in many FDA-regulated products including food, cosmetics, devices, and drugs. A third project on the impact of nanosized titanium oxide on human mesenchymal stem cell adipogenic differentiation is in progress. Several different sizes of nano titania with different crystal structures were studied *in vitro*. Cellular uptake was confirmed by optical and electron microscopy; there is size- and crystal structure-dependent inhibition of hMSC adipogenesis by TiO_2 nanoparticles at high physiological doses. Further mechanistic studies should reveal the nature of these interactions and would address if they pose any safety concern.

Several additional projects with FDA's Center for Drug Evaluation and Research, Center for Devices and Radiological Health, and Center for Food Safety and Nutrition are in progress.

An international workshop on "Nanomaterial Physico-chemical Measurement Standards for Regulatory Consideration" was organized by NanoCore and NCTR staff as part of the Global Summit on Regulatory Science in Parma, Italy, on October 11, 2015. Scientists from many regulatory and standards agencies participated. Presentations from European Medicines Agency, European Food Safety Authority, European Chemicals Agency, Joint Research Centre, National

Institute of Standards and Technology, National Measurement Institute, and FDA highlighted the need for both reference material and documentary standards for regulatory consideration. The outcome from this workshop will be described in a white paper and will entail collaborative standards development from international participation.

Training Activities:
This year, the NanoCore training activities have been expanded. The annual hands-on training was attended by more than 16 participants this year and included trainees from all FDA Centers. This three-day hands-on training included introductory lectures on various basic and advanced nanomaterial-characterization instrumentation, methods, and their utility and limitations to aid reviewers with evaluating submissions. The hands-on portion included several size measurement techniques including dynamic light scattering, particle tracking analysis, transmission and scanning electron microscopy, and atomic force microscopy.

In addition to this training for FDA-wide researchers, two additional three-day electron microscopy training courses were offered by the NanoCore staff to NCTR post-doctoral trainees and researchers. A total of 12 participants were trained in this basic training course.

Two summer students were trained in the NanoCore this year; one undergraduate and one graduate student worked on nanotechnology projects. In addition, one collaborating scientist from Fraunhofer Institute, Germany, visited to conduct collaborative research on detection of nanomaterial in complex matrices, a topic of interest to FDA. Another guest scientist from the National Metrology Institute, Australia, is visiting the NanoCore to collaboratively develop documentary standards intended for nanomaterial characterization.

The NanoCore also continued to participate in a research consortium with research universities in Arkansas by providing a new suspension protocol for graphene this year. Since graphene is hydrophobic, any biological studies would involve suspending in aqueous buffers. This protocol helps streamline these *in vitro* studies. In addition, the purity and elemental composition were further analyzed for graphene to be used in toxicology studies at the universities and NCTR.

NanoCore personnel continued to participate in support of research studies in several NCTR divisions, providing critical characterization, stability, and

detection analyses. New studies were initiated with FDA Product Centers.

These accomplishments supported the NCTR Strategic Plan, specifically Goal 1: *Advance Scientific Approaches and Tools Required to Support Public Health* and Objective 1.1: *Integrated Product Assessment*.

FY 2016 Plans

The plans for the NanoCore in FY 2016 include addressing specific research needs that align to NCTR Strategic Plan Goal 1 (*Advance Scientific Approaches and Tools Required to Support Public Health*). The NanoCore will continue working with investigators to characterize the nanomaterials used in their toxicology studies. This includes continued development of methods to better characterize the behavior of nanoparticles in solutions and evaluation of the chemistry at the surface of the nanomaterials. The NanoCore will continue to detect and quantify the nanomaterials in the biological samples derived from toxicological studies, and will continue to develop new methods, or adapt existing methods, to detect single particles of nanomaterials in biological matrices. This includes electron microscopic methods, single particle ICP-MS, and other spectroscopic techniques, such as Raman spectroscopy. These goals and objectives are consistent with the FDA Strategic Objective 2.1 *(Increase Regulatory Science Capacity To Effectively Evaluate Products)*, generating data to support FDA regulatory decisions on the safety of nanomaterials.

One major emphasis for FY16 and beyond will be proactive collaborative development of documentary standards through collaborations from U.S. and European agencies and standards-development organizations. This effort has been initiated with support from the National Toxicology Program. The National Institute of Standards and Technology will help develop reference material standards that will result in additional documentary standards.

The NanoCore will continue to support studies at FDA's NCTR, Office of Regulatory Affairs, Center for Device and Radiological Health, Center for Drug Evaluation and Research, Center for Food Safety and Nutrition, Center for Veterinary Medicine, local universities (through FDA and a Memorandum of Understanding with the State of Arkansas), and others.

Center for Tobacco Products (CTP)/NCTR Inhalation Toxicology Core Facility (InhaleCore)

Shu-Chieh Hu, Ph.D., Director, InhaleCore
870-543-7513
Shu-Chieh.Hu@fda.hhs.gov

Introduction

The CTP/NCTR Inhalation Toxicology Core Facility (InhaleCore) at Jefferson Laboratories is a joint effort by FDA's NCTR and Center for Tobacco Products (CTP) to provide technical expertise in applied research via the inhalation route. This joint effort provides research within FDA in the field of inhalation toxicology for the purpose of supporting the authorities within the Family Smoking Prevention and Tobacco Control Act to protect public health.

The lung is the primary portal of entry for cigarette smoke, which is a combustion product containing thousands of chemical constituents, many of which are toxicants, carcinogens, and addictive compounds. Cigarette smoking is also ranked one the world's most serious public-health problems. It is well known that cigarette smoking is causally linked with many diseases including lung cancer and inflammatory lung diseases, cardiovascular diseases, and neurodegenerative diseases. Additionally, exposure to cigarette smoke during pregnancy also increases the risk of spontaneous abortion, preterm delivery, and prenatal morbidity and mortality. To determine the adverse health risks associated with humans using tobacco products, *in vivo* (within the living) inhalation toxicology studies are therefore warranted. Inhalation toxicology studies are necessary to evaluate the dose-response toxicity of chemicals that are found in tobacco, or that form during the combustion process. The primary mission for the InhaleCore Facility is to conduct these studies using the advantage of NCTR's unique capabilities in toxicological research.

The InhaleCore facility is equipped with six flow-past, nose-only inhalation exposure systems, including associated instruments for the generation of gas,

vapor, particulate, or aerosol test articles; optical and physical instruments for the quantification of test article concentration or particle size; medical grade compressed air source; and vacuum pumps. The system operates as an "enclosed system within an enclosed system" for the safety of personnel. In collaboration with CTP and NCTR scientists, the InhaleCore researchers study animal-biological responses using various endpoints after they are exposed in a well-defined environment via nose-only inhalation. These testing procedures are always in compliance with the Good Laboratory Practice (GLP; 21CFR58), as well as international test guidelines (e.g., Organization for Economic Co-operation and Development, OECD). The research outcomes provide data to inform the understanding and quantification of the adverse health risks associated with humans using tobacco products, supporting the FDA mission of regulating tobacco products.

FY 2015 Accomplishments

Following completion of a pharmacokinetic study of a carcinogenic compound found in cigarette smoke (the first compound to be studied in the facility), InhaleCore researchers studied the sub-acute inhalation toxicity of this carcinogenic compound in a rodent model. These research results provide the dose-range of the studied compound for subsequent studies. These studies will examine toxicity following 90-day repeated-dose inhalation administration.

FY 2016 Plans

In FY 2016, the CTP/NCTR InhaleCore will complete the research investigation of the first compound by conducting a subchronic inhalation-toxicity study. Upon completion, InhaleCore researchers will continue investigating compounds of interest to CTP via inhalation-pharmacokinetic studies and inhalation-toxicity studies.

The FY 2015 accomplishments and FY 2016 plans support the NCTR Strategic Plan, specifically Goal 1: Advance Scientific Approaches and Tools Required to Support Public Health and Objective 1.1: Integrated Product Assessment.

Veterinary Services

Pamela A. Mack, DVM, MS, DACLAM, Director of Veterinary Services
870-543-7659
Pamela.Mack@fda.hhs.gov

Introduction

The Veterinary Services Staff (VSS) provides professional and technical support for all animal-related research projects at NCTR. VSS administers NCTR's Animal Care and Use Program, which has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) since 1977. Included within VSS is the on-site Diagnostic/Microsurveillance Unit.

Three veterinarians, all certified by the American College of Laboratory Animal Medicine (ACLAM), one also certified by the American Board of Toxicology, and all of whom hold research degrees in addition to Veterinary Medical degrees, are charged with ensuring that healthy animals are available for research projects, providing veterinary care as needed, training research staff, and participating in projects requiring veterinary expertise.

A member of the VSS staff is the Contract Officer Representative with oversight of the contracted services for animal husbandry, diet preparation, and veterinary care of nonhuman primates. The animal care contract workforce is stable, highly trained and skilled, and boasts a high percentage of certified employees in their respective disciplines. The VSS Supervisory Veterinarian is a member of NCTR's Institutional Animal Care and Use Committee (IACUC), serving as the Institution's Attending Veterinarian.

VSS oversees the operation of five animal facilities on campus consisting of over 114,000 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 highly trained and American Association for Laboratory Animal Science (AALAS)-certified animal care staff provides a wide variety of husbandry and

technical services in support of NCTR's AAALAC-accredited Animal Care and Use Program. A staff of four microbiologists provides superior on-site microsurveillance and diagnostic services, ensuring the animals used in research projects at NCTR are free of pathogens that could compromise the research program. Monitoring of animal facility sanitation/sterilization practices, feed, water, and bedding prevents research-animal exposure to microbial pathogens via these sources. In addition, this group supports the provision of exceptional veterinary care via on-the-spot diagnostic services (bacteriology, serology, parasitology, and molecular biology) facilitating the prompt development of strategic treatment actions.

The Diet Preparation Facility is a well-equipped, large-scale formulation services unit. All animal diets received at NCTR are processed through the Diet Preparation Facility. The majority of dosed diets, dosed water, gavage solutions, and topical creams used in experiments performed at the Center are prepared in this facility. Dosed-feed production capability is 200,000 kg per year. Diets can be mixed with test articles in solution or solid-state in concentrations as low as 0.1 parts-per-billion. In addition, test articles can be mixed in the animals' drinking water to exacting standards in concentrations as low as one microgram per milliliter.

FY 2015 Accomplishments

VSS provided oversight and management of all NCTR laboratory-animal facilities. Staff personnel were responsible for breeding, rearing, acquiring, and quarantining all experimental animals used on-site. Personnel submitted annual reports (USDA, Office of Laboratory Animal Welfare, AAALAC) assuring compliance with federal regulations relative to our Animal Care and Use Program and participated in semi-annual program reviews, facility inspections, and experimental protocol reviews as part of the NCTR IACUC proceedings.

The Veterinary Care program, administered through VSS, provided veterinary medical care and surgical services to NCTR's research animals, including oversight of policies and procedures for animal procurement and transportation, preventive medicine, health and genetic monitoring, environmental enrichment, surgical protocols, anesthesia of laboratory animals, pain management, and euthanasia. Veterinarians also served as principal investigators or co-investigators on several protocols including rodent-breeding operations, animal-procedures training, and the sentinel-animal program.

A significant accomplishment this year was completion of the cryopreservation of the NCTR Sprague Dawley Rats in October of 2014 and the transgenic mouse strain in September 2014. These projects provide preservation against the loss of several invaluable animal models.

Animal Care/Diet Preparation/Veterinary Care Services Contract

During FY 2015, contract personnel supported an average daily census of 31 experiments. These experiments entailed husbandry services for an average daily census of 2140 rodents, 189 nonhuman primates, and 400 zebrafish. A variety of technical procedures were performed on many experiments including tattooing, tumor palpations, biological sample collections, administration of test articles, oral gavage (20,341 procedures), behavior assessments on rats and rhesus monkeys (38,099 measurements), application of topical-dosed creams (149,469 applications), rodent breeding operations, quarantine/stabilization of rodents, physical and pregnancy examinations of nonhuman primates, anesthesia of nonhuman primates for MicroPET, microchip implantations, and humane euthanasia. An on-site rat-production operation supplied animals for several experiments. An ongoing AALAS training program ensured the maintenance of a high percentage of certified staff. Currently 83% of animal-care and diet-preparation staffs are AALAS-certified and two members of the animal-care management group are Certified Managers of Animal Resources.

Contribution to FDA's Strategic Goals

VSS contributes to the FDA Strategic Goals through its support of all animal-care services that support the animal-based research projects in the various research divisions and as principal investigators and co-investigators on research projects. The VSS plays a critical support-services role in NCTR's biomedical research program. VSS personnel interact with individuals from every research division on a daily basis, providing expertise in animal care, diet preparation, laboratory animal medicine, and microbiology. These services are provided by highly trained, skilled, and dedicated individuals whose contributions enhance the quality of the research conducted by NCTR scientists.

FY 2016 Plans

The triennial site visit for continued AAALAC accreditation will occur in FY16. The Animal Care/Diet Prep/Veterinary Care Services contract will be solicited and awarded in FY16.

Cooperative Research and Development Agreements

NCTR actively pursues and maintains partnerships with nongovernmental organizations, nonprofit organizations, and private companies through Cooperative Research and Development Agreements (CRADAs). The FY 2015 and FY 2016 CRADAs supporting NCTR research projects include those listed below.

Toxicology Excellence for Risk Assessment (TERA)

Addendum: Development of a Method To Use In vivo Mutagenicity Data To Address the Question as to Whether a Specific Chemical Induces Cancer Via a Mutagenic or a Non-Mutagenic Mode-of-Action (E0722911)

University of Illinois

Addendum: Phytoestrogens and Aging: Dose, Timing, and Tissue (E072102)

In 2008, NCTR formed a Women's Health Research program within the Office of the Director and also formed a seminar series to promote and coordinate women's health research. It is critical that we understand the differences specific to women that may influence disease manifestations and the benefits and adverse outcomes of treatment. The NCTR women's health mission is to foster research excellence regarding the influence of sex/gender on the health of women, then to apply these research findings to address the health challenges and policies of FDA. The Women's Health Research Group is an interdivisional working group of scientists working on an active and innovative research program that focuses on understanding:

- the molecular basis of drug efficacy and safety
- how genetic, epigenetic, sex/gender, diet, and other environmental factors influence drug efficacy and safety as it relates to women's health
- computational framework for drug sensitivity.

FY 2015 Accomplishments

This group also coordinates women's health research projects funded by NCTR, FDA's Office of Women's Health (OWH), and extramural grants and partnerships to ensure the research fills knowledge gaps in the safety and efficacy of FDA-regulated products as they relate to gender differences in improving women's health.

In 2015, FDA's OWH Director of Research and Development continued to increase NCTR's visibility in its development of the Women's Health Roadmap for Research. The development of the Roadmap identified priority areas in which OWH would focus to advance regulatory decisions related to the toxicity, safety and/or efficacy of FDA-regulated products for women. The OWH's overall goal is to provide a science-based framework to build women's health science into all of FDA's research activities. NCTR visibility within the OWH research model increases NCTR collaborative leverage with other FDA Centers and increases NCTR's capacity in women's health-related research. NCTR scientists working in several NCTR research divisions participated in scientific meetings, both nationally and internationally, and workshops sponsored by other FDA centers

and OWH, NIH, the Society for Women's Health Research, and the Organization for the Study of Sex Differences. A critical focus of this participation was on developing successful strategies for engaging women and minorities in clinical trials and the importance of promoting interdisciplinary collaborative research to address the gaps in scientific knowledge about women's health.

In September 2015, NCTR hosted its Annual Women's Health Research Day that focused on the theme, "*The Roadmap to Women's Health: New Strategies for Advancements in the Future for Regulatory Science.*" Two keynote speakers presented research in regulatory mechanisms in uterine diseases and the role of epigenetics in triple-negative breast cancer (TNBC) as targets for new drug discoveries. FDA's OWH Director of Research and Development, presented its Women's Health Future Roadmap for Women's Health Research for FDA Regulatory Impact. In addition, the 2016 Goals and Objectives for OWH intramural-leveraged funding were presented. Large disparities remain in women's health and mortality rates among certain ethnic groups in breast cancer, autoimmune diseases, cardiovascular disease, and cervical cancer. These disparities continue to increase despite major advances in overall survival outcomes for these diseases.

NCTR 2015 OWH awardee accomplishments are listed below:

- Blood pressure (BP) threshold for cardiovascular disease (CVD) risk: an assessment of sex-based criterion revealed interesting findings. This study systematically reviewed existing literature and developed a database for BP and ambulatory blood pressure monitoring (ABPM) measurement, hypertension, CVD risk, and outcome. The meta-analysis studies pertaining to BP and cardiovascular risk suggested that each 10 mmHg in systolic blood pressure (SBP) increases CVD risk by approximately 15% for men and 25% for women. Multiple meta-regression analyses showed that the risk of CVD per 10 mmHg SBP increment in women was 1.10 times higher in men than women (P=0.002). By setting the reference SBP at 115 mmHg, which is considered as healthy for both women and men, it was found that the extra risk incurred by men with an SBP of 140 mmHG, mapped to an SBP level of 133, 132, and 131 mmHg for females aged 50, 60 and 70, respectively. The regulatory impact of this study can be used to determine whether current definitions of hypertension classification need to be modified, whether future hypertension trials need to be stratified by sex, age, or menopausal status for drug development, and whether reviewers need to consider sex-based differences in their review of hypertension trials.

- Methodology can detect low-level mutant subpopulations and provide a quantitative oncomutation profile of breast tumors and precisely define the importance of each mutation in breast carcinogenesis. The data derived from this study is used to test the underlying hypothesis that the somatic mutational profile of TNBC is unique among the breast cancer subtypes. The outcome of this study defined genetic characterization of TNBC in terms of which potentially drugable molecules are most often mutated and what proportion of cells within a tumor carry each mutation. Specifically this study has continued to demonstrate 1) ductal carcinomas have measureable levels (>10-5) of PIK3CA H1047R mutation, 2) PIK3CA H1047R exists as mutant subpopulations in ductal carcinomas meaning they have levels of PIK3CA H1047R mutation that would go undetected by DNA sequencing, and 3) PIK3CA H1047R MF measurements in TNBC are significantly lower than that measured in a normal breast. In FY15, TNBC breast tissues additionally showed a significant increase in the BRAF codon 600 GAG mutation, as compared to that measured in normal breast.

- Probabilistic model development and analysis were successfully conducted using Monte-Carlo methods and cluster-computation applications. The resultant probabilistic model predictions provide a good representation of the maternal thyroid-hormone levels and urinary iodide levels observed in the pregnant population of the U.S. and worldwide. Global-sensitivity analysis and probabilistic analysis of the deterministic PBPK-BBDR pregnancy model has been accomplished and results published.

- Drug-induced proarrhythmia (irregular heartbeat) is a major safety issue in drug development. Women are at a higher risk (~2 folds) than men for drug-induced QT prolongation and Torsades de Pointes (TdP), a rare but lethal heart rhythm problem, which can cause the heart to stop beating. Due to the absence of appropriate tools, few studies have investigated whether genetic differences between men and women have any effects on drug-induced irregular beats. Sex hormones are believed to play a predominant role in determining the sex differences of drug-induced TdP. An *in vitro* model for high-throughput screening and risk assessment of torsadogenic drugs using a hormone-free medium and a serum-free medium for iCells from CDI and Axiogenesis was developed. It was demonstrated that dofetilide-induced proarrhythmia can be detected in

both male and females cells. Furthermore, this study demonstrated that more dofetilide-induced irregular beats were in females versus male cells.

- Demonstrated the clinical significance of three biomarkers, FoxP3, TNFSF13B(BAFF), and IL-18 in lupus. Decreased FoxP3 expression, increased BAFF (TNFSF13B) and IL-18 expression in PBMCs, and serum levels were observed in lupus patients compared to age-matched healthy controls. Differences were noted in BAFF expression and serum levels according to ethnicity, age, and the disease stage of the patients (modest versus severe). These differences suggest differences in therapeutic response of BENLYSTA (Belimumab), the FDA-approved human monoclonal antibody that is specific for soluble human BAFF. Furthermore, decreased FoxP3—which is an essential transcription factor for regulatory T-cells that are considered the guardians of peripheral tolerance—suggests less suppressive activity for autoreactive T-cells. Therefore, both autoreactive T- and B-cells can generate autoantibodies in lupus patients. In addition, these studies demonstrated elevated levels of IL-18 in lupus-patient serum. High IL-18 levels have been associated with lupus nephritis, and could be considered as a biomarker for early damage to the kidney in lupus patients.

- Confirmed methodology that evaluates the effects of potential drug-delivery nanomaterials on *Candida albicans* infection of vaginal epithelial cells.

- Demonstrated evidence that drug-delivery nanoparticles were inducing apoptosis in the vaginal epithelial cells. As further evidence, an assay to measure nuclear-chromatin condensation showed that graphene-oxide nanoparticles induced this feature of apoptosis in the cells, but *C. albicans* suppressed it in the VK2 vaginal epithelial cells. Tumor necrosis factor alpha production by VK2 vaginal epithelial cells was induced by PLGA-PEG and GO-PEG nanoparticles, which is consistent with other results we reported previously indicating the pro-inflammatory and apoptosis-inducing effects of these nanomaterials.

FY15 and FY16 NCTR Projects Supported by OWH

A Pilot Study for Evaluating Genetic Influences on Sex Differences of Drug–Induced Proarrhythmia (E0754001)

Blood Pressure Threshold for Cardiovascular Disease Risk: An Assessment of Sex-Based Criterion (E0754201)

Evaluation of Methods Used to Measure Growth of Staphylococcus aureus and the Production of Toxic Shock Syndrome Toxin-1 as Influenced by Menstrual Tampons (E0754401)

Evaluation of Methods Used to Measure Growth of Staphylococcus aureus and the Production of Toxic Shock Syndrome Toxin-1 as Influenced by Menstrual Tampons (E0754401)

Oncomutation Profile of Triple Negative Breast Cancer: Additional Studies in African American Women (E0743801))

Minority Health Research

FDA's Office of Minority Health (OMH) Program started in 2013 as required by the Affordable Care Act to support FDA's mission. OMH also works to support the HHS Office of Minority Health's efforts, to eliminate racial and ethnic disparities, to improve minority health, and to improve the quality of health care that minorities receive.

Minorities are under-represented in clinical research and trials, particularly those in therapeutic areas, which affect minorities disproportionally, such as:

- diabetes
- cardiovascular disease
- hypertension
- stroke
- AIDS
- lupus
- certain cancers, such as triple negative breast, prostate, and pancreatic cancers.

NCTR scientists are currently conducting research on triple negative breast cancer using oncomutation profiles and epigenetic regulation of specific genes. Triple-negative breast cancer mortality rates are disproportionally higher in African-American women. Scientists conducting research on pancreatic cancer, which affects African-Americans at a higher rate and an increase is predicted for the disease in 2013. Pancreatic cancer has poor prognosis and a high mortality rate. Scientists at NCTR during 2015 demonstrated the relevant polymorphisms in drug transporters, in chemoresistance of specific drugs. NCTR scientists are conducting extensive research on biomarkers and potential new epigenetic therapeutic targets in lupus which is known to affect African American and Hispanic women at higher rates. In addition to research, NCTR scientists in 2015 participated in forums at national meetings on health disparities and worked with organizations in the community through health fairs and seminars in educating the public on diseases affecting their communities at alarming rates. Moving forward, NCTR will promote and coordinate research studies within NCTR to improve minority health.

In FY15 NCTR scientists demonstrated potential ethnic differences in inflammatory biomarkers in those with Alzheimer's Disease.

- Ethnicity differences in levels of several cytokines have been identified in brain tissue from those with Alzheimer's Disease.
- Levels of Aβ appear to differ with ethnicity as well.
- These results may partially explain the increased severity of Alzheimer's Disease in African Americans.

Scientists obtained a grant from OMH to investigate the epigenome-wide profile in peripheral blood mononuclear cells from African Americans and European Americans systemic lupus erythematosus patients with SLE Disease Activity Index scores between 5 (modest) and 10 (severe). The expected outcome of this study is to generate a large-volume dataset that will facilitate multidisciplinary evaluation of epigenetic profiles of genes linked to lupus, and evaluate differences between two major ethnic subgroups of lupus patients. Results will contribute to FDA's regulatory mission on gathering and understanding information on intrinsic factors that affect disease and response to medical products effectiveness and safety.

Division of Biochemical Toxicology Summary of Activities

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

Introduction

The Division of Biochemical Toxicology conducts fundamental and applied research designed specifically to define the biological mechanisms of action underlying the toxicity of products regulated by, or of interest to, the FDA. This research centers on quantifying the toxicities and carcinogenic risks associated with specific chemicals and introducing new risk-assessment techniques to enable regulatory agencies to evaluate better the risks associated with exposure to chemicals. The risk-assessment research is firmly rooted in mechanistic and exposure assessment studies focused on the understanding of toxicological endpoints, an approach that allows greater confidence in subsequent risk assessments.

NCTR scientist conducting research in the Division of Biochemical Toxicology.

Research within the Division capitalizes on scientific knowledge in the areas of biochemistry, organic and analytical chemistry, cellular and molecular biology, nutritional biochemistry, toxicology, phototoxicology, computational methods, and pharmacology. Division investigators work in close collaboration with scientists in FDA Product Centers, the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP), and academia to address FDA's regulatory needs.

FY 2015 Accomplishments

A major theme within the Division continues to be toxicological assessments on compounds nominated by the FDA for evaluation by the NIEHS/NTP. This focus reflects NCTR's superb animal facilities supported by a multidisciplinary

staff of scientists with strong mechanistic-research experience, which allows sub-chronic and chronic toxicological assessments to be conducted in a rigorous manner, often in compliance with FDA's Good Laboratory Practice guidelines. These studies currently serve as the benchmark by which toxicological assessments are made by the FDA, other federal agencies, and international regulatory bodies. In addition to providing basic information on toxicological endpoints, such as cancer, these experiments form the basis for mechanistic and exposure assessment studies to ascertain whether or not the response detected in the experimental model is pertinent to humans.

A principal area of research within the Division is the assessment of toxicities associated with exposures to dietary contaminants and dietary supplements. During FY 2015, Division investigators published an NTP technical report on the carcinogenicity of glycidamide, a metabolite of acrylamide, a water-soluble α,β-unsaturated amide that is produced in the baking and frying of starchy foods, including French fries, potato chips, and bread. The results, combined with data from previous mechanistic studies, provide strong support for the concept that acrylamide is activated to a carcinogen through metabolism to glycidamide. These findings will allow the FDA's Center for Food Safety and Applied Nutrition (CFSAN), which nominated acrylamide to the NTP for a toxicological assessment, to establish the risk of dietary exposures to acrylamide.
In further investigations of foodborne carcinogens and at the request of CFSAN, experiments continued to characterize the risks associated with exposure to furan. Furan is another dietary contaminant produced during the cooking of many common foods, including coffee, baked or fried cereal products, canned and jarred foods, baby food, and infant formula. The carcinogenicity of furan has been assessed in mice and rats; however, the risk to humans from dietary exposure to furan cannot be estimated because the lowest dose of furan tested in rats resulted in nearly a 100% tumor incidence. With funding from the NIEHS/NTP, a reassessment of the carcinogenicity of furan was conducted. During FY 2015, an NCTR final report was completed describing the results of the bioassay. In addition to conducting the bioassay, mechanistic studies are being performed with the goal of providing a solid foundation to establish the risks to humans from dietary exposure to furan. During FY 2015, a manuscript describing the toxicokinetics of furan was accepted for publication as were manuscripts reporting epigenetic changes in the livers of rats exposed to furan.

An additional area of investigation within the Division is the elucidation of potential toxicities associated with dietary exposures to putative endocrine-disrupting chemicals. Much of this emphasis has been placed on bisphenol A, to

which there is ubiquitous exposure from food products and other environmental sources. This research effort, which is supported by CFSAN and FDA's Center for Devices and Radiological Health (CDRH) and funded by the NIEHS/NTP, aims to address conflicting data in the literature regarding non-monotonic dose-response effects of bisphenol A at doses below the currently accepted no-observed-adverse-effect level. During FY 2015, the in-life phase of a two-year chronic bioassay was completed. In addition, collaborations continued with 13 NIEHS academic grantees, who are examining additional endpoints that have been reported to be associated with exposure to bisphenol A. Division investigators published a manuscript describing gene expression and global DNA methylation reproductive tissues from rats exposed to a wide range of doses of bisphenol A. Division scientists also published manuscripts describing the pharmacokinetics of bisphenol A in humans and these data have been used to develop a new physiologically based pharmacokinetic model.

During FY 2015, Division investigators continued conducting a 2-year dermal carcinogenicity study on triclosan, a broad-spectrum antimicrobial agent present in a wide variety of antibacterial soaps, deodorants, toothpastes, cosmetics, fabrics, plastics, and other products, which was nominated by the FDA's Center for Drug Evaluation and Research (CDER) to the NIEHS/NTP. In addition, manuscripts describing absorption and metabolism of triclosan and effects of triclosan on the activation of mouse and human peroxisome proliferator-activated receptor α were accepted for publication.
In the event of a bioterrorism attack on a food-production facility, chemical decontamination methods will be needed that have been tested and proven to be effective. With support from the Food Protection and Defense Institute (FPDI), Division investigators, in collaboration with scientists at CFSAN and the Institute for Food Safety and Health, performed experiments to evaluate the thermal inactivation of ricin, a potent bioterrorism agent, in milk using commercially available pilot-scale pasteurizing equipment. Additional tests were performed to evaluate the efficacy of chemical agents used for “clean in place” procedures to inactivate ricin *in situ*. The results from this study were provided in an FPDI final report.

Adjuvants are an important component of vaccines because they allow reduced amounts of antigen to be used to achieve an acceptable level of immunological protection. In the case of pandemic influenza or the use of select agents in a biological attack, the ability to use lesser amounts of antigen per administered dose would be critical in protecting as large a population as possible. Some adjuvant-antigen vaccines, however, have been associated with adverse events

that may be related to adjuvant immuno-stimulation. As part of an effort to model immunological mechanisms of efficacy and safety, Division investigators, in collaboration with scientists at FDA's Center for Biologics Evaluation and Research, acquired pharmacokinetic data on adjuvants containing α-tocopherol. These results are currently being prepared for publication.

Pyrrolizidine alkaloid-containing plants are widespread in the world and are probably the most common poisonous plants affecting livestock, wildlife, and humans. During FY 2015, Division investigators used a newly developed ultrahigh-performance liquid chromatography mass spectrometry method to detect and quantitate blood pyrrolizidine alkaloid-protein adducts in humans. They also published a manuscript demonstrating that glutathione conjugates of pyrrolizidine alkaloids are electrophilic in nature and can react with DNA.

Skin care products containing vitamin A congeners are among the most widely used agents for the mitigation of fine wrinkles, hyperpigmentation, and tactile roughness of photo-damaged and chronologically aged skin. Retinyl palmitate is the major storage form of vitamin A in the skin and is commonly incorporated into cosmetic creams and lotions. Retinyl palmitate absorbs ultraviolet light from sunlight, which could result in phototoxicities. At the request of CFSAN and with funding from the NIEHS/NTP, Division investigators conducted a one-year photo-co-carcinogenicity study of retinyl palmitate. During FY 2015, a draft pathology report was prepared.

A strong emphasis within the Division continues to be determining whether epigenetic changes (*e.g.*, DNA methylation) induced by carcinogens and found in tumors play a causative role in carcinogenesis or are merely a consequence of the transformed state. As part of these investigations, Division scientists have assessed the potential role of epigenetic changes as early markers of carcinogenicity. They have demonstrated that the tumor response in a mouse model of chemically induced fibrosis-associated liver carcinogenesis was associated with marked epigenetic changes rather than mutations in known cancer-related genes. These findings suggest that the assessment of carcinogen-induced epigenetic alterations, in addition to genetic changes, may substantially improve the safety evaluation of products of interest to the FDA and facilitate novel approaches for identification of subpopulations susceptible to exposures. Division investigators also have shown that epigenetic alterations may be a hallmark in autoimmune diseases, such as systemic lupus erythematosus (SLE). Specifically, changes in DNA methyltransferases, specific promoter methylation of critical genes in innate immunity, and modulation of specific microRNAs were

identified in blood samples from SLE patients. Division scientists also initiated studies to assess the potential of circulating microRNAs as sensitive biomarkers of toxicity induced by FDA-regulated products.

During FY 2015, Division investigators continued experiments with antiviral drug oseltamivir (Tamiflu) in support of a study sponsored by the FDA's Medical Countermeasures Initiative. Division scientists also published a manuscript on quantitative global-sensitivity analysis demonstrating the use of novel approaches to identify sources of uncertainties and variabilities and evaluating their interaction effects on model outputs for complex computational models.

Brominated vegetable oil (BVO) is a food additive used by the food industry to stabilize emulsions of citrus oils in soft drinks. Studies in the literature suggest that exposure to BVO may be associated with a range of toxicities. At the request of the CFSAN, Division investigators have designed studies in a rat model that aim to clarify certain aspects of the toxicological profile of BVO. During FY 2015 a 28-day dietary exposure pilot study was initiated. The outcome of this study will be used to refine the design of a subsequent 90-day Good Lab Practice study.

FY 2016 Plans

In FY 2016, Division of Biochemical Toxicology investigators will:

- Prepare a NTP report on a chronic bioassay of the food contaminant furan.
- Prepare a draft report on the toxicities associated with a sub-chronic exposure to silver nanoparticles.
- Prepare draft reports on 13-week and one-year photo-co-carcinogenicity studies on retinyl palmitate.
- Prepare a draft report on the sub-chronic study to evaluate the toxicities of melamine in combination with cyanuric acid in adult rats.
- Prepare a draft pathology report for a two-year chronic bioassay to characterize the toxicities of bisphenol A in rodent models, with special emphasis on perinatal exposures.
- Complete the in-life phase of a two-year chronic study to investigate the toxicities of topically applied triclosan.
- Continue to investigate the potential of pyrrolizidine alkaloid-protein

adducts to serve as biomarkers of pyrrolizidine alkaloid exposure in livestock and humans.

- Continue to develop methods for the rapid detection of potential bioterrorism agents in foods.
- Predict effective thermal inactivation conditions for foodborne novel *Staphylococcal enterotoxins* using advanced computational methods and perform thermodynamic studies to test predictions made *in silico*.
- Continue to investigate the role of epigenetic and microRNA alterations as potential biomarkers for noninvasive evaluation of exposure to genotoxic and non-genotoxic compounds of interest to the FDA.
- Continue investigations on the pharmacokinetics of vaccine adjuvants containing squalene and α-tocopherol.
- Continue studies to investigate the induction of arrhythmia in induced pluripotent stem cell-derived human cardiomyocytes.
- Using computational approaches, continue to investigate the effects of thyroid-active chemical mixtures on thyroid hormone homeostasis in pregnant women and their fetuses.
- Using computational approaches, continue to investigate the utility of animal models in determining the effects of pregnancy on the pharmacokinetics of oseltamivir (Tamiflu) in humans.
- Initiate studies on the time- and dose- response relationship of miRNA dysregulation in radiation-induced heart disease, and the potential of using circulating miRNAs as biomarkers for early diagnosis and monitoring of radiation-induced heart disease.
- Initiate photo-co-carcinogenicity studies on the diuretic hydrochlorothiazide.
- Continue to conduct sub-chronic studies with *Aloe vera* whole-leaf preparation components, specifically aloin.
- Continue to conduct studies on brominated vegetable oil, an additive used in the food industry to stabilize emulsions of citrus oils in beverages.
- Continue to conduct studies on nattokinase and lumbrokinase, which are naturally-occurring fibrinolytic enzymes that are promoted as health supplements for human use.
- Continue to conduct pharmacokinetic studies to develop physiologically based pharmacokinetic models for nicotine across different species.

- Using computational approaches, investigate the utility of *in vitro* models in predicting the *in vivo* toxicity.
- Continue studies to determine the reversibility of the nephrotoxicity elicited by combined exposures to melamine and cyanuric acid.
- Extend studies of potential non-reproductive toxicities of the plasticized di(2-ethylhexyl)phthalate after intravenous exposure as occurs from multiple medical devices.
- Investigate the induction of adverse effects resulting from the interactions between subclinical vitamin deficiencies and hormonally active agents, such di(2-ethylhexyl)phthalate.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by the Division of Biochemical Toxicology contributes to FDA Strategic Goals 2.5 (Advance Medical Countermeasures and Emergency Preparedness), 3.1 (Advance Food Safety and Nutrition), and 3.2 (Promote Public Health by Advancing the Safety and Effectiveness of Medical Products).
A major emphasis of the Division's research is to ensure the safety of food products. This is accomplished in close coordination with the CFSAN and other FDA Product Centers, which identify research needs and data gaps that guide the design of the Division's studies. For example, Division investigators have conducted bioassays and mechanistic studies to assess the risk of dietary exposures to acrylamide, a known rodent carcinogen and neurotoxicant that has been identified in coffee and baked and fried starchy foods—notably French fries, potato chips, and bread. A similar research strategy was applied to furan, another contaminant in food. Evaluations are also being conducted on bisphenol A, a chemical derived primarily from food-contact uses to which there is ubiquitous environmental exposure, and on *Aloe vera*, a natural product incorporated into dietary supplements. As part of the Division's efforts to ensure the safety of foods, assays are being developed and applied to detect the biological activities of potential bioterrorism agents, for example ricin and abrin, in various food products. Division investigators are also conducting studies to assess the toxicities associated with exposure to melamine, cyanuric acid, and pyrrolizidine alkaloids, contaminants that have been found in certain food products.

Computational tools are being developed within the Division to integrate toxicological, mechanistic, and pharmacokinetic data for safety assessments.

Physiologically based pharmacokinetic models of bisphenol A in rats, nonhuman primates and humans have been developed to reduce the uncertainty in predicting human health risks from exposure to bisphenol A. Computational analyses have been employed for the assessment of pharmacokinetics and interspecies extrapolation across nonhuman primates and humans for toxicities associated with methylphenidate. The results suggest that continued pharmacovigilance is prudent to monitor the safe use of this drug. Division scientists developed a computational model to evaluate the effects of perchlorate exposure and dietary iodide status on the hypothalamic-pituitary-thyroid axis of pregnant women and their fetuses. Model simulations indicate that environmentally relevant perchlorate exposure levels are far lower than the levels required to cause hypothyroxinemia in a typical pregnant woman. Division investigators are developing population-based models, using the model developed for iodide and perchlorate for an average pregnant woman, to demonstrate the methodological capabilities of computational models for assessing the dose-response relationship in a population of pregnant women. As a part of this investigation, Division scientists have developed approaches, such as global sensitivity analyses, to identify the sources of model uncertainties and variabilities and their interaction effects on model outcome.

Division investigators are also conducting computational studies to evaluate approaches for addressing the issue of co-exposure of pregnant women to a mixture of chemicals. Division investigators have developed a highly sensitive, precise, and accurate liquid chromatography-isotope dilution tandem mass-spectrometry methodology for the quantification of the antiviral drug oseltamivir (Tamiflu) and its carboxylic acid metabolite in nonhuman primate serum in support of a protocol sponsored by the FDA's Medical Countermeasures Initiative. Division investigators have developed a highly sensitive, precise, and accurate liquid chromatography-isotope dilution tandem mass spectrometry methodology for the quantification of the antiviral drug oseltamivir (Tamiflu) and its carboxylic acid metabolite in nonhuman primate serum in support of a protocol sponsored by the FDA's Medical Countermeasures Initiative.

Division of Bioinformatics and Biostatistics Summary of Activities

Weida Tong, Ph.D., Director
870-543-7142
Weida.Tong@fda.hhs.gov

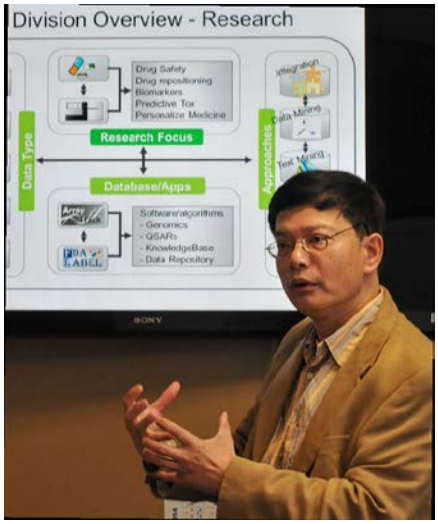
Introduction

The Division of Bioinformatics and Biostatistics develops integrated bioinformatics and biostatistics capabilities to address demands in biomarker development, drug safety, drug repositioning, personalized medicine, and risk assessment. Its capability is directed towards integration with FDA business processes to ensure NCTR linkages with FDA Product Centers are strengthened, and that NCTR informatics capabilities continue to evolve to be capable of meeting future FDA requirements.

The division is comprised of three branches:

1) Bioinformatics
2) Biostatistics
3) Scientific Computing

The **Bioinformatics Branch** conducts bioinformatics and chemoinformatics research in the fields of predictive toxicology, precision medicine, biomarker development, drug safety, and drug repositioning. Most research projects of this group are in collaboration with scientists within NCTR, across FDA Product Centers, and in the larger scientific community. A goal of this group is to develop methods and standards for the analysis and integration of diverse data derived from various technologies including emerging genomic methods (such as next-generation sequencing and microarrays), classical in-life parameters and public data sources. One of the



Weida Tong, Ph.D.

key endeavors of this group is to construct knowledge bases in the specific areas of FDA's responsibility to provide a data-driven decision-making environment for enhanced safety evaluation and personalized medicine. In addition, this group is taking an active role in supporting bioinformatics needs of other FDA Centers.

The **Biostatistics Branch** conducts peer-reviewed research and provides statistical support related to FDA's mission to protect and promote public health. The research statisticians develop new and improved statistical methods for risk/safety assessment aimed at FDA's goal of improving product safety and efficacy. A team of statisticians is dedicated to providing statistical support to the National Toxicology Program (NTP)-funded studies and NCTR scientists regarding the design, conduct, analysis, and interpretation of results of studies for safety and efficacy of regulated products.

The **Scientific Computing Branch** provides support in the areas of software and database development; high performance computing; systems integration; and IT asset management and procurement. The branch represents NCTR on FDA committees and ensures compliance with HHS and FDA information-technology policies.

FY 2015 Accomplishments

Bioinformatics Branch

- The amount of data present in the public domain and generated at NCTR is very difficult to interpret manually. Thus, bioinformatics approaches are being applied to identify connections that can be used for hypothesis-based research and to offer a new venue towards data-driven decision-making systems. One such project is the development of the Liver Toxicity Knowledge Base (LTKB). The goal is to collect all clinical parameters of each drug that has been reported to cause liver injury. Then that information would drive identification of potential biomarkers that can be easily and cheaply obtained from preclinical models including *in vitro* studies, toxicogenomics and *in silico* parameters. In FY 2015, an enhanced version of the LTKB database was developed, and made publicly available. The new version contains a large number of data for drugs along with the predictive models that can be accessed for prediction online. Most of the accomplishments in this project were published in peer-reviewed journals with high impact factors. This work continues.

- The rapid advancement of emerging genomic technologies and the application of these new data streams to assess safety and efficacy of FDA-regulated products raises concerns about their reliability and robustness in informing precision medicine and supporting regulatory decision-making in FDA. To address such concerns, we have led an international consortium of regulatory agencies, academia, pharmaceutical companies, and genomics-platform providers. The consortium was formed to resolve issues with MicroArray Quality Control (MAQC) projects and provides the basic parameters for fit-for-purpose application of these new data streams in regulatory environments. The solutions have been made publically available through peer-reviewed publications in literature. We completed the first three MAQC projects to assess the issues and challenges of applying microarrays, genome-wide association studies, and RNA sequencing (RNA-Seq) in clinical use and for regulatory application. Building on the success of the previous MAQC projects we started a follow-up study, named Sequencing Quality Control Phase 2 (SEQC2) as MAQC-IV, aiming to develop quality-control metrics and benchmark bioinformatics approaches for analysis of whole-genome sequencing and targeted-gene sequencing data to achieve best practices and standard analysis protocols and to apply these newer methods in regulatory settings and for use in precision medicine. The concept paper of the project has been approved.

- Rare diseases, although affecting a small percentage of the population, is often chronically disabling and life-limiting with few treatment options. Among the ~7000 rare diseases reported in various sources, only approximately 500 orphan drugs have been approved. FDA has implemented various policies to improve treatment options for rare diseases. As a result, in the last 10 years, over one-third of all new drug approvals by FDA were for rare diseases. We employed diverse bioinformatics approaches with integrated data related to rare diseases to uncover the underlying mechanism of rare diseases and explore the potential application of the existing marketed drugs for the treatment of rare diseases. In 2014, we published work to decipher miRNA transcription factor feed-forward loops to identify drug-repurposing candidates for cystic fibrosis (CF). We found 48 drug candidates can be repurposed for potential treatment of CF; 26 were confirmed with literature reports and/or existing clinical trials relevant to the treatment of CF patients.

Biostatistics Branch

The Biostatistics Branch research efforts focused on statistical methods to analyze toxicological, epidemiological, and molecular data; and to develop and apply data-mining methods for pattern identification and signal detection of high-dimensional data. Branch scientists conduct both individual research within the division and collaborative research with scientists from other NCTR divisions, and other FDA centers. Major research accomplishments include the following:

- Statistical methods for risk-factor identification and characterization—statistical models and procedures have been developed to analyze microarray gene expression, single nucleotide polymorphism, copy-number variation, and next-generation sequencing data for improved evaluation of safety and efficacy of FDA-regulated products. Algorithms are developed to identify individual genes and biological pathways associated with an individual's response to a treatment. Survival-risk prediction models have been developed and investigated for cancer patients for treatment selection. Statistical procedures are being developed to assess gender difference in blood pressure threshold for cardiac risk.

- Statistics and data-mining techniques for large-scaled data inference, clustering, biclustering, and classification algorithms have been developed for subgroup identification and prediction. These techniques have been applied to the development of prognostic, predictive models, and predictive enrichment classifiers in personalized medicine, serotype identification, and characterization in outbreak investigation, and identification of drug subgroup to adverse-event subgroup association in the FDA Adverse Event Reporting Systems.

- Novel methodologies development on next-generation sequencing (NGS) data analysis of bacterial pathogen. A bioinformatics pipeline was developed and implemented for sequence acquisition and genetic-diversity analysis from NGS data. The developed pipeline provides an effective bioinformatics tool for genetic-diversity clarification and marker-sequences discovery which will enhance the NGS data analysis and its applications on pathogen identification, source tracking, and population genome evolution.

The Biostatistics Support Team has continued to support National Toxicology Program (NTP)-funded studies. In addition, the Support Team performs statistical protocol review for all proposed NCTR studies, and conducts

IACUC study reviews for animal studies, statistical analysis, and support for non-NTP funded studies.

Scientific Computing Branch

The Scientific Computing Branch was responsible for supporting the software and database needs of the NCTR research and management staff and the governance of NCTR's IT infrastructure and IT projects.

- Completed updates on several IT infrastructure components. This included a new Oracle test-database environment, new versions of Oracle Application Express (ApEx) and Oracle Weblogic to enable new features and enhanced applications in the production and development environments.

- Completed a communication and collaboration application at the request of the Office of Research. The REACH application allows NCTR staff to enter information about themselves, their research interests and experience, and to search similar information about others at NCTR.

- Completed a new Document Tracking application and trained staff within the Research Divisions. The new application is web-based, user-friendly and compatible with current infrastructure components.

- Completed two software applications for the Office of Management. The Advanced Acquisition application provides a uniform location for management analysts to enter acquisition information. The Undelivered Orders application allows customers to enter and modify information regarding the status of delivery and payment. The applications aid the workflow and ensure proper planning and management of assets.

- Completed control and data-collection software for two automated rat mazes for the Division of Neurotoxicology .

- Contributed to a manuscript for the Division of Neurotoxicology.

- Represented NCTR on a number of FDA-level committees to help ensure the needs of the research and support staff are met.

- Organized multiple presentations to science, technology, engineering, and math students at the University of Arkansas at Pine Bluff to foster and encourage collaboration.

FY 2016 Plans

In FY 2016, the Division of Bioinformatics and Biostatistics will continue to emphasize a unified approach for development of safety and efficacy assessments of medical products and foods. New studies will begin to discover biomarkers of tobacco-related injury. To accomplish its mission, the Division of Bioinformatics and Biostatistics will continue with efforts to:

- Expand the Liver Toxicity Knowledge Base in the following three aspects:
    - Integrate analysis of diverse data that represent a broad range of biological complexity.
    - Integrate analysis of different predictive models for an enhanced performance of predictive modeling.
    - Conduct additional experimentation using next-generation sequencing for identifying better translational biomarkers for drug-induced liver injury in humans.
- Complete the full protocol for SEQC2.
- Regarding the drug-repurposing project, we will study potential reuse of oncologic drugs for the treatment of rare diseases. Cancer research has been the focus in the biomedical field, leading to many oncologic drugs in clinical use. In contrast, very few treatment options are available for rare diseases although they are progressive, disabling, and life-threatening. Therefore, we will investigate the potential use of oncologic drugs for the treatment of rare diseases.
- Develop decision models for clinical assignments of patients based on the patient's genomic features and disease phenotypes.
- Evaluate blood-pressure threshold for cardiovascular-disease risk to assess potential sex-based criterion.
- Develop data mining, visualization, bioinformatics methods, and tools for NGS data analysis to fully achieve the benefit of NGS technologies on public health, especially on microbial pathogen detection and surveillance.
- Develop a novel data-mining method by applying topic modeling and other machine-learning algorithms to the FDA's Substance Registration System

databases for classification of products and for detecting adverse-event safety signals.

- Analyze data generated from NCTR and National Toxicology Program collaborative studies.
- Replace the SASABM and WinLIMS commercial applications with newer software that is compatible with the current IT infrastructure and HHS IT security policies.
- Complete the update of the development, test, and production Oracle database environments including WebLogic, APEX, and Oracle Business Intelligence.
- Replace at least 15% of the legacy applications used by NCTR research and management staff.
- Create and Implement new software-development standard operating procedures to address the Enterprise Performance Life Cycle requirements from HHS.
- Complete the validation of at least two components of the Protocol Data Collection System.
- Deploy new Multispecies Behavior System droids and data-collection panels in collaboration with NCTR's Division of Neurotoxicology and the Bionetics contract staff.
- Develop an NCTR IT Strategic Plan in cooperation with the Executive Officer and members of the Center IT Board.
- Complete the conversion of term positions to permanent positions within the division.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by the Division of Bioinformatics and Biostatistics contributes to FDA's Strategic Priority 2.1, (*Advance Regulatory Science and Innovation*) and to FDA Strategic Goal 3.1 (*Advance Food Safety and Nutrition*), and Goal 3.2 (*Promote Public Health by Advancing the Safety and Effectiveness of Medical Products*).

The division provides expert advice and innovative research to the other FDA Centers, thus contributing to FDA's mission of advancing public health. Research

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

Innovation will continue to be a hallmark of this division and will include the development of newly integrated and knowledgebase methods to enable discovery of new types of biomarkers, new methodologies for safety evaluation and monitoring, and new ways of interpreting and integrating the massive and diverse data. The continued development of new bioinformatics tools will allow reviewers to easily access information from both the private and public domain—thus enhancing the FDA review process. Novel computational models will continue to be developed that predict drug safety and efficacy. These new methods will increase the number of safe and effective medical products.

The Division's **Biostatistics Branch** is helping to develop and refine preclinical and clinical trial designs, endpoints, and analysis methods. Scientists are:

1) Continuing to refine preclinical /clinical trial design and statistical methods of analysis to address issues such as, multiple endpoints, biomarker and subgroup identification, prediction algorithm, and patient selection.
2) Continuing to refine the use of modeling and simulation in preclinical/clinical trial design to enhance the effectiveness of studies.

Examples include 1) developing decision models for clinical assignments of patients based on the patient's genomic features and disease phenotypes; analysis of a number of high-dimensional datasets, QT prolongation and cardiotoxicity studies; as well as the development of a knowledge base for rapid threat assessment of enteric foodborne pathogens.

The **Scientific Computing Branch** supports NCTR efforts by developing and maintaining innovative software tools necessary to perform regulatory research. The combination of technical skills, research experience, and institutional knowledge will enhance collaborations and allow for efficient management of NCTR's information-system assets.

Division of Genetic and Molecular Toxicology Summary of Activities

Robert H. Heflich, Ph.D., Director
870-543-7493
Robert.Heflich@fda.hhs.gov

Introduction

The Division of Genetic and Molecular Toxicology (DGMT) conducts research to improve the safety assessments conducted on products regulated by FDA. The Division's goals are to promote public health by providing FDA with the expertise and tools necessary for comprehensive assessments of genetic risk and by strengthening approaches to integrate knowledge of genetic risk into regulatory decision-making. Division research is directed towards improving existing methods, and towards developing and validating new methods for evaluating high-priority issues related to the toxicity of food additives, human and animal drugs, biological therapies, nanomaterials, dietary supplements, tobacco products, botanicals, and medical devices. In collaboration with scientists from other FDA Centers, DGMT utilizes the methodologies it develops to better understand the potential toxicity of specific high-priority products of concern to FDA regulators.

As experts in the field of genetic toxicology, DGMT scientists maintain a leadership role in regulatory assay development and validation. Division scientists are actively involved in national and international efforts to harmonize genetic-toxicology tests and to improve their interpretation and use for regulatory safety assessments. Most recently, division scientists have been developing and validating next-generation sequencing (NGS) methods for whole-genome mutation detection in rodents and humans. Division scientists actively participate in the Organization for Economic Cooperation and Development (OECD) expert workgroups that are revising the current Genetic Toxicology

Research scientist working in a Division of Genetic and Molecular Toxicology laboratory.

Test Guidelines and are working to develop additional guidelines for genetic-toxicology testing. Division scientists frequently provide expert advice to FDA Product Centers, other government agencies, academia, and industry. DGMT scientists also are active participants in the FDA GeneTox Network and interagency workgroups.

Research Themes:

1) Development, Validation, and Maintenance of Regulatory Genetic-Toxicology Assays
2) Chemical-Specific Research
3) Development of New Paradigms for Regulatory Decision-Making that Integrate Measures of Genetic Risk with Biomarkers of Toxicity

    - Develop more relevant biological models
    - Develop more comprehensive approaches to monitoring genetic variation using technologies such as NGS
    - Develop better ways of evaluating data to determine human risk

FY 2015 Accomplishments

DGMT scientists actively participate in FDA and international working groups that form consensus on how to conduct regulatory genetic-toxicology testing. International working groups include those of the International Workshop for Genotoxicity Testing (IWGT), the OECD, and the International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI). Division scientists will continue to be involved in discussions concerning the appropriate strategies for conducting risk assessments of FDA-regulated products.

Specific FY 2015 research accomplishments involving developing, validating, and maintaining standard regulatory assays are listed below.

- Conducted research to develop a model for exposing suspension cultures of mammalian cells to whole smoke generated by a cigarette-smoking machine.

- Published "New Approaches to Advance the Use of Genetic Toxicology Analyses for Human Health Risk Assessment and Regulatory Decision Making" as a result of an ILSI/HESI collaboration.

- Participated in OECD Working Groups to update existing genetic toxicology

Test Guidelines, and to develop guidance for nanomaterials evaluation.

- Contributed to the development and validation of a new OECD Test Guideline on the *in vivo* Comet assay.

- Received OECD approval to develop and validate an OECD test guideline for the rodent *Pig-a* gene mutation assay for regulatory-genotoxicity safety assessment.

- Developed and validated a new transgenic hairless-albino mouse model as a mutational model for assessment of photocarcinogenicity.

- Conducted research evaluating the usefulness of standard *in vitro* assays for assessing the genotoxicity of tobacco products.

- Developed a high-fidelity NGS method called Mutational Analysis with Random DNA Identifiers to detect *Pig-a* gene mutations in heterogeneous CD48-deficient T-cell populations derived from carcinogen-treated rats.

- Published a manuscript on the utility of a transgenic hairless albino-mouse mutational model for use in photogenotoxicity and photocarcinogenicity studies, and for assessing the safety of nanoparticles in cosmetics.

- Published manuscripts on the *in vivo* alkaline Comet assay and enzyme-modified Comet assay for measuring DNA-strand breaks and oxidative DNA damage induced by FDA-relevant food contaminants and pharmaceuticals.

- Published manuscripts on the mutagenic mode-of-action of a model oxidant and lung carcinogen, vanadium pentoxide.

The Division employed standard genotoxicity assays to generate chemical-specific testing data that can be used by the FDA Product Centers as described below.

- In collaboration with FDA's Center for Tobacco Products (CTP), conducted research with standard assays to evaluate the genotoxicity caused by chemicals present in cigarette smoke and by cigarette whole-smoke solutions

- Published manuscripts on detecting mutations in the endogenous *Pig-a* gene of CD48-deficient T-lymphocytes from carcinogen-treated rats.

- Performed *in vivo* genotoxicity assessment of acrylamide and glycidyl methacrylate by measuring *Pig-a* gene mutation, MN induction, and DNA damage using the Comet assay in treated rats.
- Conducted research and published a manuscript on the toxicity and adverse clinical effects of *aloe vera* in human cells.

- In collaboration with CTP, published manuscripts on the quantitative analysis and *in vitro* genotoxicity of chemical constituents of tobacco smoke.

- Published manuscripts on the genotoxicity of chemical agents, such as acrylamide and glycidyl methacrylate, estragole, usnic acid, acrylamide and glycidamide, TEMPO, DMBA, ginkgo biloba, aristolochic acids, nutraceuticals, engineered nanomaterials, ethylene oxide, vanadium pentoxide, *aloe vera*, silver ions, and silver nanomaterials.

Progress made in FY 2015 in developing new paradigms for regulatory decision-making that integrate measures of genetic risk with biomarkers of toxicity is described below.

- In collaboration with CTP, conducted research with human *in vitro* airway cultures to assess the toxicity caused by chemicals present in cigarette smoke and by cigarette whole-smoke solutions.

- Conducted research using allele-specific competitive blocker-polymerase chain reaction (ACB-PCR) technology to develop and validate specific cancer-driver mutations as biomarkers of cancer risk.

- Published a characterization of low-frequency *KRAS* mutations in lung adenocarcinomas and described their potential impact on the efficacy of personalized cancer therapeutics.

- Conducted research to develop an *ex vivo* primary lung-tumor spheroid model, to be used in assessing acquired resistance to molecularly-targeted, personalized cancer therapeutics.

- Conducted research on the development of a new method using microRNA expression as a biomarker for identifying carcinogens.

- Continued research on the evaluation of microRNAs in blood and urine for the detection of chemical-induced carcinogenicity.

- In collaboration with CTP, developed methodology for histochemical analysis and measuring tight junction integrity, protein oxidation, extra-cellular matrix proteins, cilia beating, and mucus production in cultures of human-airway cells as endpoints of tobacco-product exposure.

- Conducted *in vivo* genotoxicity studies on three agents as part of a large multi-organization project—funded by a Cooperative Research and Development Agreement (CRADA) with Toxicology Excellence for Risk Assessment (TERA)—to evaluate the use of *in vivo* mutation data to inform cancer mode-of-action.

- Published a manuscript on the continued progress in developing the *Pig-a* gene mutation assay for international regulatory use and a book chapter on new and emerging genotoxicity tests and approaches for testing FDA-relevant chemicals and drugs.

- Developed NGS methodology to evaluate DNA sequence changes in a pool of flow cytometry-sorted *Pig-a* mutants.

FY 2016 Plans

Specific plans include:

- Complete a validation study on a tobacco smoke-generating machine and *in vitro* cell exposure modules to perform CTP collaborative studies.

- Start a new CTP-sponsored project using the *in vivo* Comet assay to study the genotoxicity of inhalation exposure to tobacco-related compounds in Sprague Dawley rats.

- Conduct an FDA Office of Women's Health (OWH)-funded project determining the oncomutation profile across breast-cancer subtypes, including triple-negative breast cancer in humans.

- Conduct an OWH-funded project comparing oncomutation profile of breast cancers in African American and Caucasian women.

- In collaboration with the University of Arkansas for Medical Sciences, begin developing a human reticulocyte *PIG-A* assay for use in monitoring gene mutation in cancer patients receiving platinum-based antineoplastic therapy.

- Conduct a CTP-sponsored project measuring the toxicity and inflammation induced by whole smoke generated from a tobacco smoke-generating machine in human *in vitro* airway cultures.

- Perform work addressing a nanotechnology Collaborative CORES initiative: determining whether the current genetic-toxicology assays are appropriate for evaluating the toxicity of nanomaterials.

- Initiate new projects evaluating a new high-throughput approach for assessing nanomaterial genotoxicity and identifying potential nanomaterial biomarkers.

- Start a project to set up and maintain a publically available database of all *Pig-a* data as part of the OECD test-guideline validation process for the *Pig-a* gene mutation assay.

- Conduct RNA-sequence data analysis and prepare a manuscript for publication cataloging mutations identified in human B-lymphoblastoid lines used in genetic-toxicology testing.

- Start a new project to measure somatic oncomutations as biomarkers for translating preclinical safety data to human cancer risk.

- Evaluate microRNAs in blood and urine as biomarkers of chemical-induced mutagenicity and carcinogenicity.

- As part of a Memorandum of Understanding between the State of Arkansas and FDA, perform research on the genotoxicity of the nanomaterial, graphene.

- In collaboration with FDA's Center for Drug Evaluation and Research (CDER) scientists, evaluate the hypothesis that rare oncogene mutations in human tumors are responsible for the failure of personalized-medicine therapies by tracking mutation subpopulations *in vitro* in spheroid cultures generated from human-lung tumors.

- Conduct research investigating the impact of strain and age on the background frequency of cancer mutations in rodent models.

- Investigate the use of NGS to measure low frequency cancer-relevant mutations in normal and tumor tissue, and start a new project to determine if NGS can be used to detect mutations from genotoxic carcinogens.

- Collaborate with TERA, using a transgenic mutational cell line, GDL-1, to investigate the mutagenicity of vinyl acetate monomer and acetaldehyde.

- Develop *in vitro* and *in vivo* methods to assess the global as well as gene-specific DNA methylation status using a single-cell gel electrophoresis assay.

- Evaluate the ability of a human peripheral blood-mononuclear cell system to act as an immunomodulater in an *in vitro* assay for the immunotoxicity of nanomaterials.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by DGMT contributes to the following FDA Strategic Priorities, excerpted from the 2014-2018 Plan:

Goal 1 – Enhance Oversight of FDA-Regulated Products

- Objective 1.1: Increase the use of regulatory science to inform standards development, analysis, and decision-making:
    - advancing regulatory science by advancing the development of predictive safety models
    - translating new technologies into real-world diagnostics, treatment and cures

Goal 2 – Improve and Safeguard Access to FDA-Regulated Products to Benefit Health

- Objective 2.1: Increase regulatory science capacity to effectively evaluate products
    - Increasing collaboration and information sharing with colleagues in industry, academia, and other regulatory bodies
    - Supporting public private partnerships to advance regulatory science
    - Improving the efficiency and validity of safety evaluations of food ingredients and dietary supplements

Goal 4 – Strengthen Organizational Excellence and Accountability

Objective 4.1: Recruit, develop, retain, and strategically manage a world-class workforce

- Promoting cross-disciplinary regulatory science training, especially in the international arena
- Promoting opportunities for continuous learning and career development

The Division provides expert advice and innovative research to the FDA Product Centers, thus contributing to FDA's mission of advancing public health through improvements to regulatory science. Several research projects involve the development of new and innovative technologies and approaches that support safety reviews conducted by FDA's Product Centers. Other research explores approaches that may benefit the implementation of personalized-medicine strategies, the development and validation of new methods to assess genetic risk, and bacterial and/or tissue-culture approaches used to detect potential genotoxicity and to generate hypotheses concerning the basic mechanisms of genotoxicity.

Genetic toxicology is concerned with the ability of chemicals to alter genetic material. FDA currently requires that petitioners provide data evaluating the potential genetic toxicity of their products as a part of the product-approval process. Due to scientific evidence indicating that genetic damage is important in tumor development, this information is used in the evaluation of new molecular entities for possible carcinogenic activity.

Division scientists recently have begun developing and evaluating tissue-equivalent human *in vitro* models that may provide more relevant data for assessing human risk than traditional bacterial- and rodent-based *in vitro* systems. Division scientists also specialize in the development and validation of new *in vivo* mammalian systems and develop strategies by which these models are best employed for risk assessment. An increased understanding of mutational mechanisms, combined with test systems that detect genetic damage in a manner relevant for assessing human risk, will provide FDA with better information for making regulatory decisions. As new assays are validated, division scientists will continue to work with international scientists to assure the harmonization of protocols and the development of guidelines to assess genetic hazards.

Genomic technologies are beginning to provide new tools for making better public-health decisions. The scientific and medical communities have benefited

by an increased understanding of the genetic material and how it functions in both humans and rodents. Utilizing this information, new molecular technologies, such as NGS, are being rapidly developed and can be used to evaluate structural and functional changes in both rodents and humans. The division is using new technologies, in combination with more traditional approaches, to address various research questions.

While current technologies in the field of genetic toxicology generally evaluate single endpoints, newer approaches are providing the opportunity to detect alterations in a number of endpoints simultaneously. In the future, these new approaches 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 evaluate chemicals concurrently for their ability to cause cancer, to impact the nervous system, to cause birth defects, and to modify immune function, providing additional insight to the individual responses and increasing the efficiency and effectiveness of the regulatory process.

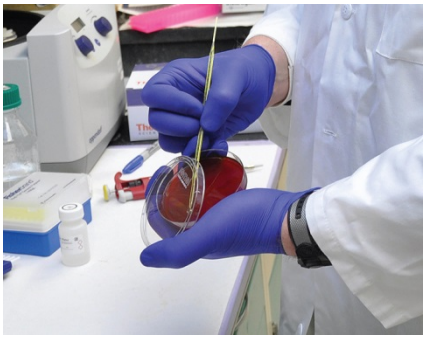
DGMT is committed to recruiting and retaining experts in the field of genetic toxicology, as well as providing continuing-education training opportunities to current staff. Several staff members have completed a graduate certificate program at the University of Arkansas for Medical Sciences that was developed through a Memorandum of Understanding between the State of Arkansas and FDA. One staff member is currently working toward a Masters of Science in Regulatory Science at the University of Maryland Center Of Excellence in Regulatory Science and Innovation. This program was developed through a collaborative effort between FDA and University of Maryland, Baltimore and funded by a grant from FDA. DGMT also offers hands-on training through special employment opportunities, and current DGMT staff include a number of Postdoctoral Fellows and an FDA Commissioner's Fellow who are receiving intensive training in the field of genetic toxicology. In the past year DGMT also has hosted scientists from China and Mexico for short-term training opportunities.

Division of Microbiology Summary of Activities

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

Introduction

The Mission of the Division of Microbiology is to provide specialized expertise to perform fundamental and applied research in microbiology in areas of FDA's responsibility to address critical issues in support of FDA's mission. The Division of Microbiology research projects are based on expertise of division staff and consultation with scientists from other FDA Centers, regulatory agencies, academia, industry, and NCTR science advisory board-committee members.



Scientist working with microbiology culture.

During FY 2015, the Division of Microbiology scientists engaged in research addressing a variety of FDA issues with special emphasis on: 1) *Host-Microbiome Interactions* by evaluating interactions between the human microbiota, antimicrobial agents, food contaminants, food additives, food supplements, nanomaterials, and other FDA-regulated products; 2) *Antimicrobial Resistance* by determining resistance mechanisms and factors that contribute to the development and dissemination of resistant pathogens; 3) *Foodborne Pathogens and Virology* by developing approaches to detect, characterize, and better understand the physiology of foodborne bacteria and viruses; 4) *Environmental Biotechnology* by improving risk assessments of priority pollutants— including polycyclic aromatic hydrocarbons—and FDA-regulated products; and 5) *Specialized Research addressing Women's Health, Tobacco Products and Nanotechnology.*

Selected accomplishments for FY 2015 in each of the areas are highlighted below, along with division plans for FY 2016.

FY 2015 Accomplishments

Host-Microbiome Interactions

A diverse population of microbes including bacteria, fungi, and viruses, collectively termed the microbiome, resides in various parts of the body including the mouth, gastrointestinal (GI) tract, vagina, and skin. Scientists in the Division of Microbiology are working to address various aspects of the host-microbiome niche. Division scientists examined the effects of silver nanoparticles (AgNP) on the intestinal bacterial (*in vitro*, *in vivo* and *ex vivo* systems) as well as viral (*in vitro* and *ex vivo*) populations. Exposure to small size AgNP led to the greatest shifts in bacterial and viral subpopulation as evidenced by 16S sequencing and viral sequencing, respectively. The antimicrobial properties of AgNP and the evidence linking human health to gut commensal microbiota are both well-established, so such research will allow the FDA to evaluate both potential host response and microbiota health effects of GI exposure to AgNP. Additional studies, in consultation with FDA's Center for Veterinary Medicine (CVM), are in progress to evaluate the safety of antimicrobial veterinary-drug residues in food with respect to their impact on the human-intestinal microbiota. This study will determine if residual amounts of tetracycline at various levels impact the human-GI tract microbiota by assessing shifts in the microbiota populations, the selection of antimicrobial-resistant bacteria and whether the GI bacteria degraded/inactivated the drug. Results of these studies are providing data as part of the safety assessment for the evaluation of the microbiological risks of veterinary-drug residues in food.

Antimicrobial Resistance

Antimicrobial resistance is a significant public-health concern globally due to the potential of treatment failure for severe infections. To address this important area, the White House released its National Strategy for Combating Antibiotic-Resistant Bacteria (CARB) in 2014. Urgent and serious threats identified in the CARB plan include carbapenem-resistant *Enterobacteriaceae*, *Clostridium difficile*, extended spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, drug-resistant *Salmonella*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). The Division of Microbiology is undertaking or is planning to conduct research to address these threats.

Research examining the emergence of extended-spectrum β-lactamase (ESBL)- and carbapenem-resistant *Escherichia coli* from companion animals has continued to explore the potential for the dissemination of these urgent public-health

threats. Plasmid DNA isolated from these bacteria was sequenced; and multiple antibiotic and antiseptic virulence and regulatory genes were detected. These genes could pose risks to public health because of the close proximity between humans and their pets. Another area the division has focused on is the genetics of resistance in the prominent foodborne pathogen *Salmonella enterica*. These studies have examined the roles of plasmids in emergence of ESBL-resistant *Salmonella* isolates from imported foods, as well as multidrug-resistant *Salmonella* from domestic foods and food-animal sources. *Salmonella* isolated in Mississippi from ground turkey demonstrated greater drug resistance than isolates from chicken meat. Another study showed that on a particular turkey farm that did not use antibiotics, turkeys and feed appeared to be the major reservoirs of multi-drug resistant *Salmonella*, which also harbored multiple virulence genes. The presence of resistance genes on plasmids allows for the potential spread of resistance from a resistant organism to one less resistant. Division researchers evaluating the impact of antimicrobial exposure on plasmid transfer found that for some *Salmonella* strains exposed to different antimicrobial agents, there was an exposure concentration-dependent impact on the efficiency of resistance plasmid transfer among bacteria. Additional research in the Division focused on identifying the genetic basis of multidrug resistance in *E. coli* and *Vibrio parahaemolyticus* isolates from imported seafood.

To evaluate the MRSA and VRE genetics, division scientists completed whole-genome sequencing of four *S. aureus* and three *Enterococcus* isolates from patients with serious and life-threatening healthcare-associated infections, which yielded important data on pathogenicity and antimicrobial resistance. Additional research is ongoing in collaboration with FDA's Center for Drug Evaluation and Research (CDER) to study the contamination of pharmaceutical products, such as the disinfectants benzalkonium chloride and chlorhexidine, by the *Burkholderia cepacia* complex. This study will provide data to help FDA determine the proper usage and storage of antiseptics and improve the efficient detection methods of these potential pathogens that contaminate pharmaceutical products.

Food Safety and Virology

The Division of Microbiology has a long history of contributing to several areas of human food safety. These contributions continue in several areas related to understanding the molecular epidemiology and dissemination of pathogens, improving pathogen-detection methods, and gathering fundamental knowledge on how pathogens develop increased ability to colonize hosts and cause diseases. Division researchers, in collaboration with colleagues in FDA's Center for Food Safety and Nutrition (CFSAN), analyzed over 250 Shiga-toxin producing

Escherichia coli (STEC) representing various serotypes and found they separated into eight distinct clades based on their overall gene content. Genes contributing to the greatest diversity among the isolates were those involved in transport, iron acquisition, and motility, as well as virulence, antimicrobial resistance, antiseptic resistance, and multidrug transporter genes. Other division researchers studying *Listeria* monocytogenes molecular epidemiology have found that serovars 1/2a and 1/2b were predominant in strains isolated from foods, while 1/2a and 4b were predominant in the environment. Pulsed-field gel electrophoresis analysis also demonstrated a high degree of genetic diversity among the isolates, which is important to help evaluate potential routes of food contamination. Proteomic analyses of *Campylobacter jejuni* under different stress conditions demonstrated that several genes were differentially expressed, which may allow enhanced colonization of the poultry host. In addition, *C. jejuni* isolates appear to survive much longer in raw chicken juices than other growth media, which may indicate an increased potential risk to consumers.

Division scientists have worked on optimizing methods to isolate bacteria, fungi, and their DNA from consumer products to conduct microbial-population genetics studies and examine for potential product contamination. Total genomic DNA from *Bacillus cereus* isolated from dietary supplements was submitted for sequencing in collaboration with CFSAN. Enterotoxigenic strains of *B. cereus* fell into a different genomic group from other strains, which may help to understand other *Bacillus* species that are enterotoxigenic. Additionally, work with *Clostridium perfringens* demonstrated that isolates from various sources were genetically diverse. Strains from domestic animals were more closely related to each other than to other strains, but clinical strains with distinct genetic backgrounds could be associated with any of the closely related groups.

Several projects have focused on the ability of pathogens, especially *Salmonella*, to cause more severe illness. Studies of the potential function of cytolethal distending toxin B in *S. Javiana* showed that strains that encoded the toxin, induced cytoplasmic distension and nuclear enlargement in intestinal cells and macrophages. Plasmid-encoded genes also contributed to increased virulence. For example, transmissible plasmids from *Salmonella* that encode a specific type-4 secretion system are likely to alter mRNA expression of cytokines in host cells, leading to increased virulence during infection. Another group of plasmids, termed incompatibility group FIB plasmids, have been studied to identify plasmid-associated antimicrobial resistance, virulence, and transfer-associated genes that may affect the bacterial phenotype and the potential for transmission of resistance to other bacteria. Additional research has shown that during co-

infection of *Salmonella* and Norovirus, *Salmonella* blocks macrophage cell death caused by Norovirus, possibly prolonging Norovirus infection. Additional Norovirus studies demonstrated that titanium dioxide (TiO2)-nanoparticle pretreatment of cells increased Norovirus replication and inflammation. These results, if extrapolated to human infection, may indicate that TiO2 exacerbates the disease caused by Norovirus infection.

Environmental Biotechnology

The division has had a long and respected history of conducting research to address public-health needs related to the environmental contamination of FDA-regulated products and understanding the role of microorganisms in the biotransformation of compounds that affect human health. For example, division scientists have utilized genome analyses to build connections between the dynamics and evolution in polycyclic aromatic hydrocarbon (PAH) metabolism in the genus *Mycobacterium.* The biological impact of BP Deepwater Horizon crude oil from the Gulf of Mexico on *M. vanbaalenii* has been studied using genomic, proteomic, ecological, and metabolic approaches. BP crude oil contains carcinogenic PAHs, whose presence in seafood is monitored by FDA. These studies which were highlighted by the American Society of Microbiology have revealed the molecular and physiological mechanism of degradation and detoxification of the toxic compounds.

Additional work in the division is focused on understanding the biotransformation of steroids, flavonoids, and other organic compounds, which have been used to produce new experimental drugs. Many biotransformation reactions are mediated by cultures of fungi, such as *Aspergillus niger*. Other research has focused on understanding how Orange II and Sudan III azo dyes in cosmetics affect global gene expression in the skin bacterium, *Staphylococcus aureus*. This was shown by microarray and quantitative real-time polymerase chain reaction (PCR) technology. In the presence of Orange II, most differentially expressed genes in *S. aureus* are down- or up-regulated by antibiotics, reactive oxygen species, and alkaline shock. In the presence of Sudan III, most differentially expressed genes (especially transporter genes) are up-regulated, suggesting that the azo dyes damage the cell wall or membrane.

Women's Health

Over the last fiscal year, scientists from the division, in collaboration with FDA's ORA, CDRH, and CDER have investigated several important areas of research related to women's health. Menstrual toxic-shock syndrome is caused by a toxin

produced by *Staphylococcus aureus* and occurs primarily in young women during menstruation and with tampon use. Although guidance documents from CDRH provide a number of published microbiological methods to be used to assess growth of and toxin production by *S. aureus*, there is no accepted reference method or standard to conduct these tests. Scientists within the division have systematically examined these methods with a variety of tampons to determine the feasibility of generating a microbiological standard of testing. Such a standard would promote an increased level of assurance to the consumer, the manufacturers and CDRH reviewers.

In addition, scientists within the division have examined the safety of using nanoparticles as intravaginal drug-delivery systems. Several types of nanoparticles, including biodegradable polymers, are taken up during an active yeast infection by vaginal epithelial cells in tissue culture and could potentially increase cellular damage and inflammation during infection. Studies are now underway to elucidate the pro-inflammatory responses in mice. Emerging nanotechnology for intravaginal drug delivery, which promises to improve treatment of diseases in women, could worsen inflammation during vaginal yeast infections, prompting this study of immune responses to intravaginal nanomaterials during yeast infections modeled in mice.

Tobacco Product Research

Scientists within the division have evaluated the effects of smokeless tobacco products on the metabolism and function of oral bacteria. The use of smokeless tobacco products poses a health risk due to potential increased risk of the development of oral-pharyngeal cancers. Research studies have involved carrying out the optimization of methods used to isolate bacteria and fungi from these products and to isolate DNA for microbial population-genetics studies.

Nanotechnology

Due to the advancement in nanotechnology, the use of nanomaterials (NMs) as biocide agents or adjuvants has gained momentum. It is expected that this widespread use of nanoparticles may result in increased human-health risk. The projects described here are part of a multi-faceted approach to develop new experimental models for safety testing of nanoparticles. Development of these protocols is in line with FDA Strategic Priorities to evaluate the safety of nanomaterials in consumer products and to develop testing alternatives to animal models.

Division scientists completed a project examining the effects of silver

nanoparticles (AgNP) in an *in vitro* cell culture-based model of intestinal permeability. Exposure to AgNP, especially those of smaller sizes, causes changes in the permeability of the intestinal epithelial barrier and modulates the gene expression of cell-cell junctions. In another study, scientists from NCTR and the University of Massachusetts-Lowell studied the impacts of NMs on enhancing the antibacterial activity of traditional antibiotics on *Pseudomonas aeruginosa*. They showed that silicon, titanium, and zinc oxide NMs with smaller size and large surface area have higher ability to enhance antimicrobial effectiveness of traditional antibiotics against wild type *P. aeruginosa* strains. The efflux pump seems to be associated with the antimicrobial-enhancing ability of NMs.

Since nanocrystal- or nanoparticle-based drug applications are submitted to various FDA centers for approval, division scientists have collaborated with other FDA centers to address the knowledge gap. Under the Collaborative Opportunities for Research Excellence in Science (CORES) grant funded by the Office of the Chief Scientist—and carried out by NCTR, CDER, and CDRH—scientists showed that exposure of mice to silver, gold, and silica NMs will not make them prone to bacterial infections. Another ongoing study in collaboration with CDER and the University of Connecticut is to establish a nonclinical model and perform risk assessment of FDA-regulated drug-nanocrystals. *In vitro* and *ex vivo* intestinal models will be utilized to examine the immunotoxicity of microsized parent drugs and reformulated nanocrystal drugs. The results should help FDA in reviewing the nano-based medicines and developing standards for the pharmaceutical industry.

FY 2016 Plans

Host-Microbiome Interactions

The Division of Microbiology's FY 2016 research plans related to the microbiome and host interactions include undertaking a risk assessment of the xenobiotic compounds through a National Toxicological Program-funded project. The results of this project will help to determine the effects of xenobiotics on the microbiome and gut-associated immune responses. Some examples of the selected compounds for this assessment are arsenic and triclosan. Additional studies are also being planned to evaluate the impact of residue levels of tetracycline and erythromycin on the gastrointestinal microbiota. Scientists from FDA's NCTR and Center for Biologics Evaluation and Research (CBER) will collaborate to study how certain types of bacteria in the human-intestinal

microbiome influence intestinal epithelial cells and dendritic cells to inhibit or advance *Clostridium difficile* infections, and better understand the mechanisms of fecal-microbiota transplantation. Furthermore, studies will be conducted to investigate effects of intestinal-bacteria metabolism on genotoxicity of food contaminants, such as Sudan dyes, the metabolic relationship between Sudan dyes and intestinal microbiota, and potential health risks in consuming foods contaminated with the dyes. Efforts to evaluate the effect of nanoparticles and nanodrugs on the intestinal microbiota are planned. These studies will support FDA Strategic Goal 1 (*Enhance Oversight of FDA-Regulated Products*). Division scientists will continue to evaluate the effect of smokeless tobacco products on oral microbiota at the physiological and metabolomic levels and conduct bioinformatics data analysis to determine the effects of smokeless tobacco products and tobacco-specific nitrosamines on oral bacterial ecology of animals. This project supports FDA Strategic Goal 3 to *Promote Better Informed Decisions About the Use of FDA-Regulated Products*.

Antimicrobial Resistance

The Division of Microbiology's FY 2016 research plans related to antimicrobial resistance include further evaluation of plasmid-associated antimicrobial resistance and virulence in *Salmonella* using DNA-sequence data analysis and *in vitro* assessment. A project is planned to develop bioinformatics tools to characterize virulence and plasmid genetics and examine factors that influence plasmid transfer. These studies should contribute to protecting public health by identifying potential ways to minimize the development and spread of virulence and antimicrobial resistance in foodborne pathogens. In addition, upcoming work is planned to examine antimicrobial resistance and biofilm development in *S. aureus* contaminating implanted medical devices that are imbedded with antibiotics. Similarly, scientists are planning to study the role of efflux pumps in antibiotic resistance, biofilm formation, and colonization by *E. coli* associated with urinary-tract infections. These studies should improve understanding of factors that contribute to urinary tract and medical device-associated infections and of biofilm biology.

These antimicrobial resistance-associated projects address several of the FDA Strategic Goals, including to *Enhance Oversight of FDA-Regulated Products* (Goal 1) by providing data to the FDA's regulatory centers on factors that contribute to the development of resistance. In addition, the studies help to *Improve and Safeguard Access to FDA-Regulated Products to Benefit Health* (Goal 2) by providing improved methods for recovery of microorganisms from pharmaceutical products and identifying ways to potentially limit the dissemination of

antimicrobial resistance. Divisional projects also *Promote Better Informed Decisions About the Use of FDA-Regulated Products* (Goal 3) by providing data on how antimicrobial selective pressure can lead to the development of antimicrobial resistance, which can help with the judicious use of the drugs.

Food Safety and Virology

For FY 2016, the division plans to help develop bioinformatics tools to characterize virulence and plasmids genetics and the development of a multilocus sequence-typing method for rapid detection of microorganism in different foods. The division will also further investigate the effect of nanoparticles in virus and recombinant FimH (fimbriae) of *Salmonella* on Norovirus replication. These studies will support FDA Strategic Goal 2 to *Improve and Safeguard Access to FDA-Regulated Products* by improving methods to evaluate drug and regulated products safety; and Strategic Goal 3 to *Promote Better Informed Decisions About the Use of FDA-Regulated Products* by providing data on potential disease exacerbation by food products and additives.

Environmental Biotechnology

New initiatives planned for FY 2016 include assessing the biotransformation of plant-derived terpenoids by fungi to produce new compounds with antibacterial, antifungal, antiparasitic, antiviral, or antitumor activity. New detection methods for *Clostridium difficile* infections and *Mycobacterium chelonae* contamination of tattoo inks will also be developed and evaluated. The division's environmental biotechnology research will help address the FDA Strategic Goal 2 to *Improve and Safeguard Access to FDA-Regulated Products to Benefit Health* by examining the utility of biotransformed terpenoids as potential therapeutics. The ongoing and new environmental biotechnology research will address Strategic Goal 3 to *Promote Better Informed Decisions About the Use of FDA-Regulated Products* by improving the detection methods for the contamination of consumer products and by continuing projects that help our understanding of the environmental fate and toxicity of compounds before FDA approval of new drugs and other medical products, as indicated by FDA's CDER and CVM "Guidance for Industry" documents.

Women's Health

These multi-year projects to address the needs of women's health will continue in FY 2016 by working to advance the microbial testing approaches for tampons and examine the pro-inflammatory responses to nanoparticles in model systems. These studies contribute to FDA's Strategic Goal 2 to *Improve and Safeguard Access to FDA-Regulated Products to Benefit Health* through the evaluation of the safety

new drug- delivery approaches and the identification of optimal safety-assessment approaches for testing menstrual tampons. They also address FDA Strategic Goal 3 to *Promote Better Informed Decisions About the Use of FDA-Regulated Products* by providing consumers and regulators with a better understanding of microbial-growth and toxin-production dynamics in tampons.

Tobacco Product Research

Studies are underway to quantify nitrate, nitrite, nicotine, and tobacco-specific nitrosamines (TSNAs) content in smokeless tobacco products and will examine the effects of smokeless tobacco products and TSNAs on oral bacterial ecology of animals. The data generated by these ongoing studies will provide important scientific evidence on carcinogen dynamics to aid in making effective regulatory decisions related to smokeless tobacco products which will support FDA Strategic Goal 3 to *Promote Better Informed Decisions About the Use of FDA-Regulated Products*.

Nanotechnology

The division's FY 2016 research related to nanotechnology will involve screening human skin, intestinal and vaginal microbiota (bacteria and viruses), as well as host toxicity (using *in vitro*, *ex vivo* and *in vivo* models) in the presence of nanoscale (metal- and carbon-based) materials. For example, studies will utilize *in vitro* assays of human-skin microbiota in the presence of nanoparticles from cosmetics to determine their effect on human-skin microbial ecology, which should enhance our knowledge of the safety and toxicity of nanomaterials and provide data for FDA safety assessments. Another project will investigate the effects of nanoparticles used in dentistry on the oral microbiota. Overall, the nanotechnology studies will enhance our scientific knowledge of the safety and toxicity of nanomaterials and provide data to be considered for safety assessment. Because the division's nanotechnology research spans the spectrum of more fundamental evaluation of the NM-exposure effect on the host and its microbiome to applied studies assessing the impact of nanoparticles on specific pathogens, the research supports FDA Strategic Goals 1, 2, and 3. For example, studies to establish science-based minimum standards for conducting hazard analysis of products containing AgNP is a step towards FDA readiness to evaluate innovative technologies for product assessment and will help *Enhance Oversight of FDA-Regulated Products* (Goal 1). Similarly, understanding the migration of nanomaterials from consumer-use products may lead to exposure of mucosal surfaces and impact intestinal health; thus studies of nanotechnology will need to consider these effects to *Improve and Safeguard Access to FDA-Regulated Products to Benefit Health* (Goal 2). In addition, research on nanoparticle

exposure to bacterial susceptibility will help in developing standards and regulatory-guidance policy for the pharmaceutical industry, which will *Promote Better Informed Decisions About the Use of FDA-Regulated Products* (Goal 3).

Division of Neurotoxicology Summary of Activities

Merle G. Paule, Ph.D., Director
870-543-7203
Merle.Paule@fda.hhs.gov

Introduction

The National Institute of Mental Health estimates that one in four American adults suffer from a diagnosable mental disorder in any given year and that one in three will experience some form of mental disorder during their lifetime. Fifty-million Americans have a permanent neurological disability that limits their daily activities. The number of persons suffering from Alzheimer's, Parkinson's, and other neurodegenerative diseases is increasing dramatically as our population ages. Disability from depression alone exceeds that of diabetes, hypertension, gastrointestinal, and lung diseases *combined*. Conservative estimates put the financial cost of brain-related dysfunction in the U.S. at well over half a trillion dollars per year. Thus, diseases and disorders of the brain represent enormous societal burdens, both economically and in terms of human suffering. The known and suspected causes of brain-related disorders include exposures to chemicals—including therapeutic drugs and drugs of abuse—food additives, food products, cosmetic ingredients, pesticides, and naturally occurring substances. Each year millions of children are exposed to anesthetics and sedatives that have been shown in pediatric-animal models to cause significant nerve-cell death and subsequent brain dysfunction. Nanomaterials are entering our world at an ever-increasing pace, yet little is known about their potential toxicity. Addictive behaviors associated with tobacco products continue to take their toll.

The number of FDA-regulated chemicals that can affect the nervous system runs well into the thousands and chemicals that are known or suspected causes of brain-related disorders are vital to the national economy and our quality-of-life. Our challenge, thus, is to determine at what levels of exposure and under what conditions these compounds can be used effectively while minimizing risk.

The ultimate goals of the Division of Neurotoxicology are to understand the

biological pathways relevant to the expression of neurotoxicity and identify relevant, yet practical, biomarkers. Developing methods to help identify potential toxicities is critical for the assessment of neurotoxic risk, the development of informed safety guidelines, and the development of protective and therapeutic strategies.

The strategies employed for achieving these goals often involve multidisciplinary approaches that capitalize on the expertise of division personnel which includes: neurochemistry, molecular neurobiology, neuropathology, neuro and behavioral pharmacology, neurophysiology, experimental psychology, and multi-modal bio-imaging. Fortunately, technological advances are continuing to provide new tools with which to better study and understand the causes and pathologies associated with brain-related diseases and to better define the biological pathways involved. Efforts to develop sensitive, higher-throughput systems for screening potential neurotoxicants have been developed (brain cell cultures, zebrafish, rodent-neural stem cells) or are well underway (human and nonhuman primate-neural stem cells). Methods are being employed to assess the addiction potential of tobacco-product constituents. These advances will provide the tools to assess the risks associated with the use of regulated products and inform actions to protect and improve public health.

Preclinical MRI machine — one instrument used in the NCTR Bio-Imaging Facility.

FY 2015 Accomplishments

In partnership with FDA's Center for Drug Evaluation and Research (CDER) colleagues, division staff continued studies on the neurotoxicity associated with pediatric general anesthetics utilizing both *in vitro* (rodent and human neuronal-cell cultures) and *in vivo* (rat and nonhuman primate) approaches by expanding studies to include the commonly used anesthetic agents sevoflurane and propofol. The data obtained contribute to the regulatory needs of FDA and, importantly, help to identify strategies that may prevent or reduce anesthetic-induced neurotoxicity. This year studies utilizing a Positron Emission Tomography (PET) imaging signal for visualizing aspects of brain inflammation

in a non-invasive fashion have shown that sevoflurane, like other general anesthetics, also causes activation of inflammatory responses in the brain for at least several days— and likely weeks—after exposure. We are, thus, continuing to define the time course—in both rodents and primates—of neuroinflammation associated with pediatric exposures to general anesthetics and to begin to explore ways to minimize such effects. The utilization of PET imaging brings us closer to our goal of being able to translate preclinical findings to the clinical setting. Coupling Computerized Tomography technology with PET technology brings enhanced mapping detail to the process and new instrumentation to augment that approach was brought on line this year.

In studies exploring the biological mechanisms associated with pediatric anesthetic-induced neurotoxicity we have continued to demonstrate in virtually all of our models that the antioxidant and mitochondrial-stabilizing agent, acetyl-L-carnitine, exerts significant neuroprotective properties when given prior to and during pediatric anesthesia. Studies designed to assess the effects of developmental exposures to acetyl-L-carnitine, with and without general anesthetics, on brain function in our nonhuman primate model are ongoing. In these studies we are employing NCTR's battery of cognitive functions tests [the Operant Test Battery (OTB)] that are also being used in collaborating clinics studying children who have experienced general anesthesia at a young age. Preliminary data indicate that acetyl-l-carnitine can prevent some of the adverse cognitive deficits associated with pediatric general anesthesia, but that it may, itself, have unwanted side effects at the doses that were used. As reported previously for the pediatric general anesthetic, ketamine, early data show that general anesthesia induced by isoflurane plus nitrous oxide during early brain development also results in long-lasting cognitive function deficits in nonhuman primates.

The use of animal-neural stem cells has figured prominently in much of our work on pediatric general anesthetics and other agents with a view towards further elucidating the cellular and subcellular effects underlying exposure-associated neurotoxicity. This year we have incorporated human-neural stem cells into these studies and are now providing information not only about how anesthetics might impact nerve-cell growth, differentiation, and proliferation but also about species comparability and the ability to extrapolate animal findings to human systems. The early data suggest that human-neural stem cells are approximately equal to rodent-neural stems cells in their sensitivity to the adverse effects of general anesthetics.

Employing Magnetic Resonance Imaging (MRI) with a very powerful magnet (7 Tesla), prototypic neurotoxicants were used in studies to induce classic neuropathology and the MRI was used to obtain information in living animals about the location, onset, severity, and time-course of notable changes in MRI signals. In initial publications, the case is being made that this approach demonstrates that the use of MRI in such a fashion will provide critical information for helping to detect neurotoxicity and details were provided for the neurotoxicant, kainic acid. By identifying areas of abnormal MRI signals in the brain, subsequent neuropathological assessments using tissue slices and traditional staining techniques can be targeted towards those areas, thereby maximizing chances of detection while minimizing effort. In addition, each animal can serve as its own control and be imaged repeatedly in a non-invasive manner, thus, reducing the number of animals needed and providing comprehensive life-cycle information on brain tissue responses to chemicals.

Significant progress was made in the development of novel nervous-tissue stains to aid in the evaluation of brain pathologies, in particular, those associated with the deposition of Alzheimer's disease-type plaques in brain. To advance studies related to Alzheimer's disease neurodegeneration, a transgenic rat model has been acquired by the Division and an on-site colony is being developed to provide this valuable research animal to NCTR researchers. Early assessments indicate that this rat model more closely approximates the disease in humans than do the more commonly used transgenic mouse models.

The use of the dye, Fluoro-Jade C (for labeling dead and dying nerve cells), was previously only known to be effective in fixed tissue. It has now been shown to also work in fresh (unfixed) tissue as well as in living cells in culture. When confirmed in other systems, these observations will demonstrate that it may now be possible to assess the health of living cells in a rapid and high-throughput fashion. Changes in gene and protein expression in sick cells identified in this manner can then be assessed: this is not possible in fixed tissue.

At exposures matching or near human therapeutic levels, we continue to see few adverse effects of chronic methylphenidate (MPH) treatment on cognitive function in a nonhuman-primate model, even after several years of exposure. PET imaging studies, designed to examine the integrity of important neurotransmitter systems throughout the brain, have begun to determine if such treatment affects basic brain metabolism. In light of the current widespread use of MPH to treat Attention Deficit and Hyperactivity Disorder, such studies are critical in providing important information about the safety of its long-term

administration. Cognitive functions in children are also being assessed using the NCTR OTB —the same instrument used with our nonhuman primates. These studies are being carried out at our laboratory at nearby Arkansas Children's Hospital and in laboratories at the Mayo Clinic where the effects of pediatric general anesthesia are being studied. Such studies are exemplary of translational neuroscience and highlight the divisions' cross-species capabilities for assessing cognitive function.

A nonhuman-primate behavioral pharmacology laboratory to support the needs of the Center for Tobacco Products has come online and initial studies are underway. This laboratory will assess the ability of tobacco-product constituents to actually produce and maintain addictive behaviors. Studies are underway to develop physiologically-based pharmacokinetic models to help define and quantitate exposures. Concomitant PET-imaging studies are being conducted to explore aspects of neurochemical changes associated with exposure to nicotine and other tobacco-product constituents.

In partnership with the Health and Environmental Sciences Institute (HESI) of the International Life Sciences Institute (ILSI), a protocol using the prototypic neurotoxicant— trimethyltin—was begun in an attempt to identify biomarkers of neurotoxicity having potential clinical utility. The focus is, thus, on those biological signals that are available using minimally-invasive sampling techniques, such as the collection of bodily fluids and imaging. This effort involves partners from government, industry, and academia and has benefited from extensive collaborative consultation.

FY 2016 Plans

Division efforts in FY 2016 will focus on studies addressing the following:

- The effects of *developmental* exposures to pediatric general anesthetics on subsequent complex brain function in rodent and nonhuman primate models.

- The effects of *adult* exposures to general anesthetics on subsequent complex brain function in rodent and nonhuman primate models.

- Expanding the utilization of *in vitro* models to include nonhuman primate-neural stem cells, blood-brain barrier models, and the zebrafish developmental-neurotoxicity model to study prototypic neurotoxicants, including general anesthetics.

- The efficacy and toxicity of a variety of potential anti-Alzheimer's agents using transgenic mouse and rat models.

- The development and use of novel histochemical tracers to monitor the health of important nervous-system structures in both fresh and fixed tissue: incorporation of Rare Earth Metals.

- Utilization of state-of-the-art imaging capabilities to provide new insights into the events contributing to neurotoxicity and neuroprotection using markers of cell death and neuroinflammation.

- Identification of MRI approaches to support regulatory science efforts by developing standards for using MRI/Magnetic Resonance Spectroscopy (MRS) signals to direct traditional neuropathological assessments: begin biomarker-qualification process.

- The relationship between performance of the tasks that comprise the NCTR OTB and clinically-relevant psychological tests to further assess the translatability of the OTB: multisite studies.

- Nonhuman-primate behavioral pharmacology studies to support Center for Tobacco Product studies on the ability of tobacco-product constituents to engender and/or maintain addictive behaviors.

Contributions to FDA's Strategic Priorities/Goals

The bulk of the research conducted by the Division of Neurotoxicology directly supports NCTR's Strategic Goal of Advancing Scientific Approaches and Tools Required to Support Public Health, a goal which directly supports FDA's Core Mission and Goals.

The development of sophisticated imaging approaches, alternative preclinical models (rodent models of Alzheimer' s and Parkinson's disease, zebrafish, neural stem cells, and *in vitro* blood-brain barrier models), cross-species metrics of brain function, and the integration of omics approaches to identify novel markers of neurotoxicity all support FDA's Goal 1 (Enhance Oversight of FDA-Regulated Products) and Objective 1. 1 (Increase the use of regulatory science to inform standards development, analysis, and decision-making). Developing and

refining animal models and providing techniques for monitoring and detecting adverse effects associated with the use of regulated products are clear objectives under this FDA goal.

The Division's work on the toxicity of general anesthetics and sedatives used in a pediatric context and its studies on the effects of developmental exposures to methylphenidate and other agents also supports the FDA Goal 2 (Improve and Safeguard Access to FDA-Regulated Products to Benefit Health)and Objective Goal 2.2 (Improve the Effectiveness of the Product Development Process) and the priority to "Expand Efforts to Meet the Needs of Special Populations" by conducting research directly relevant to children. Our training of students, visiting scientists, and postdoctoral fellows contributes to all of these goals.

Division of Systems Biology Summary of Activities

William B. Mattes, Ph.D., D.A.B.T., Director
870-543-7681
William.Mattes@fda.hhs.gov

Introduction

The Division of Systems Biology mission is to solve problems of food, drug, and medical-product safety using systems-biology approaches and innovative technology. This includes the development and evaluation of new technologies and the identification of new biomarkers to support the FDA mission. The Division is divided into three branches:

1) Biomarkers and Alternative Models Branch
2) Innovative Safety and Technologies Branch
3) Personalized Medicine Branch

The division is comprised of a multidisciplinary group of toxicologists, molecular biologists, analytical chemists, computational modelers, and more. The goals of the division are to use systems-biology approaches and emerging science and technology to:

- Identify mechanisms of toxicity and new translational biomarkers that a) improve the safety evaluation of drugs and other FDA-regulated products b) improve the safety of FDA-regulated medical interventions.

- Develop and evaluate innovative methods to detect unsafe products, advance the identification of infectious-disease contamination, and enhance diagnostic procedures.

NCTR scientist conducting bacterial detection analysis.

- Determine the impact of differences in the responses of species and human sub-populations on current assessments of drug safety and efficacy.

- Evaluate the potential of "agents of interest" to the FDA to induce developmental toxicity. This is accomplished by the use of well-established methods and development of new approaches including use of stem cells.

FY 2015 Accomplishments

Mechanisms and Biomarkers of Tissue Injury

- **Liver**
  Liver injury caused by drugs remains not only a major reason for failure during the drug- development process (and risk for clinical-trial subjects), but also a patient-management issue with approved medications. Existing tests for liver damage are sensitive, but not specific, and do not provide information regarding the severity of the damage or the likelihood of repair. Furthermore, there are several drugs where the standard measurements in preclinical tests do not adequately predict human safety. Thus, new biomarkers and understandings of toxicity mechanisms are needed.

  1. A new class of drugs, mostly used for anticancer treatment, is the tyrosine kinase inhibitors (TKIs). Despite their development as "targeted" therapies, interfering with specific signaling pathways, many elicit adverse reactions in patients, including liver and heart injury. In an ongoing effort to comprehensively explore the mechanisms of toxicity for these agents, three have been examined in primary rat hepatocytes. Regorafenib, crizotinib, and ceritinib all induce cellular damage in primary rat hepatocytes at therapeutic levels; regorafenib appears to do so through specific mitochondrial injury.

  2. Former work in the Division of Neurotoxicology demonstrated that certain microRNA (miRNA) species in urine of treated rats allowed detection of hepatotoxicity. To investigate the translational nature of such biomarkers, we have undertaken two studies exploring miRNA responses in humans under conditions of hepatotoxicity. In collaboration with Dr. William Lee and the Drug-Induced Liver Injury Network we have examined urine miRNAs in patients studied by the Acute Liver Failure

Study Group. While the results are preliminary, it seems that miRNA patterns can discriminate between survivors and non-survivors. In another clinical study, serum and urine samples were obtained from 1) healthy pediatric patients, 2) those exposed to therapeutic doses of acetaminophen (APAP) and 3) those that had overdosed with APAP. Significant increases in the serum and/or urinary level of several species of miRNA were observed upon APAP overdose. Our results suggest that miRNAs might provide needed information to those developing drugs and to clinicians managing patients undergoing drug-induced injury.

3. In a metabolomics analysis of pediatric patients who were admitted for APAP overdose, we observed significant increases in bile acids (glycodeoxycholic acid, taurodeoxycholic acid, and glycochenodeoxycholic acid) compared to healthy controls. Variability in bile acids was greater among hospitalized children receiving low doses of acetaminophen than in healthy children with no recent acetaminophen exposure. While not as accurate as APAP-protein adducts in discriminating children with APAP overdose from those with low-dose APAP, these biomarkers provide some indication of liver injury.

- **Heart**

  Doxorubicin is a potent anticancer drug that can cause chronic heart damage in humans. Furthermore, a new class of targeted anticancer drugs, the tyrosine kinase inhibitors (TKI), also shows the ability to cause heart damage similar to that caused by doxorubicin. There is a need to understand how these drugs cause such damage so as to better evaluate new drugs and manage treatment with existing drugs.

  1. A mouse model of drug-induced cardiac injury has been developed. Changes in the expression levels of genes and miRNAs (genomics) and proteins (proteomics) in the heart were associated with drug-induced cardiac injury and identified as candidate biomarkers of early cardiac injury and may lead to a new understanding of the mechanism behind doxorubicin toxicity. Metabolomics analysis of the plasma and tissue revealed potential biomarkers in both samples that preceded overt heart damage. In particular, 3 of 7 short acylcarnitines— including carnitine— were increased in plasma but decreased in the heart early in doxorubicin treatment.

  2. These investigations have been extended to examination of miRNA in the plasma of the treated mice. Numerous miRNAs were found to increase

very early in doxorubicin treatment, preceding overt heart damage. Further study will confirm if these may serve as early biomarkers of heart injury.

3. To conduct parallel experiments in a cultured-cell system a model using human- heart cells derived from pluripotent stem cells was developed. These cells show characteristics of heart cells including rhythmic beating. Doxorubicin treatment of these cells do cause toxicity, and examination of the cell-culture medium showed that certain miRNAs were released from the cells very shortly after treatment, and before other signals of toxicity were measured. This system has the potential to not only explore early biomarkers but also to screen the toxicity of drugs such as TKIs.

4. Since mitochondrial dysfunction has been implicated in the toxicities of many therapeutic drugs, mitochondria-specific oligonucleotide microarrays (MitoChips) for rodents, nonhuman primates, and humans were developed. Unlike other microarrays, these microarrays consist of both mitochondrial and nuclear genes-encoding mitochondrial proteins to obtain important insights into the interaction between mitochondrial and nuclear genomes during drug-induced toxicities or disease conditions. Theses arrays are being used to explore the mitochondrial impact of several cardiotoxic and hepatotoxic drugs.

Innovative Technology

- **Infectious agents and antibiotics**
  Rapid identification of infectious agents in FDA-regulated products continues to be an important public-health need.

  1. RAPID-B™ is a technology that was developed by researchers within this division and has been licensed to a commercial entity. This is a flow cytometric-based approach using a field-tested machine. A successful internal FDA level-3 validation of the *E. coli* O157 (a variant of a bacterium that causes severe food poisoning) was performed in collaboration with FDA's Office of Regulatory Affairs' Arkansas Regional Laboratory.

  2. The RAPID-B™ was successfully adapted to identification of prions in human blood samples using nanospheres.

3. Initial studies have shown success in detecting *Listeria* monocytogenes and *Listeria* spp. using genetic probes.

4. A novel mass spectrometry-based system, SpecID, was developed to rapidly develop "fingerprints" of pathogenic strains, useful in characterizing food-pathogen outbreaks.

- **Stem cells**

In the past, *in vitro* cell-culture testing was limited to immortalized cell lines, or primary cells isolated from tissues. In the case of cell lines, there is always the question of relevance of cellular processes to those of normal tissues, and in the case of primary cells there is always the question of de-differentiation in culture. Stem-cell cultures offer the possibility of utilizing well-characterized differentiated cells and/or monitoring differentiation in culture.

1. The mouse Embryonic Stem Cell Test has been used to examine agents that induce developmental toxicity. The current assay uses differentiation to cardiomyocytes as an endpoint. We have examined the use of differentiation to osteoblasts as a parallel endpoint. Our results suggest that differentiation to osteoblasts may provide confirmatory information in predicting embryotoxicity.

2. In another study, human induced pluripotent stem (iPS) cell-derived cardiomyocytes have been evaluated for their ability to evaluate the potential of regulated products for cardiotoxicity. The toxicity of mainstream cigarette smoke condensates (CSCs) was assessed in iPS-derived cardiomyocytes with cellular function assays and cardiomyocyte-specific endpoints. The CSC treatments reduced cell viability and resulted in dose-dependent changes in the beat rate as assessed by a real-time cellular- impedance measurement. Global gene-expression analysis of cardiomyocytes treated with CSCs using next-generation sequencing identified dysregulation of genes for multiple cardiac ion channels, including major genes from potassium and calcium channels.

- **Computational modeling**

  To improve early screening of molecules for unwanted toxicity, new *in silico* modeling approaches are being developed.

1. We have continued to improve upon our patented modeling approach (3D-QSDAR), to models that are more accurate and useful than previously

published models. We built and statistically validated 3D-SDAR models of phospholipidosis, and binding to the hERG channel protein. Note that drug-induced phospholipidosis, observed preclinically and clinically, is a concern for FDA, and compound binding to the hERG channel protein has been shown to have a role in cardiac dysfunction. Thus, both models have the potential to augment *in silico* screening and/or regulatory appraisal of New Molecular Entities.

2. The 3D-QSDAR models offer a unique advantage in being able to predict a chemical toxicophore, and a fascinating finding is that the toxicophores predicted by the hERG channel and phospholipidosis models have true similarity. The possibility that channel binding of some nature may play a mechanistic role in phospholipidosis is being explored.

3. Patients carrying certain human leukocyte antigens (HLAs) alleles may develop adverse drug reactions (ADRs) after taking specific drugs. Peptides play an important role in HLA related ADRs as they are the necessary co-binders of HLAs with drugs. A network analysis-based method to understand and predict HLA-peptide binding was developed and is being further explored.

Species and Sub-population Comparisons and Personalized/Precision Medicine

Since NMEs are evaluated for safety and efficacy on the basis of both non-clinical and clinical data, it is essential to evaluate how responses in animal models recapitulate those in various human populations. Conversely, to enable personalized/ precision medicine, the uniqueness of sub-populations and individuals and how this affects responses (both in terms of efficacy and toxicity) must be better understood. In addition to the rat and mouse studies reported above that evaluated individual differences in response to drugs that induce liver damage, other studies were performed.

- **Species Comparative Responses**
  An important question for both research and regulatory practice is which drugs show concordance in human and animal adverse responses and which do not. An approach to data mining FDA submission documents was developed that allows direct comparisons between clinical and preclinical reports of toxicity. For both antiviral drugs and the tyrosine kinase inhibitors examples may be found where there is concordance between animal and

human responses. On the other hand, drugs where the animal responses do not predict the human response present opportunities for investigative species-specific mechanisms.

- **Sex and Age Comparative Responses**
  1. Sex-related differences have been indicated in the development of cardiotoxicity induced by doxorubicin. However, mechanism(s) underlying differences in susceptibility to doxorubicin between the sexes is still unclear. A mouse model of sex-related differences in doxorubicin was developed that showed a greater sensitivity to doxorubicin in males than females.

  2. We previously conducted a study of the mRNA, miRNA, and DNA methylation levels of selected organs of untreated rats, assessed at various time points during their life cycle and in both sexes. Analysis of the miRNAs in kidney showed age effects predominated over sex effects, with 2-week miRNA expression being much different from other ages.

  3. To identify the potential role of mitochondria in sex-related differences, the same dataset was analyzed for the basal-expression levels of mitochondria-related genes in the hearts of male and female rats. The expression of genes involved in fatty acid metabolism was significantly different between the sexes in young and adult rat hearts. In old rats, a majority of genes involved in oxidative phosphorylation had higher expression in females compared to males.

  4. Given that tyrosine kinase inhibitors have numerous organ-specific toxicities, we hypothesized that differences in transcriptional profiles of the kinome (i.e. protein kinases) may be responsible for differential susceptibility of specific tissues to the adverse effects of TKIs. Preliminary analysis of the dataset revealed that a majority of these protein kinases showed significant difference in the expression level between at least two organs in both male and female rats.

- **Sub-Population Comparative Responses**
  It is well known that genetic differences between individuals in the genes coding for drug-metabolism enzymes can lead to differential responses to certain drugs. What is now becoming clear is that such genes can be controlled by a number of micro RNAs and that differential expression of these miRNAs may lead to individual susceptibilities. The role of miRNA in

controlling several drug-metabolism and drug-transport proteins was examined, and experiments confirm the role of certain miRNAs in the regulation of the CYP2B6, CYP2C19, ABCC6, ALDH5A1, and SLC22A7 genes.

- **Developmental Toxicity**
  2-hydroxy-4-methoxybenzophenone (HMB; oxybenzone) is an ultraviolet (UV)-absorbing compound used in many cosmetic products as a UV-protecting agent and in plastics for preventing UV-induced photodecomposition. HMB has been detected in over 97% of randomly collected human-urine samples and in the urine from premature infants, and it may have estrogenic potential. Rats were exposed to various doses of HMB in feed throughout pregnancy and lactation, and while some parameters were affected at the highest dose, at possible human-exposure levels, HMB does not appear to be a significant reproductive toxicant.

FY 2016 Plans

- New tyrosine kinase inhibitors (TKIs) are continually being developed, and as such it is critical to understand the mechanisms of their toxicity and biomarkers that may be used to screen for toxicity. We plan to examine the hepatotoxicity using *in vitro* models from several species, and applying systems-biology tools to understand the pathways affected. We will carry out in parallel, experiments examining the cardiotoxicity of certain TKIs both *in vitro* and using the mouse-cardiomyopathy model. Further data mining of the mouse, rat, and human kinome can provide clues as to species and target organ specificity of action.

- It is critical that new biomarkers of tissue damage be translational, i.e. useful in preclinical testing and in humans. To this end, new attempts on creating collaborations to access useful human samples will be made.

- Equally important is the development of new approaches to improve safety evaluation of FDA-regulated products. The use of stem cells will be further explored to examine the utility of cells derived from males or females and those derived from relevant subpopulations.

- We plan to expand the application of our *in silico* modeling approaches to areas that may provide screening opportunities, such as mutagenicity. Also, using data developed as part of the Tox21 program of the National Center for

Advancing Translational Sciences we will determine the relationship between the phospholipidosis and hERG models.

- We plan to advance our proteomics capabilities by exploring a new, multiplexing technology using DNA aptamers that act effectively like antibodies. In addition to providing a new tool for biomarker discovery, this effort will provide valuable information to regulatory divisions as this tool enters the clinical diagnostic realm.

Contributions to FDA's Strategic Priorities/Goals

The work performed in the Division of Systems Biology contributes to the FDA Strategic Priorities. The work being done on identifying biomarkers of drug-induced organ injury, developing new stem cell assays, and computational modeling all support FDA Objective 2.1 (*Increase regulatory science capacity to effectively evaluate products*) as well as Objective 2.2 (*Improve the effectiveness of the product development process*). These efforts and those focused on the improvement in the detection of bacteria and prion contamination of FDA-regulated products also support Objective 1.1 (*Increase the use of regulatory science to inform standards development, analysis, and decision-making*). Finally, the efforts being made in understanding precision medicine, and the role of genetics, sex, and age on drug-induced tissue damage support Objective 2.2 (*Improve the effectiveness of the product development process*).

NCTR Objective 1.1 – Integrated Product Assessment

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

Participation in Data Quality Task Group (DQTG) to Foster Development of Consensus Quality Control (QC) Standards in Metabolomics Data Acquisition (S00787)

Responsible Division: Systems Biology

Objective(s):

The goal of the DQTG efforts would be to move toward validation exercises for analytical QC techniques with multiple international metabolomics groups. By using the greater metabolomics community as the source of analytical QC techniques and criteria information, the DQTG should be able to reach consensus decisions on what samples of QC is used and how those QC samples are evaluated.

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

Distribution of an Adjuvant Containing Squalene and Alpha-Tocopherol in Mice (E0751401)

Responsible Division: Biochemical Toxicology

Collaborating FDA Center: CBER

Objective(s):

Provide to CBER, experimental pharmacokinetic data in mice for an oil-water adjuvant designated AS03 that will be used to validate an *in silico* Physiologically Based Pharmacokinetic model.

PI: Binienda, Zbigniew K., Ph.D.

Assessment of Iron-Oxide Nanoparticle (NP)-Induced Neurotoxicity in Cell Cultures and Whole-Animal Models (E0739401)

Responsible Division: Neurotoxicology

Collaborating FDA Center: CFSAN

Objective(s):

1) Determine if acute or chronic exposure of different sizes of iron-oxide NPs produce specific changes in the mitochondrial function, cell death, and generation of reactive oxygen species in different regions of rat and mice brain using *in vivo* microdialysis.
2) Determine if acute or chronic exposure to iron-oxide NPs produce significant changes in neurotransmitter concentrations in different regions of mice/rat brains using microdialysis.
3) Determine if acute or chronic exposure of different sizes of iron-oxide NPs produce alterations in the brain-free fatty acid levels.
4) Determine if acute or chronic exposure to different sizes of iron-oxide NPs produce changes in lipid peroxidation and/or in antioxidant enzyme activity (catalase, superoxide dismutase, glutathione peroxidase) and glutathione levels in mice and rat brains.

5) Determine if acute or chronic exposure of different sizes of iron-oxide NPs produce selective pattern of deposition and damage in different regions of rat and mice brain using *in vivo* MRI.

PI: Boudreau, Mary D., Ph.D.
A 13-Week Dosed Water Study to Determine the Potential Toxicity of Aloin in the Cecum and Large Intestine of F-344 Rats (E0219501)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CDER
Objective(s):

1) Evaluate whether or not drinking water administration of aloin (aloin-A and aloin-B) to F-344 rats exerts similar effects in the rat large intestine, when administered at concentrations similar to those in the previous studies conducted at NCTR on *Aloe vera* whole leaf extract.
2) Evaluate aloin for dose trend effects in the F-344 rat and incorporate dose concentrations that bracket the maximum level of aloin (10 mg aloin/kg) that is permitted in commercial products certified under the International Aloe Science Council program.
3) Evaluate the toxicity of drinking water administration of Senna in the F-344 rat.
4) Examine the effects of exposure to aloin and Senna extract on different populations of bacteria in the rat gastrointestinal tract.

PI: Boudreau, Mary D., Ph.D.
A Toxicological Evaluation of Nanoscale Silver Particles in Rodents (E0217001)

Responsible Division: Biochemical Toxicology
Collaborating FDA Centers: CDRH, CFSAN
Objective(s):

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

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

An Evaluation of the Effect of Vehicle Cream on the Photococarcinogenicity of Retinyl Palmitate in SKH-1 Mice (E0218501)

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

1) Determine the stability and homogeneity of retinyl palmitate in the *Aloe vera* control cream.
2) Evaluate the photococarcinogenicity of retinyl palmitate when incorporated into the *Aloe vera* control cream applied to the skins of SKH-1 mice in the absence and presence of simulated solar light (SSL).
3) Determine the photococarcinogenicity of disopropyl adipate as the filler ingredient in the *Aloe vera* control cream in the absence and presence of SSL.

PI: Cao, Xuefei, Ph.D.

Dose-Response Genotoxicity of Ethylmethane Sulfonate (EMS) in Mice Using the Pig-a and Transgenic gpt Delta Assays (E0739001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):

1) Use sensitive genotoxicity endpoints with low background frequencies to increase the sensitivity of the assays for detecting low-dose effects.
2) Measure genotoxicity using a design to detect the maximum responses.
3) Measure the effects of EMS exposure in neonatal as well as adult animals.
4) Measure genotoxicity in the major target tissues for EMS carcinogenicity.

PI: Cao, Xuefei, Ph.D.

Evaluating the Toxicity and Inflammation Produced by Cigarette Smoke Using Human *In vitro* Airway Models (E0754901)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Divisions: Biochemical Toxicology, Systems Biology
Collaborating FDA Center: CTP
Objective(s):

1) Evaluate the toxicological effects of cigarette smoke.
2) Identify the most informative intracellular molecular biomarkers for cigarette smoke toxicity. Cigarette smoke-induced changes in mRNA and miRNA expression and DNA methylation status will be assessed in tissue samples.
3) Identify extracellular molecular biomarkers for cigarette smoke toxicity. Cigarette-smoke-induced changes in miRNA expression will be evaluated by analysis of basal medium and secreted mucus.

PI: Chelonis, John, Ph.D.

Effects of Sevoflurane Exposure on Complex Brain Function in Adult Rhesus Macaques (E0759401)

Responsible Division: Neurotoxicology

Objective(s):

1) Determine the effects of sevoflurane exposure on the performance of adult monkeys in tasks designed to measure motivation and learning.
2) Determine the effects of sevoflurane exposure on the performance of adult monkeys in tasks designed to measure short-term memory, time perception, and visual stimulus discrimination.

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

Application of Biometrical Procedures for National Toxicology Program (NTP) Projects (S00175)

Responsible Division: Bioinformatics and Biostatistics

Objective(s):

In response to requests from NCTR scientists, modify and/or apply statistical techniques to the design, conduct, analysis, and interpretation of NTP studies to identify and assess the cancer and noncancer risks of potentially toxic substances.

PI: Chen, Tao, Ph.D.

Do Engineered Silver Nanomaterials (Ag-ENMs) Varying by Size and Coatings Behave Differently Than Bulk Silver in Their Ability To Induce Genetic Damage? (E0750101)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Office: Office of Scientific Coordination

Objective(s):

1) Evaluate the Ames test and mouse lymphoma assay, in addition to the *in vitro* micronucleus assay.
2) Investigate Ag-ENMs of various sizes and compare to bulk silver results.

PI: Chen, Tao, Ph.D.

Evaluation of the Applicability of *In vivo* Micronucleus Assays for Assessing Genotoxicity of Engineered Nanomaterials (E0731001)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Divisions/Office: Biochemical Toxicology, Microbiology, Office of Scientific Coordination

Collaborating FDA Center: CFSAN

Objective(s):

1) Assess the genotoxicity of four types of nanoscale materials, carbon nanotubes, nanoscale titanium dioxide, nanoscale gold, and nanoscale silver in three standard tests suggested by FDA; *Salmonella* Ames test, mouse lymphoma assay,

and *in vivo* mouse micronucleus assay.

2) Evaluate the possible mechanisms of nanomaterial-induced genotoxicity using a transgenic mutation system comet assay, and genomic analysis.

PI: Delclos, Kenneth B., Ph.D.
Evaluation of Molecular, Morphological, and Functional Endpoints in NCTR Sprague-Dawley Rats Treated with Bisphenol A (BPA) Administered by Gavage to Sprague-Dawley Rats from Gestational Day 6 Until Birth and Directly to Pups from Postnatal Day (PND)-1; Continuous and Stop Dose (PND-21) Exposures (E0219101)

Responsible Division: Biochemical Toxicology
Collaborating Office: Office of Scientific Coordination
External Partner: National Toxicology Program
Objective(s):
Evaluate a range of molecular, morphological, and functional endpoints in rats dosed orally with a wide range of BPA doses in a chronic toxicology study.
Determine if any effects observed are predictive of long-term toxic effects evaluated in the companion chronic toxicology study or reveal potential effects undetected by standard toxicological evaluations.

PI: Delclos, Kenneth B., Ph.D.
Two-Year Chronic Toxicology Study of Bisphenol A (BPA) Administered by Gavage to Sprague-Dawley Rats from Gestational Day-6 until Birth and Directly to Pups from Post-Natal Day (PND)-1, Continuous and Stop Dose (PND-21) Exposures (E0219001)

Responsible Division: Biochemical Toxicology
Collaborating Office: Office of Scientific Coordination
External Partner: National Toxicology Program
Objective(s):
Characterize the long-term toxicity of orally administered BPA, including developmental exposure, in the NCTR Sprague-Dawley rat over a broad dose range. In addition, animals generated in this study will be assigned to separate protocols for assessment of a range of molecular, morphological, and functional endpoints to determine if these endpoints are predictive of long-term toxic effects or reveal potential effects undetected by standard toxicological evaluations.

PI: Doerge, Daniel R., Ph.D.
Human Biomonitoring for Bisphenol A (BPA)(E0743101)

Responsible Division: Biochemical Toxicology
Objective(s):

1) Develop and implement sensitive and selective analytical methodology

to measure BPA from blood and urine samples from children and adults with known exposures.

2) Integrate human biomonitoring data with pharmacokinetic data from animals and humans to produce a physiologically based pharmacokinetic model for BPA to empower FDA to reach science-based decisions about risks, particularly to children and fetuses, from medical devices, food contact materials, and other environmental exposures.

PI: Doerge, Daniel R., Ph.D.
Human Biomonitoring for Exposure to Bisphenol A (BPA) and Potential Replacement Products (E0747101)

Responsible Division: Biochemical Toxicology
External Partner: National Institute of Environmental Health Sciences
Objective(s):

1) Provide human biomonitoring data for BPA and its structural analogs that are potential replacement products in adults exposed occupationally to thermal-paper cash register receipts. The routes of administration (dermal/oral) are likely key determinants of internal exposure to the active unconjugated form of BPA and/or possible replacements. These data will be used for physiologically based pharmacokinetic modeling along with existing pharmacokinetic data from experimental animals and humans.
2) Provide estimates of concentrations of active BPA aglycone in potential target tissues of developing fetuses and children for BPA and/or structural analogs from all possible exposures, particularly from food and medical devices, so FDA can make science-based decisions on risks from BPA and possible replacement products.

PI: Doerge, Daniel R., Ph.D.
Human Studies of Isoflavone Safety and Efficacy (S00607)

Responsible Division: Biochemical Toxicology
External Partners: University of Miami, Wayne State University
Objective(s):
Conduct bioanalytical analysis of soy isoflavones (and metabolites) in support of clinical trials at the University of Miami and Wayne State University.

PI: Fang, Jia-long, Ph.D.
Two-Year Dermal Carcinogenicity Bioassay of Triclosan in B6C3F1 Mice (E0219401)

Responsible Division: Biochemical Toxicology
Collaborating Division/Office: Bioinformatics and Biostatistics, Office of Scientific Coordination
External Partner: National Toxicology Program
Objective(s):
Evaluate the chronic toxicity/carcinogenicity of triclosan administered dermally to mice for 104 weeks.

PI: Ferguson, Sherry A., Ph.D.
Methylphenidate (Ritalin) Exposure During Pregnancy: Assessment of Neurotoxicity in Offspring (E0731801)

Responsible Division:
Neurotoxicology
Collaborating Divisions:
Biochemical Toxicology, Genetic and Molecular Toxicology, Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Quantify the neurobehavioral toxicity associated with pre- and early postnatal treatment with methylphenidate in rats.

PI: Ferguson, Sherry A., Ph.D.
Neurobehavioral Effects of Bisphenol A Across Age and Sex (E0219201)

Responsible Division:
Neurotoxicology
External Partner: National Toxicology Program
Objective(s):
Advance development of testing platforms in the area of food safety.

PI: Fisher, Jeffrey W., Ph.D.
PBPK Models for Bisphenol A (BPA) (E0742601)

Responsible Division: Biochemical Toxicology
Collaborating Division:
Neurotoxicology
Objective(s):

1) Create physiologically based pharmacokinetic (PBPK) models for BPA in mouse, rat, and rhesus nonhuman primate of adult, neonatal, pregnant (mother and fetus), and lactating (mother and neonate) laboratory animals. These models will be used to calculate internal measures of dose for both active and inactive forms of BPA.
2) Create human PBPK models for BPA (adult, child, pregnant mother and fetus, and lactating mom and infant) using data from the nonhuman primate, mouse, and rat, and limited human information from literature. The human suite of models will be used to extrapolate the internal toxic doses of BPA in laboratory animals to humans. The PBPK models will also be used to extrapolate dosimetry from regions of observation to low levels of exposure to BPA for which no experimental data exist.

Interpret biomonitoring data for BPA in urine and blood.

PI: Foley, Stephen L., Ph.D.
Microbial Populations and the Development of Tobacco-Specific Nitrosamines in Moist Snuff Products (E0756801)

Responsible Division:
Microbiology
Collaborating Division:
Biochemical Toxicology
Collaborating FDA Center: CTP
Objective(s):

1) Determine the population changes of bacteria in moist snuff products marketed over a one-year timeframe,

with special emphasis on species that are known nitrate reducers.

2) Determine the population changes of bacteria within batches/lots of moist snuff products over a three-month timeframe.
3) Compare the levels of tobacco-specific nitrosamines in smokeless tobacco products with different bacterial populations present.
4) Determine the impact of storage temperature on the production of TSNAs and bacterial populations.

PI: Goodwin, Amy K., Ph.D.
Aspects of Nicotine Self-Administration in the Nonhuman Primate (E0753701)

Responsible Division: Neurotoxicology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Collaborating FDA Center: CTP
Objective(s):

1) Set up a surgical suite for implantation of intravenous (IV) catheters in squirrel monkeys and a nonhuman primate behavioral pharmacology laboratory for conducting the self-administration studies, drug mixing, and related tasks.
2) Purchase, quarantine, and habituate 12 early adolescent male squirrel monkeys and 12 adult male squirrel monkeys.
3) Compare the acquisition and maintenance of nicotine self-administration across decreasing doses in adolescent and adult squirrel monkeys.
4) Investigate and compare the abuse liability of the non-nicotine tobacco product constituents myosmine and anatabine in squirrel monkeys using a substitution procedure.
5) Investigate and compare the effects of the non-nicotine tobacco product constituents myosmine and anatabine on responding for nicotine in squirrel monkeys.
6) Describe the pharmacokinetics of IV self-administered nicotine and other tobacco constituents (myosmine and anatabine).
7) Describe and compare alterations in dopamine levels in the midbrain associated with IV-administered nicotine and other tobacco constituents (myosmine and anatabine).

PI: Goodwin, Amy K., Ph.D.
Nicotine Nonhuman Primate Pharmacokinetic Study (E0753711)

Responsible Division: Neurotoxicology
Collaborating Division: Biochemical Toxicology
Collaborating FDA Center: CTP
Objective(s):
Collect pharmacokinetic data for nicotine and relevant metabolites (cotinine and trans-3-hydroxycotinine) in three adult and three adolescent experimentally naïve squirrel monkeys from blood and urine samples to allow for

development of a physiologically-based pharmacokinetic model.

PI: Gough, Bobby J., Ph.D.
The Impact of a Glial Modulator (PPF) on Methamphetamine (METH)-Induced Dopamine (DA) Dynamics: A Microdialysis Study in Rats and Mice (E0743301)

Responsible Division: Neurotoxicology
Objective(s):

1) Simultaneously measure DA and its metabolite levels in the caudate nucleus of rats and mice using dual online injection.
2) Determine effect PPF will have on METH-evoked DA levels.
3) Determine the protective nature of PPF against METH-induced neurotoxicity in both species. Results of pilot study will indicate possible future studies.
4) Strengthen FDA abilities in microdialysis and further validate the use of mice in neurochemical studies.

PI: Gu, Qiang, Ph.D.
Proteomic Assessment of the Cytotoxic Effects of Nanoparticles (NPs) on the Blood-Brain Barrier (BBB) (E0746001)

Responsible Division: Neurotoxicology
Collaborating Divisions/Office: Biochemical Toxicology, Systems Biology, Office of Scientific Coordination
Objective(s):

1) Describe alterations in expression and/or phosphorylation of proteins that are involved in apoptosis, inflammation, oxidative stress, and tumor-genesis signaling pathways in the cells that form BBB following NP exposure using cutting-edge proteomic approaches.
2) Proteomic changes will also be correlated with conventional cytotoxicity and BBB permeability assays. Additional nanoparticles may also be studied if early findings warrant.

PI: Guo, Lei, Ph.D.
Develop Methods for the Evaluation of Smokeless Tobacco-Associated Carcinogenesis (E0748801)

Responsible Division: Biochemical Toxicology
Collaborating Division: Microbiology
Collaborating FDA Center: CTP
Objective(s):

1) Evaluate and compare the carcinogenic activity of smokeless tobacco products.
2) Investigate animal models for comparing and evaluating carcinogenic activities (especially oral-cavity tumor induction) of smokeless tobacco products.
3) Test the hypothesis that tobacco-specific N-nitrosamines (TSNA) are major contributors to carcinogenic activity of smokeless tobacco.
   a. Determine and quantify the major carcinogenic alkaloid-

derived TSNA (NNK and NNN) in each product.

b. Detect and quantify NNK-and NNN-derived DNA adducts in various samples, such as liver, pancreas, blood, and oral tissue, collected from animals administered NNK, NNN, or smokeless tobacco.

4) Determine gene expression and DNA methylation profiles at whole genome level and for specific pathways, such as DNA damage/repair, for biomarker discovery and mechanism elucidation.
5) Determine effect of smokeless tobacco products or TSNA (chemical in smokeless tobacco) on oral microbiota of the animals.

PI: Guo, Lei, Ph.D.

Study of Drug-Induced Liver Toxicity Using State-of-the-Art *In vitro* Liver Models, Including Primary Rat and Mouse Hepatocytes and Stem Cells (E0732101)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Genetic and Molecular Toxicology, Systems Biology
Objective(s):

1) Obtain signature-gene and protein-expression patterns of each cell type for comparison to toxin-induced changes.
2) Determine the contribution of each cell type to overall liver toxicity from agent exposure once these isolated cell types are reliably available.
3) Provide training to give confidence in the integrity of liver cells following perfusion, separation, and culture of the liver cells.

PI: Guo, Xiaoqing, Ph.D.

Development of Methods To Expose Cells in Culture to Volatile Chemicals (E0754301)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Collaborating FDA Center: CTP
Objective(s):

1) Develop and demonstrate the reproducibility of a cell culture exposure protocol for volatile test articles by:
   a. Developing a test procedure for the Mouse Lymphoma Assay utilizing suspension cells in culture, the CH Technologies Jaeger-Baumgartner 30-Port Cigarette Smoking Machine, VitroCell exposure chambers, and whole cigarette smoke as the test article.
   b. Demonstrating the reproducibility of the exposure conditions for the MLA by conducting exposures with different concentrations of whole smoke over a period of six months.

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

In vitro Analysis of Factors Influencing CYP1A2 Expression as Potential Determinants of Sex and Interindividual Variation: Role of Hormones and Epigenetics (E0739301)

Responsible Division: Biochemical Toxicology

Objective(s):

1) Determine the effect of cigarette smoke condensate (CSC) or I3C on CYP1A2 expression in selected liver and lung cell lines.
2) Determine the effect of combining menthol with CSC.
3) Determine the effect of CSC (with and without menthol) or I3C on DNA methylation and histone modification in CYP1A2 as epigenetic regulatory mechanisms in liver and lung cells.
4) Determine the effect of hormones (eg., estrogen, testosterone, growth hormone) on CYP1A2 expression as factors underlying sex differences in expression.

PI: Heflich, Robert H., Ph.D.

ADDENDUM to E0739001: Dose-Response Genotoxicity of Ethylmethane Sulfonate (EMS) in Mice using the Pig-a and Transgenic gpt Delta Assays (E0739021)

Responsible Division: Genetic and Molecular Toxicology

Objective(s):

Replace the dams used in a previous experiment so we can repeat the neonatal dose-response study in Experiment 3. This experiment was not conducted properly due to a failure of the dosing pump that was not noticed until several daily doses had been administered, making data from this experiment unreliable.

PI: Hiranita,Takato, Ph.D.

Assessment of Effects of Tobacco Product Constituents on Extracellular Dopamine Levels in the Nucleus Accumbens in Rats (E0753801)

Responsible Division: Neurotoxicology

Collaborating FDA Center: CTP

Objective(s):

1) Set up a surgical suite [for implantation of intravenous (IV) catheters and intracranial probes in rodents] and a rodent neurochemical and behavioral pharmacology laboratory [for brain microdialysis/locomotor activity (LMA) studies].
2) Assess the capacity of IV injections of S(-)-nicotine and non-nicotine tobacco product constituents (anatabine, harmane, myosmine, norharmane and their vehicles) by themselves to alter:
    a. extracellular levels of dopamine (DA) in the nucleus acumens shell
    b. concurrent spontaneous LMA in drug naïve rats.
3) Assess the capacity of IV injections of the non-nicotine tobacco product constituents anatabine, harmane,

myosmine, norharmane and their vehicles to alter S(-)-nicotine-stimulated:

a. extracellular levels of DA in the nucleus acumens shell
b. spontaneous LMA in drug naïve rats.

PI: Hong, Huixiao, Ph.D.

High-Throughput Screening Tobacco Constituents for Addiction Potential Using Docking of Nicotinic Acetylcholine Receptors (E0754801)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CTP
Objective(s):
Develop *in silico* models for screening chemicals in tobacco products and smoke that have potential to cause addiction. More specifically, docking analyses will be conducted on all tobacco constituents using three-dimensional (3D) structures of '4ß2 and '7 of which the ligand binding sites will be modeled by using all crystal structures of the complexes of nAChRs bound with ligands that are available in the PDB (Protein Data Bank). The docking results then will be used for the purpose of predicting addiction potential of the more than 8000 chemicals that have been identified in tobacco products to assist the FDA regulatory decision making or to help the design of follow-up experiments for identifying addictive tobacco constituents.

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

NCTR/Office of Regulatory Affairs (ORA) Nanotechnology Core Facility—NTP IAG SUPPORT (S00715)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Office: ORA
External Partner: National Toxicology Program
Objective(s):

1) Support the needs of NCTR to characterize nanoscale materials used in toxicology tests and to detect these materials in biological samples.
2) Support the needs of ORA/Arkansas Regional Laboratory to detect and characterize nanoscale materials in FDA-regulated products.

PI: Hu, Shu-Chieh, Ph.D.

13-Week Nose-Only Inhalation Toxicity Study of NNK in Rats (E0753101)

Responsible Office: Office of Scientific Coordination
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Collaborating FDA Center: CTP
Objective(s):

1) Set up a surgical suite for implantation of intravenous (IV) catheters in squirrel monkeys and a nonhuman primate behavioral pharmacology laboratory for conducting the self-administration studies, drug mixing, and related

tasks.

2) Purchase, quarantine, and habituate 12 adolescent squirrel monkeys and 12 adult male squirrel monkeys.
3) Compare the acquisition and maintenance of nicotine self-administration across decreasing doses in adolescents and adults.
4) Investigate and compare the abuse liability of the non-nicotine tobacco product constituents myosmine and anatabine in squirrel nonhuman primates using a substitution procedure.
5) Investigate and compare the effects of the non-nicotine tobacco product constituents myosmine and anatabine on responding for nicotine in squirrel monkeys.
6) Describe the pharmacokinetics of IV self-administered nicotine and other tobacco constituents (myosmine and anatabine).
7) Describe and compare alterations in dopamine levels in the midbrain associated with IV-administered nicotine and other tobacco constituents (myosmine and anatabine).

PI: Hu, Shu-Chieh, Ph.D.
14-Day Nose-Only Inhalation Toxicity Study of NNK in Rats (E0753401)

Responsible Office: Office of Scientific Coordination
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Collaborating FDA Center: CTP
Objective(s):
Evaluate the biological responses in rats following nose-only inhalation exposure of NNK for 14 days.

PI: Hu, Shu-Chieh, Ph.D.
Pharmacokinetic Analysis of NNK in Sprague-Dawley Rats (E0752501)

Responsible Office: Office of Scientific Coordination
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Collaborating FDA Center: CTP
Objective(s):
Evaluate the pharmacokinetic parameters of NNK in rats following a single-dose administration of test substance via intraperitoneal injection, nose-only inhalation exposure, and oral gavage, respectively.

PI: Hu, Shu-Chieh, Ph.D.
Support of CTP/NCTR Inhalation Toxicology Core Facility (S00785)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Center: CTP
Objective(s):
The objective for the CTP/NCTR Inhalation Toxicology Core Facility (Inhalation Core) are to conduct inhalation toxicology studies on behalf of the FDA Center for Tobacco Produces (CTP). The intent of this project/support is to account for the inter-study efforts that are required for the inter-study processing and validation of components of the equipment used

in the Inhalation Core.

PI: Inselman, Amy L., Ph.D.
Effect of Oxybenzone on Fertility and Early Embryonic Development in Sprague-Dawley rats (Segment I) (E0218601)

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

1) Examine the reproductive toxicity of oxybenzone in male and female rats. The study is designed to focus specifically on fertility and early embryonic development to implantation [ICH Guideline S5(R2) 4.1.1].
2) Compare the results of a typical Segment I, II, and III study design with results from a modified one-generation study proposed by the National Toxicology Program.

PI: Inselman, Amy L., Ph.D.
Effect of Oxybenzone on Pre- and Post-natal Development in Sprague-Dawley Rats (Segment III) (E0218801)

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

1) Determine the potential toxicity of oxybenzone to male and female rats. Compare the results of a typical Segment I, II, and III study with results from a modified one-generation study proposed by the NTP.

PI: Kanungo, Jyotshnabala, Ph.D.
Developmental Neurotoxicity Assessment of NMDA Receptor Antagonists in Zebrafish (E0752801)

Responsible Division: Neurotoxicology
Objective(s):

1) Assess the effects on Rohon-Beard sensory neurons of WT zebrafish embryos exposed to NMDA receptor antagonists (MK-801, dextromethorphan, ketamine and sevoflurane).
2) Assess their effects on the primary and secondary motor neurons and their axons using hb9:GFP transgenic embryos.
3) Post-exposure, wash-out experiments will be pursued to determine the effects of these drugs on the nervous system.
4) Determine estradiol-17' levels in control and treated embryos. Changes in gene expression for the two CYP aromatases/estrogen synthases (brain aromatase cyp19a1b and gonadal aromatase cyp19a1a) will be quantified using qPCR.
5) Perform ELISA to quantify the protein level.
6) Assess phenotype-based cell signaling mechanisms (MAPK, etc.) and neuron development-specific gene (Notch, Gli, Ngn1, NeuroD) expression.
7) Utilize neurotoxicity pathway-focused gene expression arrays

(SABioscience) to demonstrate potential genotype-phenotype correlations.

8) Reversal of noted adverse effects of these compounds on neurons will be attempted, particularly by treatment with acetyl L-carnitine.

PI: Khan, Ashraf A., Ph.D.
Detection of Cytolethal Distending toxin (cdtB), pltA and pltB Homologs of Components of the Pertussis Toxin Genes by Polymerase Chain Reaction (PCR) and Studies on Functionality of cdtB in Non-Typhoidal Salmonella spp. (E0739601)

Responsible Division: Microbiology
Collaborating Office: Office of Scientific Coordination
Collaborating FDA Center/Office: CFSAN, ORA
Objective(s):

1) Develop a duplex real-time, quantitative polymerase chain reaction (qPCR) to detect *Salmonella* and antibiotic-resistance gene markers simultaneously from food samples.
2) Validation of qPCR method at FDA, ORA laboratories.
3) Characterize plasmids, virulence genes, and integrons, for their role in virulence in multidrug-resistant *Salmonella* strains isolates from food.

PI: Khan, Saeed A., Ph.D.
Antimicrobial Properties of Zinc Oxide (ZnO) and Titanium Oxide (TiO2) Nanoparticles (NPs) Against Multidrug-Resistant Staphylococcus and Enterococcus spp. and Their Cytotoxic and Genotoxic Potential in Bacteria and Normal Human Epidermal Keratinocytes (NHEK) and Primary Intestinal Cells (E0751501)

Responsible Division: Microbiology
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination
Objective(s):

1) Study the mechanism of antimicrobial properties of TiO2 and ZnO Nanoparticles (NPs) in multidrug-resistant *Staphylococcal* and *Enterococcal* spp.
2) Study synergy between NPs and antibiotics.
3) Evaluate the cytotoxic and genotoxic potential of NPs in bacterial, and NHEK and primary intestinal-cell lines.
4) Study the transcriptomic gene expression in NHEK and intestinal-cell lines.

PI: Khan, Saeed A., Ph.D.
Does the Durable Nanoparticle Bioaccumulation in Macrophages Increase Susceptibility to Bacterial Infection? (E0753601)

Responsible Division: Microbiology
Collaborating Division/Office: Systems Biology, Office of Scientific

Coordination
Collaborating FDA Center: CDER
Objective(s):
Determine whether animals exposed to durable nanoparticles are more susceptible to *Listeria* infection as measured by the severity of disease and the length of time needed to clear the infection.

PI: Khare, Sangeeta, Ph.D.
Assessment of Size- and Shape-Dependent Toxicity of Silver Nanoparticles as Measured by Changes in the Permeability at the Gastrointestinal Surface (E0750601)

Responsible Division:
Microbiology
Collaborating Office: Office of Scientific Coordination
Objective(s):

1) Determine the effect of nanomaterials on the permeability of intestinal epithelial cells and ileal mucosa.
2) Assess tocixity of silver nanoparticles as measured by changes in the expression of genes involved in the epithelial integrity of polarized epithelial cells and ileal mucosa.

PI: Khare, Sangeeta, Ph.D.
Graphene-Induced Toxicity on the Population of Intestinal Microbiota and Gut-Associated Immune Response (E0754701)

Responsible Division:
Microbiology
Collaborating Office: Office of Scientific Coordination
Objective(s):
Evaluate the effects of graphene on the gastrointestinal homeostasis. To accomplish this goal we have subdivided the study in two phases. Phase one of the study will include *in vitro* assessment of effect of graphene; whereas phase two will include the *in vivo* study. The objective of these phases will be as follow:
Phase One (*in vitro* assessment):

1) Effect of graphene on the representative species of intestinal bacteria. The effect of graphene will be examined in a time- and dose-dependent manner.
2) Effect of graphene on the permeability of polarized epithelial cells. Graphene will be tested to assess the permeability and integrity of *in vitro*-cultured intestinal epithelial cell.

Phase Two (*in vivo* assessment):

1) Evaluate the effects of the graphene on the intestinal commensal microbiota in the orally gavaged rats. Perform a comprehensive culture-independent phylogenetic analysis of intestinal mucosa-associated microbes to analyze the effect of graphene on microbiome present in intestine in the representative from rat intestinal mucosa and feces.

2) Delineate the interaction of orally gavaged graphene at the gastrointestinal surface by assessing the gut-associated immune responses.
3) Measure by real-time polymerase chain reaction the expression of genes involved in the host innate immune response (proinflammatory and anti-inflammatory genes).

PI: Leakey, Julian E., Ph.D.
Physiologically-Based Pharmacokinetic (PBPK) Modeling of Nanomedicine; Building Clinically Relevant Standards for FDA-Regulated Nanoparticulate Drug Products (E0755401)

Responsible Office: Office of Scientific Coordination
Collaborating Division: Microbiology
Collaborating FDA Centers/Office: CDER, CVM, ORA
Objective(s):
1) Determine *in vivo* liposomal doxorubicin-release kinetics in individual tissues and blood stream by PBPK modeling.
2) Establish quantitative physicochemical property (liposomal size and content of ammonium sulfate)-biodistribution relationships of liposomal doxorubicin products by PBPK Modeling.
3) Extrapolate the PBPK model to rats and humans.
Develop a whole-body PBPK model to describe and simulate the biodistribution of liposomal vesicles and doxorubicin.

PI: Mattes, William B., Ph.D.
Understanding and Predicting Immune-Mediated Idiosyncratic Drug Reactions (IDRs): Molecular Modeling of Interactions Between Drugs, Polymorphic HLA Proteins, and T-cell Receptors (E0739501)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Apply molecular modeling approaches to better understand the underlying mechanisms of existing drug-HLA combinations known to cause immune-mediated IDRs.

PI: Meehan, Joseph F., Ph.D.
Support of Collaborative Regulatory Review and Research Projects with FDA-CDER (S00784)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Provide assistance in the development and enhancement of regulatory review and research tools at CDER, including enhancements to CDER's Data Analysis and Search Host system, development of a Pediatric Clinical Trials database, enhancement of the FDALabel system, genomics data analysis and review, and additional joint biomedical informatics projects as

requested.

PI: Mei, Nan, Ph.D.
Evaluation of the Ability of Standard Genetic Toxicology Assays To Assess the Relative Genotoxic Potential of Cigarette Smoke Condensates (E0745901)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Center: CTP
Objective(s):

1) Optimize short-term assays to evaluate their ability to initially assess the genotoxicity of cigarette smoke condensates. Based on the results from these studies, further research using commercial cigarettes and whole cigarette smoke will be designed.
2) Attain a dynamic potency range adequate to detect reductions of select harmful and potentially harmful constituents by 30, 50, and 70 percent.
3) Develop and validate a quantitative assay or assays to detect statistically significant differences in cigarette-smoke cytotoxicity over a range of biologically relevant concentrations.
4) Evaluate genotoxicity assays to determine their robustness, sensitivity, reproducibility, and accuracy.

PI: Mei, Nan, Ph.D.
Genetic Toxicology Evaluations in Support of FDA Centers for Evaluating Substances for their Genotoxic Potential (S00677)

Responsible Division: Genetic and Molecular Toxicology
Objective(s):
Provide direct research to FDA Product Centers.

PI: Mei, Nan, Ph.D.
***In vitro* Genotoxicity of Graphene-Family Nanomaterials Using FDA-Recommended Short-Term Genetic Toxicity Test Battery (E0753301)**

Responsible Division: Genetic and Molecular Toxicology
Collaborating Office: Office of Scientific Coordination
Objective(s):

1) Determine whether graphene and its functionalized derivatives are genotoxic using the standard regulatory test battery (the Ames test, the mouse lymphoma assay, and the micronucleus assay).
2) Compare the results from the three assays and provide insight into mechanisms underlying graphene's genotoxic effects.
3) If materials are mutagenic to mouse lymphoma cells, evaluate loss of heterozygosity at the thymidine kinase and three other microsatellite loci spanning the entire chromosome 11 in Tk mutants induced by graphene-family nanomaterials.
4) If the materials are mutagenic/genotoxic, determine whether the mode of action is through an oxidative pathway by measuring the intracellular ROS and reduced glutathione levels in cells immediately after exposure to

different concentrations of graphene-family nanomaterials.

5) If the materials are mutagenic/genotoxic, utilize gene expression arrays to provide additional insight into the mode of action.

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

Microbial Genetics of Non-0157:H7 Shiga-Like Toxin Producing Escherichia Coli (STEC) Insulated From Humans and Foods (E0735701)

Responsible Division: Microbiology

Collaborating Division: Biochemical Toxicology

Objective(s):

1) Obtain *E. coli* isolates from clinical, food-related outbreaks and veterinary diagnostics samples.
2) Map the epidemiological profiles of the isolates for specific genetic markers attributable to the origin of isolates and their phenotypic diversity.
3) Determine the antimicrobial-resistance profiles and potential mechanisms of drug resistance among enterohemorrhagic *E. coli* (EHEC) of various serogroups.
4) Identify the antimicrobial resistance and virulence gene determinants in the bacterial isolates that contribute to their pathogenicity.
5) Examine the role of plasmids, if any, in mitigating the transfer of drug resistance.
6) Compare the cytoxicities of selected EHEC strains to cultured RAW264.7 macrophage cells.
7) Evaluate the expression of Shiga-like toxin using *in vitro* enzyme assays.

PI: Pang, Li, Ph.D.

A Comprehensive Characterization of iPSC-CMs Models for Drug-Induced Arrhythmia Using High-Throughput Screening Assays (E0758201)

Responsible Division: Biochemical Toxicology

Collaborating Division: Systems Biology

Collaborating FDA Center: CDER

Objective(s):

1) Develop standard baseline criteria for high-throughput readouts of drug-induced arrhythmia in human induced pluripotent stem cell-cardiomyocytes (iPSC-CMs) from different suppliers.
2) Minimize variations during the drug- treatment process, iCells, Cort.4U cells, and one line of iPSC-CMs from SCT by treating them with the same hormone-free, and serum-free medium to assess the dose-response titration of 5 reference compounds: dofetilide, cisapride, sotalol, moxifloxacin, and verapamil.
3) Assess cardiotoxic responses including MEA for quantification of Field Potential Duration, beats per minute, Vmax upstroke velocity, and drug-induced arrhythmia; Fluo-4 Ca2+ imaging for assessing Ca2+ transients and homeostasis; RTCA

assay for real-time detection of changes of cell contractility and viability, the onset of irregular beat, IB20 (the minimal dose that induces approximately 20% arrhythmic beats in 3 consecutive 20-s sweeps) and BR20 (the lowest concentration that induces a reduction in beat rate of approximately 20% in 3 consecutive sweeps compared with the time-matched vehicle controls).

4) Employ quantitative gene-expression assays to assess gene expression for ion channels in different lines of cells.
5) Assess individual variance and possible sex differences in drug-induced cardiotoxic responses across a panel of non-genetically modified iPSC lines.
6) Evaluate the individual variations of drug response and potential sex differences.

PI: Parsons, Barbara L. Ph.D.

ADDENDUM to E0722901: Development of a Method To Use *In vivo* Mutagenicity Data to Address the Question as to Whether a Specific Chemical Induces Cancer Via a Mutagenic or a Non-mutagenic Mode-of-Action (MOA)—CRADA with TERA (E0722911)

Responsible Division: Genetic and Molecular Toxicology

Objective(s):

1) Establish culturing conditions for GDL1 cells and characterization of cell growth when two different sera are used in the growth media.
2) Expose GDL1 cells grown in DMEM with both FBS and HS to different doeses of a mutagen, N-ethyl-N-nitrosourea and a clastogen, mitomycin C for induction of mutations in the gpt, and spi- genes for validation of the cell line for testing vinyl acetate monomer (VAM) and acetaldehyde (AA).
3) Expose GDL1 cells cultured in DMEM with both FBS and HS to different doeses of VAM and AA for cytotoxicity and induction of mutations in the gpt, and spi- genes at doses that induce low, mid, and high cytotoxicity in GDL1 cells.
4) Further characterize several mutants if the induced mutant frequency is statistically significant, by DNA sequencing for VAM and AA-induced mutational profiles.

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

ADDENDUM to E0722901: Assessment of the Importance of K-RAS Mutation Induction in the Mode-of-Action for Lung-Tumor Development and Implications of K-Ras Mutant Subpopulations in Cancer Therapies (E0722921)

Responsible Division: Genetic and Molecular Toxicology

External Partner: Toxicology Excellence for Risk Assessment

Objective(s):

The basic mode-of-action hypothesis to be tested in this phase of the CRADA will be that vanadium pentoxide induces lung tumors in

mice by causing clonal expansion of pre-existing K-Ras mutations.

PI: Patri, Anil, Ph.D.
NCTR/Office of Regulatory Affairs (ORA) Nanotechnology Core Facility—FDA SUPPORT (S00714)

Responsible Division: Office of Scientific Coordination
Collaborating FDA Office: ORA
Objective(s):

1) Support the needs of NCTR to characterize nanoscale materials used in toxicology tests and to detect these materials in biological samples.
2) Support the needs of FDA's ORA/Arkansas Regional Laboratory to detect and characterize nanoscale materials in FDA-regulated products.

PI: Paule, Merle G., Ph.D.
ADDENDUM to E0215101: Developmental Neurotoxicity Assessment of Acrylamide in Rats— Long-Term Studies (E0215111)

Responsible Division: Neurotoxicology
Collaborating Divisions: Biochemical Toxicology, Bioinformatics and Biostatistics, Systems Biology
Objective(s):
Determine the consequences of long-term exposure to acrylamide on a variety of developmental milestones and measures of nervous-system integrity throughout life.

PI: Paule, Merle G., Ph.D.
Long-Term Consequences of Neonatal Ketamine Anesthesia in Rhesus Monkeys: Extended Cognitive Assessments (E0736401)

Responsible Division: Neurotoxicology
Collaborating Office: Office of Scientific Coordination
Collaborating FDA Center: CDER
Objective(s):

1) Continue monitoring the cognitive capabilities of rhesus nonhuman-primate subjects that were exposed to a single, 24-hour bout of ketamine-induced anesthesia during the first week of life. Data to date indicate that, compared to control animals, ketamine-exposed subjects exhibit significant deficits in several aspects of brain function, including learning, the ability to perform simple visual discriminations, motivation, and speed of psychomotor processing. Continuing these observations will provide valuable information on the ultimate time course and severity of the observed deficits.
2) Extend the functional domains that are being assessed. Performance of a temporal discrimination task (timing task), a counting task, and reversal learning tasks (cognitive flexibility) will be added to the current assessment battery.

PI: Perkins, Roger G.
CTP Scientific Enclave, Tobacco Constituents Knowledge Base, and Topic Modeling for Tobacco Industry Documents (E0753501)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CTP
Objective(s):

1) Provide CTP an external scientific enclave for collaboration.
2) Provide a chemical centric knowledge base for the > 8400 tobacco constituents.
3) Develop and validate a topic-mining tool to structure tobacco companies' document submissions along thematic topics to aid knowledge discovery in a regulatory context.

PI: Pogribny, Igor P., Ph.D.
Development and Evaluation of a Novel *In vitro* Epigenomic Screening Model System for the Hazard Identification of FDA-Regulated Products (E0755001)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CTP
Objective(s):

1) Determine the dose-dependent *in vitro* genetic and epigenetic effects of compounds regulated by FDA.
2) Characterize the specific epigenetic changes induced *in vitro* by genotoxic and non-genotoxic compounds.
3) Characterize the specific genetic and epigenetic effects of compounds regulated by FDA using an *in vitro* 3-D organotypic liver-culture model system.

PI: Rafii, Fatemeh, Ph.D.
Antimicrobial Susceptibilities of Clostridium Perfringens Strains Isolated from Different Sources and Genetic Characterizations of Resistance (E0751601)

Responsible Division: Microbiology
Objective(s):
Provide new insight into the molecular basis for the spread of drug resistance in pathogenic bacteria and determine if antibiotic-resistant environmental *C. perfringens* strains are capable of acting as reservoirs for antibiotic resistance genes for human-restricted antibiotics, which are not used in animals at all.

PI: Tolleson, William H., Ph.D.
Rapid Detection of Ribosome-Inactivating Protein Toxins in Foods (E0736101)

Responsible Division: Biochemical Toxicology
Collaborating Division: Microbiology
Collaborating FDA Center: CFSAN
Objective(s):
Provide robust methods for detecting the biological activity of the potential bioterrorism agents ricin, abrin, and shiga-like toxins, each of which is characterized as a ribosome-inactivating protein toxin, in three selected foods (spinach, apple juice, and milk).

PI: Trbojevich, Raul, Ph.D.
Study of Nanoparticles Migration from Food-Contact Nanomaterials: Characterization and Quantification of Silver Nanoparticles in Stimulants (E0736801)

Responsible Division: Biochemical Toxicology
Collaborating Office: Office of Scientific Coordination
Collaborating FDA Office: ORA
Objective(s):

1) Study migration of nanoparticles from nanocomposites used in food-contact materials.
2) Characterize and quantify silver nanoparticles in food simulants.

PI: Yang, Xi, Ph.D.
ADDENDUM to E0747701: Use of New Technologies to Develop Biomarkers of Harm for New Tobacco Products (E0744711)

Responsible Division: Systems Biology
Collaborating Divisions/Office: Biochemical Toxicology, Bioinformatics and Biostatistics
Collaborating FDA Product Center: CTP
Objective(s):

1) Assess various omics changes (genomics, metabolomics, and proteomics) in two primary lung-cell types and two cardiac-cell types. At this stage, only tobacco smoke condensate (TSC) will be tested. Once smoking machines (for *in vitro* cell-culture exposure) are purchased and running, whole smoke will be tested and this work will be added to the protocol via an addendum. Two different test cigarettes and smoking simulation conditions will be used.
2) Analyze the omics data to determine if any of the omics changes can be used as biomarkers of harm.

PI: Zhang, Xuan, Ph.D.
ADDENDUM to E0742401: Chronic Methylphenidate Administration in Rhesus Monkeys (E0742411)

Responsible Division: Neurotoxicology
Collaborating Division: Biochemical Toxicology
Objective(s):
Dosing and behavior assessments have been and will continue to be conducted in current protocol on these animals to determine the long-term influence of MPH on learning and behavior.

PI: Zhang, Xuan, Ph.D.
PET-CT Imaging Using Translatable Biomarkers for Evaluating the Central Nervous System Effects of Chronic Methylphenidate (MPH) Administration in Rhesus Monkeys (E0742401)

Responsible Division: Neurotoxicology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Objective(s):

1) Determine if prolonged treatment with MPH induces long-lasting changes in specific neurotransmitter

receptors using micro postitron emission tomography (microPET). Computed tomography will be used to localize PET findings and visualize brain structures and volumes.

2) Identify potential neurochemical alterations related to the noted behavioral changes seen in these animals using minimally invasive imaging techniques.

NCTR Objective 1.2 – Advance Regulatory Science Through the Development of New Tools and Approaches

PI: Ahn, Young-Beom, Ph.D.
Exploring Strategies for Resuscitation and Enrichment of Burkholderia Cepacia Complex Strains in Pharmaceutical Products (E0749801)

Responsible Division:
Microbiology
Collaborating FDA Center: CDER
Collaborating Division/Offices:
Systems Biology, Office of Scientific Coordination, Office of Research
Objective(s):

1) Screen and identify strains of *B. cepacia* that are difficult to cultivate from pharmaceutical water.
2) Develop a resuscitative step and enrichment technique for *B. cepacia* complex recovery.
3) Develop methodology to detect *B. cepacia* and its 16 related genomovars.
4) Evaluate the use of modern molecular technologies to identify *B. cepacia* complex.

PI: Azevedo, Marli P., Ph.D.
Development of an Infectivity Assay to Detect Human Norovirus from Contaminated Food (E0745801)

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

1) Develop alternative assays to detect infectious Norovirus from contaminated food.
2) Gain insights on Norovirus *in vitro* replication.

PI: Azevedo, Marli P., Ph.D.
Addendum to E0745801: Development of an Infectivity Assay to Detect Human Norovirus from Contaminated Food (E0745811)

Responsible Division:
Microbiology
Collaborating Divisions:
Biochemical Toxicology, Bioinformatics and Biostatistics
Objective(s):
Explore the survival of Norovirus on pre-harvest contaminated produce (fresh fruits and vegetables) and effects of sunlight exposure on Norovirus inactivation.

PI: Bowyer, John F., Ph.D.
Developing More Complete Genomic and Histological Evaluations of Vascular Damage in the Brain Meninges and Choroid Plexus After Neurotoxic Insult (E0751901)

Responsible Division:
Neurotoxicology
Collaborating Divisions/Office:

Biochemical Toxicology, Bioinformatics and Biostatistics

Objective(s):

1) Identify additional biomarkers enabling a further understanding of the functions of the MAV and choroid plexus.
2) Develop better histological methods to evaluate vascular damage in the MAV, choroid plexus, and brain.

Successful completion will enable the application of methods developed in this protocol to be applied to a future protocol that will evaluate how a damaged blood-brain barrier (BBB) interacts with various drugs with respect to increasing/altering their neurotoxicity and whether this interaction further compromises the BBB.

PI: Buzatu, Dan A., Ph.D.
Examination of a Novel Flow Cytometer as a Diagnostic Platform for Rapid Determination of Bacterial Antibiotic Resistance and the Presence of Viruses, Prions, or Parasites in Clinical Samples (E0746901)

Responsible Division: Systems Biology

Collaborating Division/Office: Microbiology, Office of Scientific Coordination

Collaborating FDA Center: CBER

Objective(s):

Examine a variety of adaptations to the diagnostic method and platform so that its performance advantages can be extended to other public-health applications.

PI: Chelonis, John J., Ph.D.
Data Repository for Behavioral and Questionnaire Data (S00762)

Responsible Division: Neurotoxicology

Objective(s):

1) Develop a data repository for behavioral and questionnaire data collected in human-subject studies.
2) Allow for hypothesis testing with increased sample sizes.
3) Test additional hypothesis that were not proposed in the original studies in which data collection occurred.

PI: Chelonis, John J., Ph.D.
Off-Site Clinical Collaborations Involving the NCTR Operant Test Battery (S00786)

Responsible Division: Neurotoxicology

Objective(s):

Develop and maintain collaborative research with outside laboratories that are using the NCTR Operant Test Battery. This research will provide validation data for the use of this instrument in human subjects as well as apply this test battery to topics of relevance to the FDA.

PI: Chelonis, John J., Ph.D.

System Test of NCTR's Multispecies Behavioral Test System (MBS) Upgrade: Development of a Fixed Consecutive Number (FCN) Task (E0751701)

Responsible Division: Neurotoxicology

Collaborating Office: Office of Scientific Coordination

Objective(s):

Provide a necessary step in the development and implementation of new MBS software to support administration of the FCN task which will provide new information about
brain function that cannot be derived from the current Operant Test Battery tasks.

PI: Chen, Tao, Ph.D.

Development and Evaluation of Exposure Dosimetry Methods to Optimize the Standard *In vitro* Mammalian Genotoxicity Assays for Assessing Engineered Nanomaterials (ENMs) (E0745701)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Divisions/Office: Neurotoxicology, Systems Biology, Office of Scientific Coordination

Objective(s):

1) Evaluate whether the *in vitro* mammalian genotoxicity assay is suitable for assessing the genotoxicity of nanomaterials.
2) Explore the possible mechanisms underlying genotoxicity of ENMs by conducting genomic analysis.
3) Identify potential improvements to the assay and general strategies for evaluating nanomaterials.
4) Examine whether the suitable methods and other experiences learned from the micronucleus assay are applicable to other genotoxicity tests, such as mouse lymphoma assay and *in vivo* micronucleus assay.

PI: Chen, Tao, Ph.D.

Evaluation of MicroRNAs (miRNAs) in Blood and Urine for Detection of Chemical-Induced Carcinogenicity (E0753001)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination

Objective(s):

1) Determine miRNAs in blood and carcinogenic target tissues that respond to exposure of carcinogens, and the best time for sampling of their expression after treatments in rats.
2) Determine miRNA profiles from the blood and target tissue samples of rats treated with different mode-of-action carcinogens, such as alkylating agents, aneugens, clastogens, and non-genotoxic carcinogens at the appropriate sampling time determined by Objective 1.

3) Determine the functions and pathways of the dysregulated miRNAs by the carcinogen treatments and examine whether the miRNA changes can be anchored to the carcinogens with the known mode-of-actions and whether the changes in blood relate to those in the target tissues.
4) Establish specific miRNA biomarkers in blood for assessing different types of carcinogens.

PI: Chen, Minjun, Ph.D.

Development and Evaluation of Predictive Models for the Management of Risk of Drug-Induced Liver Injury (DILI) in the Investigational New Drug (IND) Phase (E0760501)

Responsible Division:
Bioinformatics and Biostatistics
Collaborating Divisions:
Biochemical Toxicology, Systems Biology
Collaborating FDA Centers: CDER, CVM
Objective(s):
The overall goal of this project is to develop more accurate and reliable predictive models for DILI to support regulatory decision during the review process.

1) Closely work with clinical hepatologists to improve the DILI annotation for marketed drugs.
2) Collect data specifically focused on the mechanistic understanding of DILI to guide an integration of diverse experimental datasets.
3) Apply advanced bioinformatics methodologies to mine the data and develop models with improved predictive power.
4) Incorporate the data derived from the emerging technologies, such as high-content screening assay, toxicogenomics, next-generation sequencing technology, and stem cell-based assays will be incorporated into the Liver Toxicity Knowledge Base. The integration of diverse DILI data or models will provide opportunities to develop more robust and powerful predictive models to improve the DILI research.
5) Validate the regulatory utility of the predictive models and the reference database using perspective and retrospective approaches.
6) Work with outsides DILI researchers to perform independent validations after identifying promising new biomarkers and/or models.
7) Develop a publicly-available software product in this project based on the collection of DILI data and predictive models. We hope that the developed product will be eventually utilized and referenced by FDA when DILI events are encountered in the review process.

PI: Desai, Varsha G., Ph.D.
Development and Application of a Mitochondria-Specific Gene Array (Mitochip) for the Investigation of Clinical and Non-Clinical Predictive Biomarkers of Toxicity (E0739701)

Responsible Division: Systems Biology
Collaborating Divisions: Bioinformatics and Biostatistics, Neurotoxicology
Collaborating FDA Centers: CDER, CDRH
Objective(s):

1) Develop MitoChip for various mammalian species, including rat, nonhuman primate, and human.
2) Conduct transcriptional profiling of mitochondria-related genes using mitochondria-specific gene arrays to investigate the mechanisms of drug toxicities and degenerative diseases associated with mitochondrial dysfunction.
3) Characterize species-specific transcriptional profiles to predict risk of drug toxicity or disease-onset in different mammalian species.

PI: Desai, Varsha G., Ph.D.
Development of Predictive Mitochondrial Biomarkers for Drug-Induced Cardiotoxicity Using a Systems Biology Approach (E0733201)

Responsible Division: Systems Biology
Collaborating Divisions/Office: Biochemical Toxicology, Bioinformatics and Biostatistics, Office of Scientific Coordination
Collaborating FDA Center: CDER
Objective(s):

1) Measure heart rate and variability, using ECGenie.
2) Measure cardiac troponin T, creatine kinase MB, and cardiolipin levels in plasma as indicators of doxorubicin-induced cardiac-tissue damage.
3) Identify morphological changes in cardiac mitochondria in left ventricular region by electron microscopy.
4) Use omics for heart-analyte profiling: transcriptional profiling of ~906 mitochondria-related genes using MitoChip; protein profiling by 2D-HPLC/MS/MS, and measurement of endogenous metabolites by nuclear magnetic resonance and mass spectrometry.
5) Measure expression levels of 906 mitochondria-related genes in whole blood using MitoChip.
6) Measure levels of creatinine, creatine, lactate, Krebs cycle intermediates, small ketone bodies in plasma using metabolomics.
7) Integrate genomic, proteomic, and metabolomic endpoints in the heart tissue to define the molecular basis of doxorubicin-induced cardiac toxicity and also correlate omics data to genomic findings obtained in whole blood.

PI: Ding, Wei, Ph.D.
Validation of the *In vivo* Comet Assay for Pre-Market Submissions and Preparation of Detailed Review Paper To Assist in the Development of a New Organisation Economic Cooperation and Development Guideline (E0750401)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Center: CFSAN
Objective(s):

1) Review existing literature and identify critical information gaps.
2) Conduct limited research at NCTR.
3) Combine all information to prepare a detailed literature review that will serve as a background document for the new Organisation Economic Cooperation and Development guideline for the Comet assay.

PI: Dobrovolsky, Vasily N., Ph.D.
Development of a High-Throughput Assay for Measuring *In vivo* Mutation in an Autosomal Gene (E0741301)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Objective(s):

1) Develop a high-throughput *in vivo* mutation model that detects mutations induced by a range of mechanisms, including gene mutation, large deletions, and loss of heterozygosity.
2) Evaluate the basic properties and sensitivity of the model in experiments employing well-characterized mutagens.

PI: Fang, Hong, Ph.D.
Support and Development of FDALabel Database Application (S00788)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Center: CDER
Objective(s):

1) Support FDA, particularly CDER reviewers and research scientists with use of the current FDALabel database.
2) Maintain the FDALabel server and keep the drug label data and information up to date.

PI: Fisher, Jeffrey W., Ph.D.
Computational Toxicology for Safety and Risk Assessment (S00780)

Responsible Division: Biochemical Toxicology
Objective(s):
Assist other Centers in FDA (e.g., CFSAN and CDRH) and research organizations outside FDA (e.g., P & G and U.S. EPA).

PI: Fu, Peter P., Ph.D.
Determination of Cytotoxicity and Genotoxicity of Nanomaterials of Interest to the FDA and Mechanism of Action (E0752701)

Responsible Division: Biochemical Toxicology
Objective(s):

5) In conjunction with the erythrocyte sedimentation rate (ESR or SED rate)

measurements made by Dr. Yin at CFSAN, to develop a set of cell-free and cell-based *in vitro* tests that can be used to rapidly identify nanomaterials of interest to the FDA that elicit oxidative damage.

6) Determine if, in the presence of nano-metal materials, endogenous and dietary antioxidants can display pro-oxidative activity.

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

Mechanism of Tumorigenic Pyrrolizidine Alkaloids and Development of LC/ES/MS/MS Methodology for Detection and Quantification of Pyrrolizidine Alkaloids (E0728901)

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

1) Validate the proposed mechanism by which pyrrolizidine alkaloids induce tumors in rodents.
2) Develop an LC/ES/MS/MS method for detection and quantification of DHP-derived DNA adducts in rodents.
3) Develop an LC/ES/MS/MS method for detection and quantification of genotoxic pyrrolizidine alkaloids in herbal plants and herbal dietary supplements.
4) Develop an LC/ES/MS/MS method for detection and quantification of DHP-derived hemoglobin adducts in rodents.

PI: Gamboa Da Costa, Goncalo Ph.D.

Developing Methods for the Analysis of Brominated Vegetable Oils and Derivatives (E0756301)

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

1) Develop methods for the homogeneous incorporation of brominated vegetable oil (test article) and vegetable oil (control/vehicle) in rat feed.
2) Develop analytical methods to assess the concentration, homogeneity, and stability of BVO in rat feed.
3) Conduct a short-term pilot study (28 days) in rats fed a range of concentrations of brominated vegetable oil (treatment groups) or vegetable oil (control) in feed.
4) Provide an initial confirmation of observations reported in the literature, and samples of serum, organs, and tissues to validate the analytical methods.
5) Verify if the blood chemistry equipment currently available at NCTR is adequate for the quantification of bromide ion in the serum of rats.
6) Develop mass spectral-based analytical methods to quantify brominated fatty acids in a range of rat tissues. This task will involve the synthesis of commercially unavailable isotopically-labeled brominated fatty acids.

7) Evaluate the use of neutron activation analysis to determine the total elemental bromine content in selected rat organs and tissues.

PI: Gokulan, Kuppan, Ph.D.
Nonclinical Modeling and Risk Assessment of FDA-Regulated Drug Nanocrystals (E0759001)

Responsible Division: Microbiology
Collaborating Office: Office of Scientific Coordination
Collaborating FDA Center: CDER
Objective(s):

1) Help to determine the critical process and formulation parameters for the formation of stable crystalline nanoparticles.
2) Help develop a nonclinical model of the gastrointestinal tract to assess risk associated with drug nano-crystal administration. The innovative approach proposed is to utilize polarized intestinal epithelial cells and *ex vivo* intestinal tissue explants to evaluate trans-epithelial resistance of the cell-cell tight junctions, cell proliferation, and adhesive properties.

PI: Gu, Qiang, Ph.D.
Identification of Protein Biomarkers for Neurotoxicity Assessments Using a High-Throughput Antibody Microarray Approach (E0747701)

Responsible Division: Neurotoxicology
Objective(s):

1) Examine proteomic changes at both the expression and phosphorylation levels using five established *in vivo* models of neurotoxicity.
2) Identify common changes in protein expression and phosphorylation status in these animal-model systems.
3) Confirm the observed alterations in protein expression and phosphorylation status by means of other independent methods.
4) Apply the proteomic findings to a global ischemic animal model to further validate the utility of protein biomarkers for use in neurotoxicity assessments.

PI: Gu, Qiang, Ph.D.
Development of a Simple *In vitro* Approach for the Rapid Detection of Neurotoxicity (E0752401)

Responsible Division: Neurotoxicology
Collaborating FDA Center: CDER

1) Characterize Fluoro-Jade C (FJ-C) labeling *in vitro*. Experiments will be conducted in a variety of cell cultures to identify the types of cultured cells that can be labeled using FJ-C: neurons, astrocytes, oligodendrocytes, microglia, brain-capillary endothelial cells, or other non-neuronal cells. In addition, optimal concentrations and incubation durations will be determined for FJ-C use *in vitro* and we will determine whether FJ-C itself can be neurotoxic in culture media.
2) Validate FJ-C labeling *in vitro*.

Experiments will be performed using several well-known neurotoxicants such as tetrodotoxin, lead, mercury, cadmium, ethanol, biphenyl compounds and others to confirm FJ-C labeling for neurodegeneration *in vitro* and to determine dose-dependent and time-dependent effects of these toxic compounds on FJ-C labeling as reference parameters of neurotoxicity *in vitro*.

3) Develop an FJ-C-based *in vitro* approach for high-throughput determination of neurotoxicity. Experiments will be designed to combine FJ-C labeling, multi-well culture plates, and high-content time-lapsed recordings to achieve the goal of simple, fast, multiplexed, efficient, and accurate screens and analyses of neurotoxic compounds.
4) Explore the mechanism underlying FJ-C labeling. Studies will be undertaken in attempts to identify the 'death' molecule(s) that bind FJ-C.

PI: Hanig, Joseph P., Ph.D.
Development of Magnetic Resonance Imaging (MRI) and Informatics Techniques for Tissue Sampling to Guide and Confirm Classical Neuropathology (E0741801)

Responsible Division:
Neurotoxicology
Collaborating FDA Center: CDER
Objective(s):
Use MRI and informatic analysis of MRI files to screen brain samples for neuro-irregularities (presumed toxicities) that would inform and direct the plane and loci of slices or sections taken for confirmatory classical neuropathology.

PI: Hanig, Joseph P., Ph.D.
ADDENDUM to E0741801: Development of Magnetic Resonance Imaging (MRI) and Informatics Techniques for Tissue Sampling to Guide and Confirm Classical Neuropathology (E0741811)

Responsible Division:
Neurotoxicology
Collaborating Center: CDER
Objective(s):

1) Build dose-response and time-course curves of trimetyltin and hexachlorophene neurotoxicity using MRI T2 mapping.
2) Assess sensitivity and specificity of T2 mapping in relation to histopathology using Receiver Operating Characteristic curves approach.
3) Assess neurotoxicological effect of mefloquine.

PI: Hong, Huixiao, Ph.D.
Further Development and Refinement of the FDA Endocrine Disruptor (ED) Knowledge Base (EDKB) for Assessing Endocrine Disrupting Potential of Drugs and Food Additives (E0741501)

Responsible Division:
Bioinformatics and Biostatistics
Collaborating Divisions/Office:

Biochemical Toxicology, Systems Biology, Office of Scientific Coordination

Objective(s):

1) Improve EDKB by including the ED data that will be generated by NTP and NCTR as well as estrogen receptor and androgen receptor data generated at EPA, and data that have been published in the past eight years.
2) Conduct meta-analyses of the large datasets accumulated in the EDKB to gain a better understanding of chemical structure requirement and mechanisms related to EDs.
3) Investigate various chemoinformatics/ bioinformatics approaches to develop effective predictive models for assessing the potential of drugs and food additives.

PI: Howard, Paul C., Ph.D.
Analytical Assay for Photochemical Generation of Hydroxyl Radical (S00728)

Responsible Office: Office of Scientific Coordination

Collaborating Division: Biochemical Toxicology

Objective(s):

1) Provide support for analysis of the photoactivation of nanomaterials using the OH/coumarin-3-carboxylic acid assay.
2) Provide particle-size analysis for all materials being analyzed by OH method and other nanomaterials used in studies at FDA's NCTR and ORA/Arkansas Regional Laboratory.
3) Improve the assay using ultraviolet light diode laser as a replacement to the existing broad-band ultraviolet light-A source.

PI: Howard, Paul C., Ph.D.
Support of Collaborative Projects in Nanotechnology with Baylor University and University of Texas Health Science Center (S00774)

Responsible Office: Office of Scientific Coordination

Collaborating FDA Office: ORA

External Partners: Baylor University, University of Texas Health Science Center

Objective(s):

Provide analytical support for small or investigative projects in collaboration with investigators at Baylor University and University of Texas Health Science Center.

PI: Howard, Paul C., Ph.D.
Support of Collaborative Projects in Nanotechnology with FDA/Center for Drug Evaluation and Research (CDER) (S00770)

Responsible Office: Office of Scientific Coordination

Collaborating FDA Center/Office: CDER, ORA

Objective(s):

Provide analytical support for small or investigative projects in collaboration with investigators at FDA/CDER.

PI: Howard, Paul C., Ph.D.
Support of Collaborative Projects in Nanotechnology with FDA/Center for Food Safety and Nutrition (CFSAN) (S00771)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Center/Office: CFSAN, ORA
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at FDA/CFSAN.

PI: Howard, Paul C., Ph.D.
Support of Collaborative Projects in Nanotechnology with University of Arkansas at Little Rock (S00766)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Office: ORA
External Partner: University of Arkansas at Little Rock
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at University of Arkansas at Little Rock.

PI: Inselman, Amy L., Ph.D.
Establishment of Embryonic Stem (ES) Cells as an *In vitro* Model to Explore Developmental Toxicity (E0735401)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Objective(s):

1) Maintain and passage several mouse ES and human-induced pluripotent stem (iPS) cell lines in a pluripotent, undifferentiated state in the absence of feeder cells and serum.
2) Recapitulate early embryonic development by terminal differentiation of mouse ES and human iPS cells into a variety of cell types (i.e. osteoblasts).
3) Monitor this differentiation process by examining gene expression in undifferentiated ES and in cells that have undergone differentiation.
4) Provide proof-of-concept by using known teratogens and investigating gene changes in differentiated cells, such as acetazolamide treatment of differentiating osteoblasts.

PI: Khare, Sangeeta, Ph.D.
Interaction of Nanoparticles with Gastrointestinal Tract (E0744301)

Responsible Division: Microbiology
Collaborating Office: Office of Scientific Coordination
Objective(s):

1) Determine the effect of nanomaterials on the permeability of epithelial cells and establish immune correlates.
2) Delineate the interaction of nanomaterials with gastro-intestinal tract and gut-associated microbiota using *ex vivo* model (intestinal explants).
3) Establish the effect of nanoparticle on the developmental stage of

intestine and assess biodistribution of nanoparticle using zebrafish model.

PI: Leakey, Julian E., Ph.D.
Complement Assays for the Detection of Immuno-Sensitizing Activity of Nanomaterials (E0754501)

Responsible Office: Office of Scientific Coordination
Objective(s):

1) Establish two complement assays at NCTR for routine evaluation of immuno-sensitizing activity of nanomaterials.
2) Validate the assays using nanoparticles with known immunoreactivity and determine the immuno-sensitizing activity of novel nanomaterials.

PI: Leakey, Julian E., Ph.D.
Complement Assays for the Detection of Immuno-Sensitizing Activity of Nanomaterials: Influence of Euthanasia on Serum Complement Activation in Rats (E0754511)

Responsible Office: Office of Scientific Coordination
Objective(s):
Determine optimum conditions for minimizing spontaneous activation of the complement system during blood collection from rodents.

PI: Liu, Zhichao, Ph.D.
Drug Repositioning with Bioinformatics (E0747001)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination
Objective(s):
Use bioinformatics to explore repositioning opportunities of marketed drugs (prescription and over-the-counter) for various diseases (including rare and neglected diseases).

PI: Manjanatha, Mugimane, Ph.D.
Validation of a Newly Developed Transgenic, Hairless, and Albino Mice (E0727701)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Objective(s):

1) Analyze tissue-specific mutant frequency and spectra using transgenic systems to evaluate a variety of hypotheses on the mechanisms or mode-of-action for cancer induction in rodents.
2) Facilitate improvements in human-risk characterization based on extrapolation from animal data.

PI: McKinzie, Page, Ph.D.

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

Responsible Division: Genetic and Molecular Toxicology

Objective(s):

Understand the distribution and range of spontaneous oncogene-mutant frequencies in the major organs of rats and mice to provide important basic information for the validation of these oncogene-mutant frequencies as biomarkers of chemically induced carcinogenesis.

PI: Paredes, Angel, Ph.D.

Collaboration on Nanotechnology and Electron Microscopy with St. Jude Children's Research Hospital (S00779)

Responsible Office: Office of Scientific Coordination

External Partner: St. Jude Research Hospital

Objective(s):

The objective is for the NCTR/ORA Nanotechnology Core Facility to provide
analytical and electron microscopy support form small investigative projects in collaboration with investigators at St. Jude Children's Research Hospital.

PI: Petibone, Dayton, Ph.D.

Differential Transcriptomic Characterization of TK6 and WTK1 Human Lymphoblast Cells by Next-Generation RNA Sequencing (E0744001)

Responsible Division: Genetic and Molecular Toxicology

Collaborating Divisions: Bioinformatics and Biostatistics, Systems Biology

Objective(s):

1) Develop next-generation pyrosequencing capabilities for RNA-sequence analysis and genomic sequencing in order to characterize the TK6 and WTK1 transcriptosome.
2) Apply technology to the determination of the baseline whole-genome gene expression levels in each cell line.
3) Measure transcriptosome response in TK6 and WTK1 after exposure to classical positive control agents, which may include the direct-acting alkylating agent, ENU, as well as ionizing radiation. Other positive control agents may be selected during the conduct of the protocol.

PI: Sarkar, Sumit, Ph.D.

Development and Maintenance of F344-AD Tg Rat Breeding Colony (E0754601)

Responsible Division: Neurotoxicology

Collaborating Division: Systems Biology

Objective(s):

Create and maintain a breeding

colony of this unique strain of rat that will provide animals for use in subsequent protocols.

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

Efficacy and Toxicity of Potential Alzheimer's Disease (AD) Therapeutics in the AD/Tg mouse, Phase III (E0757601)

Responsible Division: Neurotoxicology

Objective(s):

1) Confirm and expand findings on the most promising AD therapeutic agents based on the previous phase I and phase II studies.
2) Resolve questions addressing the underlying modes of action of different classes of the most promising AD therapeutics. The following specific mechanistic basic science questions will be addressed:
    a) Is A-beta inoculation more effective in younger individuals prior to the development of amyloid plaques than in older animals with a robust plaque burden?
    b) Is the plaque reduction seen following dosing with anti-depressants independent of their psychotropic properties?
    c) Is the reduction in amyloid plaques seen following dosing with cannabinoids independent of their psychotropic properties?
    d) Is the reduction in amyloid plaques seen following dosing with Clioquinol the result of it being a specific copper/zinc chelator, or would more general chelators be comparably, or more, effective?
    e) Is there a correlation between the amyloid binding affinity of certain histological tracers and their potential to inhibit A-beta aggregation?
    f) Do Retinoid X receptor agonists reduce plaque burden via enhanced clearing of A-beta?
3) Evaluate the efficacy and toxicity of specific agents that are, or will be, in clinical trials in the near future. The following specific agents will be evaluated: A-beta inoculation (pre vs. post plaque development), NaEDTA (general metal chelator), citalopram (R vs. S enantomer), cannabinol and THC (CB1 and CB2 agonists), K-114 and its analogue Amylo-Glo and the Thioflavin T analogue Bis-thioflavin T and Barexatone (a retinoid X receptor agonist).
4) Determine whether the NCTR rodent micro-PET facility is capable of detecting changes in amyloid plaque burden over time.

PI: Shi, Qiang, Ph.D.

Using Cell-Free MicroRNA (miRNA) as Improved Clinical Biomarkers of Drug-Induced Liver Injury (DILI) (E0749701)

Responsible Division: Systems

Biology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center/Office: CDER, Office of Chief Scientist
Objective(s):

1) Measure the level of miRNAs in human serum, urine, and liver samples from patients experiencing DILI and normal healthy controls.
2) Compare the changes in miRNA levels between groups to identify specific miRNAs that may serve as new biomarkers of DILI.

PI: Slavov, Svetoslav, Ph.D.

Development and Validation of 3D-QSDAR Models for Prediction of the Binding Affinity of Chemicals from the ToxCast Database to the Estrogen Receptor (ER) (E0753901)

Responsible Division: Systems Biology
External Partner: U.S. Environmental Protection Agency
Objective(s):

1) Build and validate 3D-QSDAR models for ER binding.
2) Search for structural patterns in the data.
3) Decode the structure-activity relationship.
4) Provide validated models for a reliable estimation of the ER binding affinity of a diverse dataset of chemicals.
5) Identify the structural features responsible for binding to ER.
6) Provide a list of chemicals prioritized for further laboratory testing.
7) Send model and structural features to EPA for combination with other models being provided by collaborators.

PI: Sun, Jinchun, Ph.D.

Evaluate Potential Serum Metabolic Biomarkers that Predict Severity of Acute Kidney Injury (AKI) in Critically Ill Patients (E0757101)

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

1) Evaluate whether the serum AKI biomarkers discovered in the pilot study can be used, to some extent, to predict clinical outcomes in critically ill patients with AKI that required hemodialysis support.
2) Determine whether patients who recovered from AKI after dialysis have decreased levels of acylcarnitines and amino acids (methionine, homocysteine, pyroglutamate, asymmetric dimethylarginine (ADMA), and phenylalanine) and an increase in serum levels of arginine and several LysoPCs when compared to the serum levels of patients that did not recover from AKI.
3) Evaluate for serum NGAL levels using ELISA techniques to determine whether there are correlations between high serum levels of homocysteine, ADMA, NGAL, and creatinine in AKI patients.

PI: Sutherland, John B., Ph.D.
Reducing Health Risks from Antimicrobial-Resistant Bacteria by Eliminating Environmental Reservoirs of Resistance (E0738201)

Responsible Division: Microbiology
Collaborating Divisions/Office: Biochemical Toxicology, Systems Biology, Office of Research
Collaborating FDA Center: CVM
Objective(s):
Identify the specific bacteria and enzymes in the environment that are able to degrade fluoroquinolones to products without antimicrobial activity.

PI: Tolleson, William H., Ph.D.
Evaluating Conventional Methods for Thermal and Chemical Inactivation of the Bioterrorism Agent—Ricin— Contaminating Pilot-Scale Milk Pasteurization Equipment (E0746701)

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

1) Determine the residual biological activity remaining for ricin-contaminated milk processed using the range of time/temperature conditions recommended for pasteurization of raw milk using pilot-scale (4L) equipment safely within the secure, high-level biosafety level 3 food-processing facility located at the National Center for Food Safety and Technology.
2) Determine the efficacies of chemical inactivation methods developed on a laboratory-scale to decontamination of food-processing equipment on a pilot-scale.

PI: Tong, Weida, Ph.D.
Development and Refinement of the FDA Genomic Tool, ArrayTrack™™ for Advancing Pharmacogenomics and Personalized Medicine Supporting FDA's Critical Path Initiative (S00671)

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

1) Analyze data from CDER drug review offices using ArrayTrack™™ and return results to CDER collaborators.
2) Develop the functionality in ArrayTrack™™ to review non-microarray PGx data, supporting the Critical Path Initiative.
3) Develop new modules in ArrayTrack™™ to review proteomic, metabolomic, and genome-wide association studies data.
4) Develop modules to allow electronic data submission in the Voluntary Genomic Data Submission (VGDS)/Voluntary Exploratory Data Submission (VXDS) program.

PI: Tong, Weida, Ph.D.
FDA IT Support (S00699)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
External Partner: Clinical Data Interchange Standard Consortium (CDISC)
Objective(s):

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

PI: Tong, Weida, Ph.D.
MicroArray Quality Control (MAQC) Project Database (S00691)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Division: Systems Biology
Objective(s):

1) Update MAQC database when new data become available.
2) Maintain and regularly back up database at NCTR.

PI: Varma, Vijayalakshmi, Ph.D.
An Omics Approach To Investigate the Metabolic and Endocrine Effects of Fructose on Adipocytes Compared to Glucose (E0740401)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CFSAN
External Partner: University of Arkansas for Medical Sciences
Objective(s):
Identify cellular mechanisms involved in fructose-induced metabolic and endocrine regulation of human adipocytes in culture using omic technologies.

PI: Wang, Yuping, Ph.D.
Study of Translational Biomarkers for Drug-Induced Liver Injury (DILI) with Next-Generation Sequencing (NGS) (E0753201)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Division: Genetic and Molecular Toxicology
Objective(s):
Conduct a comprehensive survey of miRNA using NGS technology. The resulting findings will elucidate the molecular pathways and processes modulated by RNAs (including mRNAs, miRNAs and other non-coding RNAs) and their importance in DILI risk and phenotypes. It is anticipated that miRNA biomarkers from rat may be more predictive for human-specific DILI than mRNA alone.

PI: Xu, Joshua, Ph.D.
Developing an Intelligent Recognition System for Storage Pests Fragments Contaminating Food Products (E0759101)

Responsible Division: Bioinformatics and Biostatistics

Collaborating FDA Centers and Office: CDER, CFSAN, ORA

Objective(s):

Develop an intelligent and efficient system for ORA filth analysts to identify beetle species based on microscopic images of their elytral fragments recovered from processed food products. This system will increase the reliability, sensitivity, and throughput of microanalytical filth analysis compared with current methods used by the FDA for the identification of storage pest fragments in FDA-regulated food products. In order to achieve this goal, we have set three specific aims.

a) Expand whole insect specimens collection for the most of common stored product beetle species and develop a digital library of beetle body parts images.
b) Refine and validate image analysis and machine learning algorithms for species identification to empower risk analysis.
c) Develop a computer-aided intelligent system with a user-friendly graphical user interface to manage insect fragments image data and risk analysis. The system will integrate all image analysis and machine learning algorithms and provide an interface for image management.

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

Metabolomics and Proteomics Approaches Addressing Pre-Analytical Variability in Human Plasma Samples (E0755601)

Responsible Division: Systems Biology

Collaborating FDA Center: CDER

External Partner: Metanomics Health GmbH

Objective(s):

Establish a cooperative research and collaboration agreement (CRADA) between Metanomics Health GmbH and NCTR in the field of Quality Assurance and Quality Control that will discover biomarkers of metabolomics and proteomics sample quality related to variations in pre-analytical processing of clinical plasma samples. The metabolomics labs at the NCTR will become a tester of the proprietary metabolomics sample quality biomarkers developed at Metanomics Health.

PI: Zou, Wen, Ph.D.

Bioinformatics Methodology Development for Microbial Next-Generation Sequencing Data Analysis and Data Mining (E0745001)

Responsible Division: Bioinformatics and Biostatistics

Collaborating FDA Center: CDER

Objective(s):

Develop a simple high-throughput microarray system based in microtubes for fast and efficient

pathotyping foodborne illness-related bacteria isolates (starting with *Salmonella*), together with a unique and scalable analysis platform that leverages genomic and other data contained in the PATRIC BRC to provide a real-time analysis capability that evolves as the bacterial genome data does, over time.

NCTR Objective 1.3 – Approaches for Promoting Individualized Health and Identifying Susceptible Populations

PI: Beger, Richard D., Ph.D.
Delta Vitamin Obesity Prevention Summer Camp (E0733001)

Responsible Division: Systems Biology
Collaborating Division: Microbiology
External Partners: U.S. Department of Agriculture/Agricultural Research Service, Marvell Community Development Center, University of Arkansas at Medical Sciences
Objective(s):

1) Analyze levels of 13 vitamins in 100 children in grades 4-6 to confirm food-frequency questionnaire data showing low intakes of certain nutrients and vitamins.
2) Provide fresh fruits, vegetables, and fortified snacks to supplement low-vitamin intake for a one-month period to improve serum concentration levels of vitamins.
3) Analyze ancestry through whole-genome scans and candidate genes responsive to vitamin intake to associate individual responses with genetic polymorphisms.
4) Improve the nutrition and genetic education of the participants through lessons taught by local teachers with materials provided by NCTR, U.S. Department of
5) Area Health Education Centers
6) diabetes educator.
7) Develop health-economic analyses of the intervention.
8) Begin developing a sustainable program for improving the foods of the children in the Marvell School District by analyzing economic impact of vitamin intervention.

PI: Beger, Richard D., Ph.D.
Identification of New Mechanistic Biomarkers of Adverse Responses to Acetaminophen (APAP) (E0731301)

Responsible Division: Systems Biology
Objective(s):

1) Identify specific adduct proteins in children/adolescents receiving therapeutic doses of APAP (P-adducts) and in children/adolescents that have received APAP overdoses (T-adducts).
2) Examine metabolomic markers in these patients to address the role of redox status and energy metabolism in the study population.
3) Establish 2nd-generation biomarkers of APAP toxicity using the data generated from this study, based on specific adduct proteins, which can be used in future risk assessment studies of children receiving APAP.

PI: Camacho, Luisa Maria, Ph.D.
Circulating microRNAs as Minimally Invasive Biomarkers of Nevirapine Adverse Effects (E0758501)

Responsible Division: Biochemical Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
External Partner: University of Lisbon
Objective(s):
Perform a three-tiered case-control retrospective study to compare serum samples from female patients undergoing NVP treatment who do not show signs of NVP adverse effects (control group) with those who have manifested NVP-induced adverse effects (case group).

PI: Chang, Ching-Wei, Ph.D.
Blood Pressure (BP) Threshold for Cardiovascular Risk: An Assessment of Sex-Based Criterion (E0754201)

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

1) Review systematically existing literature and develop a database for BP and Ambulatory Blood Pressure Monitoring (ABPM) measurements, hypertension, and cardiovascular risk.
2) Conduct meta-analyses of studies in the database developed in #1 above and perform sensitivity analysis to determine thresholds for assessing cardiovascular risk.
3) Determine whether BP or ABPM measurements from clinical studies warrant modifications for sex-based thresholds for cardiovascular risk and whether this criterion should differ for pre- and post-menopausal women.

PI: Chelonis, John J., Ph.D.
ASK CHILDREN Study—Assess Specific Kinds of Children Challenges for Neurologic Devices (E0734301)

Responsible Office: Neurotoxicology
Objective(s):

1) Establish a science-based framework of recommendations to help develop more efficient strategies in evaluating pediatric products regulated by FDA.
2) Develop a framework of science-based recommendations important to help expedite pediatric prostheses to market, including recommendations for the research and development of neurologic devices.
3) Collect qualitative and quantitative self-report clinical data (through interviews) and identify scientific and medical issues associated with pediatric devices when used in children undergoing treatment, to

develop more efficient strategies for evaluating these types of products regulated by FDA.

4) Organize data that are important to developing more efficient strategies in evaluating these types of products regulated by FDA into multiple categories, including (but not limited to); device type, pediatric subpopulations, disorder or condition, and intended use.

PI: Chelonis, John J., Ph.D.
Complex Brain-Function Study in Children With and Without Major Depression (E0717701)

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

PI: Chelonis, John J., Ph.D.
Development and Validation of Interspecies Cognitive Assessments (E0735501)

Responsible Division:
Neurotoxicology
Collaborating Office: Office of Research
Objective(s):
Compare children's performance on operant tests (that have been used extensively to assess drug effects in animals) with performance on neuropsychological tests (that are typically used in clinical settings that are thought to measure similar cognitive functions).

PI: Chelonis, John J., Ph.D.
Effects of Anxiety on Complex Brain Function in Children (E0721701)

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

PI: Chen, James J., Ph.D.
Predicting Patient-Specific Treatment Outcomes: Identification and Validation of Molecular Biomarkers Using *In silico* Tools (E0748601)

Responsible Division:
Bioinformatics and Biostatistics
Collaborating FDA Centers: CBER, CDER, CDRH
Objective(s):
Develop statistical and data-mining techniques to identify personalized biomarkers to characterize individual differences in response to treatment and to understand disease progression: application to lung-cancer therapies.

PI: Chen, Minjun, Ph.D.

Biomarker Study To Improve Adjuvant Treatment for ER-positive and HER2-Negative Breast-Cancer Patients (E0748401)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Office: Office of Scientific Coordination
Objective(s):

1) Assess the utility and effectiveness of the potential biomarker in predicting treatment outcomes of chemotherapy for breast-cancer patients with positive estrogen-receptor (ER) and negative human epidermal growth factor receptor-2 (HER2) based on breast-cancer tissue arrays using immunohistochemistry.
2) Study the role of the potential biomarker in breast-cancer development at both gene and protein levels using human breast-cancer cell lines.

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

Investigation of the Mechanistic Aspects of Sex-Based Differences in Susceptibility to Doxorubicin (DOX)-Induced Cardiac Toxicity in Mice (E0759201)

Responsible Division: Systems Biology
Objective(s):
Understand the molecular basis associated with differential susceptibility to DOX toxicity between sexes in a newly established mouse model exhibiting sex-related differences in DOX cardiotoxicity.

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

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

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CDRH
Objective(s):

1) Determine the pharmacokinetics of the production of urinary metabolites of DEHP and BPA after cardiopulmonary bypass (CPB) in children.
2) In a pilot study, quantify the exposure of children to DEHP and BPA while undergoing CPB compared to critically ill children without cardiac surgery and healthy controls.
3) Evaluate the ability of urinary biomarkers to detect acute kidney injury in patients following CPB.

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

Comorbidity of Alzheimer's Disease (AD) and Type 2 Diabetes (T2DM) in African Americans: Comparison of Biomarkers of Inflammation in Human Tissues (E0754101)

Responsible Division: Neurotoxicology
Collaborating Division: Systems Biology
Collaborating FDA Office: Office of Minority Health
Objective(s):

1) Determine the potential ethnicity-related differences in cytokine profiles of African Americans and

Caucasians comorbid for AD and T2DM.

2) Examine the role of ethnicity-related insulin signaling and oxidative stress signaling in AD/T2DM tissues with specific focus on AGEs and the potential interaction with amyloid beta peptide.
3) Determine the correlations between those cytokines measured in brain tissue and those that can be measured in more accessible tissues. Endpoints measured in postmortem brain tissue provide the necessary data for fulfilling objectives 1 and 2 above; however, they cannot serve as potential biomarkers of AD. If such direct measures are strongly correlated with measures in serum or adipose tissue, then the utility of the serum markers increases substantially.

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

Preliminary Quantification of the Neurotoxicity/Neuroprotection of Selective Estrogen Receptor Modulator (SERM) Treatment in a Female Mouse Model of Alzheimer's Disease Utilizing Alzheimer Brain-Derived Amyloid ß-Protein (E0752301)

Responsible Division: Neurotoxicology

Collaborating Division/Office: Systems Biology, Office of Scientific Coordination

Objective(s):

1) Establish simultaneous use of MRS (Magnetic Resonance Spectroscopy) and microdialysis techniques.
2) Quantify the effects of raloxifene on neurochemical and behavioral endpoints.

PI: Fisher, Jeffrey W., Ph.D.

Biological Based Dose-Response (BBDR) Modeling for the Thyroid Axis in the Fetus and Neonate (E0743601)

Responsible Division: Biochemical Toxicology

Objective(s):

1) Create BBDR models for the hypothalamic–pituitary–thyroid (HPT) axis in the developing rat and human as a function of iodide status.
2) Interface the BBDR-HPT models with physiologically-based pharmacokinetic (PBPK) or thymidine kinase TK models for thyroid-active chemicals to predicted conditions (iodide status and chemical exposure) for which brain thyroid-hormone homeostasis cannot be maintained in the fetus and neonate.
3) Evaluate the possible influence of population exposures to thyroid-active chemicals on fetal and neonatal-thyroid status as a function of iodide intake using the models.

PI: Fuscoe, James, Ph.D.

Evaluation of Transcriptomics-Based Predictions of Sex and Age-Related Susceptibilities to Treatment-Induced Adverse Effects in F344 Rats (E0755501)

Responsible Division: Systems Biology

Collaborating Divisions: Biochemical Toxicology, Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):

1) Identify, using bioinformatics approaches with existing data, drugs/chemicals that may exert differences in susceptible populations (e.g., young vs. adults).
2) Conduct in-life studies to confirm or refute these predictions. These studies will result in advancing a mechanistic basis for predicting *in vivo* outcomes as well as address gaps in our understanding of sex- and age-related differences in drug-induced adverse effects.

PI: Fuscoe, James, Ph.D.

Addendum to E0755501: Evaluation of Transcriptomics-Based Predictions of Sex and Age-Related Susceptibilities to Treatment-Induced Adverse Effects in F344 rats (E0755511)

Responsible Division: Systems Biology
Collaborating Divisions: Biochemical Toxicology, Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
The Phase I bioinformatics part of the protocol has been completed and a set of approximately 40 drugs has been selected that we predict will show sex-associated differences in drug disposition and/or toxicity. Briefly, 29 genes encoding drug metabolizing enzymes (DMEs) were found to be differentially expressed (fold change > 2; $p < 0.05$) between adult male and female rats based on gene expression data. Drug substrates of these differentially expressed DMEs were obtained from Pharmapendium and 40 drugs which are metabolized by one, or at most two, differentially expressed DMEs were selected for further testing.

We will perform studies on these drugs using primary rat hepatocytes before performing *in vivo* assessments. Use of the primary rat hepatocytes will allow the efficient screening of these drugs for predicted sex-different toxicities and drug metabolism without the use of large numbers of animals. The results of such *in vitro* cell experiments will allow selection of a small number of drugs for testing in animals and will result in a highly robust test of our ability to use easily available transcriptomics data to predict sex- and age-specific susceptibilities to drug-induced adverse events.

PI: Fuscoe, James, Ph.D.

Genetic and Epigenetic Mechanisms of Sex Differences in the Kidney of a Rat Model System: Developing Safety Biomarkers for FDA-Regulated Products (E0743901)

Responsible Division: Systems Biology

Collaborating Division:
Bioinformatics and Biostatistics
Objective(s):

1) Perform whole-genome expression profiling on the 10 rat tissues of both sexes at 9 ages.
2) Perform miRNA profiling of selected tissues, including liver.
3) Perform DNA methylation profiling of selected tissues, including liver.
4) Use bioinformatics and statistical approaches to understand the genetic machinery operational at each developmental stage in each sex and relate the findings to potential susceptibility to adverse drug reactions and disease.
5) Use bioinformatics approaches to analyze the findings for potential age- and sex-related susceptibility in an animal model system to humans.

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

Animal Models of Pregnancy To Address Medical Countermeasures for Influenza and Chemical, Biological, Radiological and Nuclear Threats in the "At Risk " Population of Pregnant Women—Phase I (E0746201)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Genetic and Molecular Toxicology, Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):

1) Conduct literature search and analysis regarding animal models of pregnancy.
2) Hold public workshop.

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

ADDENDUM to E0746201: Effect of Pregnancy on the Pharmacokinetics of Oseltamivir Phosphate and Oseltamivir Carobylate in Nonhuman Primates, Phase 3 (E0746231)

Responsible Division: Biochemical Toxicology
Collaborating Divisions/Office: Bioinformatics and Biostatistics, Genetic and Molecular Toxicology, Neurotoxicology, Office of Scientific Coordination
Collaborating FDA Center: CDER
Objective(s):
Determine the pharmacokinetics of oseltamivir phosphate and oseltamivir carboxylate in nonpregnant and pregnant Rhesus macaques of Indian origin.

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

Evaluation of Effects of Black Cohosh on Risedronate Efficacy in a Peri-Menopausal Rat Model (E0758301)

Responsible Division: Systems Biology
Objective(s):

1) Establish a peri-menopausal rat model. Achieving this aim will ensure that the rats are in a perimenopausal state and will provide baseline bone mineral density (BMD) data.

2) Determine if treatment of peri-menopausal female rats with sodium risedronate or black cohosh alters BMD. Achieving this aim will allow an evaluation of the effect of risedronate and black cohosh independently on BMD.
3) Determine if the combination of black cohosh and risedronate alters BMD in a peri-menopausal rat model. Achieving this aim will allow an evaluation of the effect of the combination of risedronate and black cohosh on BMD.

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

Evaluation of Methods Used to Measure Growth of Staphylococcus aureus and the Production of Toxic Shock Syndrome Toxin-1 (TSST-1) as Influenced by Menstrual Tampons (E0754401)

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

1) Utilize the tampon sac, flask-shaking, syringe, and toroid models as published to determine the ability of a variety (based upon absorbency, wadding material and design) of market-available tampons to:
   a. enhance the growth of TSST-1 producing strains of *S. aureus* isolated from clinical cases of TSS, and
   b. increase the production of TSST-1.

   Experimentally investigate parameter differences between the aforementioned methods and the results they generate to determine a standardized method for evaluating tampons for their potential to enhance *S. aureus* growth and increase TSST-1 production.

PI: Imam, Syed Z., Ph.D.

Modulation of the Effects of Parkinson's Disease (PD) Medications by Nicotine (E0746601)

Responsible Division:
Neurotoxicology
Objective(s):

1) Evaluate the effect of nicotine treatment on the effect of PD medications in MPP+ treated DAN human dopaminergic cells and SHSY-5Y human neuroblastoma cell lines.
2) Evaluate the effect of nicotine administration on the effects of PD medications in an MPTP-treated C57BLJ6 mouse model of progressive PD.

PI: Liachenko, Serguei, Ph.D.

Gender Differences in Neuronal Reward Circuit Activation by Nicotine and Tobacco Smoke Using Magnetic Resonance Spectroscopy (E0751001)

Responsible Division:
Neurotoxicology
Collaborating Division/Office:
Bioinformatics and Biostatistics, Office of Scientific Coordination
Collaborating FDA Center/Office:
CTP, Office of Women's Health
Objective(s):

1) Determine biomarkers of smoking addiction and harm and describe the

sex differences in these biomarkers. These biomarkers can then be used to provide more effective and personalized smoking cessation treatments based on gender and level of addiction. For example, nicotine replacement therapies seem less effective in women.

2) Describe neurometabolite changes in response to acute and sub-acute exposure to nicotine or tobacco smoke.
3) Noninvasively measure neurometabolite changes—increasing the translational nature of the outcomes.

PI: Lumen, Annie, Ph.D.
Population-Based Computational Framework for Assessing Xenobiotic Disposition and Interaction Effects in Pregnant Women—Pilot Study (E0752201)

Responsible Division: Biochemical Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Office: Office of Women's Health
Objective(s):

1) Develop a population-based integrated Physiologically Based Pharmacokinetic (PBPK) Biological Based Dose-Response (BBDR) model for the hypothalamic–pituitary–thyroid axis during pregnancy to predict the pharmacokinetics of iodide, perchlorate, and their interactions on serum thyroid hormone levels of pregnant women.
2) Create an extension of the PBPK-BBDR pregnancy model to evaluate serum-thyroid hormone perturbations from concomitant exposures to multiple thyroid active agents found in food and the environment, such as thiocyanate, in addition to perchlorate and iodide, in a pregnant population.

PI: Lyn-Cook, Beverly A., Ph.D.
Clinical and Biological Significance of Three Identified Targets in Systemic Lupus Erythematosus Patient PBMCs: IL-18, TNFSF13B, and FoxP3 (E0744611)

Responsible Division: Biochemical Toxicology
Objective(s):

1) Determine expression levels of toll-like receptors 3, 7, 9, and miRNA-146a in controls and lupus patients grouped according to sex and ethnicity.
2) Correlate expression of TRLs 3,7,9 to type-1 interferon levels in lupus and control patients.
3) Determine the polymorphisms profile of 3,7,9 in lupus and control patients and correlate to expression levels.
4) Correlate expression levels of TRLs 3,7,and 9 with expression of miRNA-146a.
5) Determine if interferon regulation may be through epigenetic regulation.

PI: Lyn-Cook, Beverly A., Ph.D.
Male vs. Female Expression in Human Liver, Kidney, and Small Intestine: Microarray Analyses (E0743001)

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

1) Ascertain whether there are basic expression differences in drug metabolizing tissues (liver, kidney, and small bowel) between males and females. These studies will be conducted on untreated tissue (no drugs). The major focus will be on drug-metabolizing enzymes and transporters particularly. An assessment of major biological pathways that display sexual dimorphism will also be made.
2) Answer the question: "Do functional differences between male and female tissues exist in the liver?" Explore this with the liver sandwich assay and several drugs that display sexual dimorphism in the clinic. These drugs include paclitaxel, rosiglitazone, and pioglitazone initially (the list may expand).

PI: Lyn-Cook, Beverly A., Ph.D.
Sex and Ethnic Differences in Expression of Toll-Like Receptors (TLR-3, TLR-7, and TRL-9) in Systemic Lupus Erythematous: New Targets for Emerging Therapeutics (E0744601)

Responsible Division: Biochemical Toxicology
Objective(s):

1) Determine the expression levels of TLRs 3,7, 9, and miRNA-146a in controls and lupus patients grouped according to sex and ethnicity.
2) Correlate expression of TLRs 3,7, 9 to type 1 interferon levels in lupus and control patients.
3) Determine the polymorphisms profile of TLRs 3,7, and 9 in lupus and control patients and correlate to expression levels.
4) Correlate expression levels of TRLs 3,7, and 9 with expression of miRNA-146a.
5) Determine if interferon regulation may be through epigenetic regulation.

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

Responsible Division: Biochemical Toxicology
Objective(s):
Elucidate whether there is a sex and/or ethnic bias in expression levels of epigenetic markers in SLE patients.

PI: Myers, Meagan B., Ph.D.
Determining Oncomutation Profile of Triple Negative Breast Cancer: Information to Direct Development of Personalized Therapies (E0743801)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Center/Office: CDRH, Office of Women's Health
External Partner: University of Arkansas for Medical Sciences
Objective(s):

1) Establish which molecules should be targeted to treat the largest percentages of breast cancers.
2) Identify which mutational biomarkers should be used as diagnostics in personalized approaches to breast-cancer treatment.
3) Determine what sensitivity is needed in the measurement of those mutational biomarkers.

PI: Nakamura, Noriko, Ph.D.
Development of an Immunohistochemical Tool To Measure Degree and Distribution of Global Epigenetic Alterations in Liver Tissue Samples (E0755201)

Responsible Division: Systems Biology
External Partners: Central Arkansas Veterans Healthcare Systems, Duke University, University of Arkansas for Medical Sciences
Objective(s):

1) Develop an immunohistochemical tool to assess degrees of global DNA methylation and histone protein acetylation in the liver along with their distributions.
2) Develop an immunohistochemical approach as a tool for evaluating the alteration of epigenetic modifications using various tissues section (including the liver) from mice treated with/without acetaminophen.
3) Determine the correlations between the degree of epigenetic alterations, gender, and severity of liver injury by statistical analyses.
4) Perform and optimize three immunohistochemical stains to investigate the degree and distribution of global DNA methylation and histone acetylation in the liver in a quantifiable manner.
5) Develop a histologic scoring system to systematically assess these global epigenetic alterations for ease of comparison with future studies using human liver tissue samples.
6) Correlate the quantitative data (on DNA methylation, histone acetylation) with the severity of acetaminophen-induced liver injury.

PI: Ning, Baitang, Ph.D.
Genetic Variants in Cardiovascular Disease Risks and Drug Responses: Exome Sequencing and Variant Characterization in the Amish Population (E0752601)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Bioinformatics and Biostatistics, Systems Biology

Objective(s):

1) Identify causative alleles for four phenotypic traits that were associated with risks of cardiovascular disease, based on previously obtained genetic markers and association studies.
2) Perform validation genotyping assays in existing cohorts consisting of approximately 1000 well-phenotyped individuals to further confirm risk-association variants.
3) Conduct functional assessments using biochemical approaches to delineate mechanisms underlying the association between the variants and phenotypic traits.

PI: Ning, Baitang, Ph.D.

Whole-Genome Sequencing to Identify Genetic Susceptibilities to Carbamazepine-Induced Adverse Reactions (E0745301)

Responsible Division: Systems Biology

Collaborating Divisions: Bioinformatics and Biostatistics, Neurotoxicology

Objective(s):

1) Identify and compare genetic variants in 30 patients with Steven-Johnson Syndrome/toxic epidermal necrolysis and 10 Amish individuals with public data from the 1000 Genome Project.
2) Identify genetic variants associated with phenotypes of interest.
3) Evaluate the molecular mechanisms accounting for interindividual variations responding to carbamazepine.
4) Model patient-specific drug/human leukocyte antigen interactions to predict the outcome.
5) Assess technical performance and bioinformatics solutions of next-generation sequencing on whole-genome sequencing.

PI: Pang, Li, Ph.D.

Sex Differences in Drug-Induced QT Prolongation and Torsade de Pointes (TdP): Establishing an *In vitro* Model for High-Throughput Screening and Risk Assessment of Torsadogenic Drugs (E0754001)

Responsible Division: Biochemical Toxicology

Collaborating Division: Systems Biology

Collaborating FDA Center: CDER

External Partner: University of Arkansas for Medical Sciences

Objectives:

1) Establish the model and positive control.
2) Evaluate the sensitivity and specificity of the model and test the possibility of high-throughput screening and ranking QT prolonging drugs for the risk of TdP.

PI: Pang, Li, Ph.D.

The Role of ABC-Drug Transporters in Chemoresistance in Pancreatic Cancer (E0751101)

Responsible Division: Biochemical Toxicology

Objective(s):

1) Compare various ABC transporters' expression in normal and pancreatic

adenocarcinoma specimens and determine whether the expression of ABC transporters is correlated with clinical aggressiveness of the tumor.

2) Evaluate whether the single nucleotide polymorphisms in ABC transporters genes are associated with the abnormal expression of the efflux pumps and drug sensitivity.
3) Assess the epigenetic regulation of ABC transporters in pancreatic cancer.

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

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

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Objective(s):

1) Develop the information necessary for the rational use of oncogene mutations as quantitative biomarkers of cancer risk; specifically Allele-specific competitive blocker PCR (ACP-PCR) will be used to determine normal and pathological levels of relevant oncogene mutations in multiple human tissues and tumors.
2) Compare the information derived from human tissues with data generated in a parallel rodent protocol as an approach for incorporating carcinogenesis-relevant data into the rodent to human extrapolation necessary in cancer-risk assessment.
3) Validate a streamlined ACP-PCR methodology and develop the methodology necessary to measure oncogene mutant fraction in cell-free DNA isolated from plasma.
4) Convey to the regulatory risk-assessment community through a series of publications, the regulatory significance of the data regarding tumor-associated mutations which have and will be generated.

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

Improving the Efficacy and Development of Targeted Cancer Therapeutics by Establishing a Model to Identify Molecularly-Targeted Therapies that Prevent Acquired Resistance (E0755101)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Systems Biology
Objective(s):

1) Establish a 3D cancer tissue-originated spheroid model at NCTR, which can be used to experimentally assess the development of acquired drug resistance (expected to occur with monotherapy).
2) Assess the development of drug-resistant clones by total spheroid area, cell cycle profile, the frequency of apoptosis, and by ACB-PCR (a method that can quantify minor mutant subpopulations).

3) Determine, using the model, which combination of molecularly-targeted chemicals inhibits growth of NSCLC spheroids to the greatest extent. Treatments will include erlotinib alone and in combination with drugs/chemicals expected to target KRAS mutant cells. This project will employ a genomics approach (RNA sequencing) to identify additional genetic lesions that impact response to treatment.

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

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

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

1) Determine the role of epigenetic dysregulation in the etiology and pathogenesis of dietary NASH-induced hepatocarcinogenesis in mice.
2) Determine whether or not interstrain-specific susceptibility of mice to NASH-induced hepatocarcinogenesis is associated with differences in individual hepatic epigenetic phenotypes.
3) Determine the role of epigenetic dysregulation in the etiology and pathogenesis of NASH-induced hepatocarcinogenesis in mice induced by tamoxifen administration.
4) Determine if aberrant epigenetic markers can be used as targets for prevention of NASH-induced hepatocarcinogenesis in mice.

PI: Sarkar, Sumit, Ph.D.

Evaluation and Characterization of Blood-Brain Barrier (BBB) Pathology in MPTP-Probenecid-Induced Parkinsons Disease (PD)-Like Conditions in a Mice Model and its Potential Amelioration by Endoplasmic Reticulum Stress Reducers (Molecular Chaperones) and Other Putative Anti-PD Therapeutics (E0751201)

Responsible Division: Neurotoxicology
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination
Objective(s):

1) Determine the role of key neurovascular units in the expression of PD-like pathology.
2) Determine the ability of endoplasmic reticulum stress-reducers to alter the expression of PD-like pathology.
3) Determine the ability of antioxidant peptide SS31, N-acetyl cysteine, acetyl-l-carnitine, the orexin A receptor inhibitor SB 334 867 A, and metal chelators, such as M30, clioquinol, and VK-28 to provide neuroprotection against PD-like pathology.
4) Evaluate changes in cerebral hemodynamics, BBB permeability

and neurochemicals associated with the development of PD-like pathology.

5) Conduct behavioral assessments for those compounds shown to be efficacious in ameliorating PD pathology to quantify symptom improvement.

PI: Shi, Qiang, Ph.D.
Identifying Drugs That Cause Female-Biased Hepatotoxicity by Analyzing FDA Drug-Approval Packages/Labels and FDA-Maintained Databases and Conducting Comparative Studies in Primary Hepatocytes of Rats, Mice, and Humans (E0750201)

Responsible Division: Systems Biology
Collaborating FDA Office: Office of Women's Health
Objective(s):

1) Identify specific drugs that cause drug-induced liver injury (DILI) more often in women than in men.
2) Establish a hepatocyte culture model to study a drug's potential to induce sex-biased DILI.

PI: Shi, Qiang, Ph.D.
Preclinical Studies Investigating the Dose Range and Proof-of-Principle that Leflunomide-Induced Liver Injury (LILI) Is Enhanced by Cytochrome P450 Inhibition (E0744201)

Responsible Division: Systems Biology
Objective(s):
Develop a rat model of LILI focusing on the involvement of hepatic cytochrome P450s (CYPs). These proof-of-concept studies will help determine if a preclinical model of LILI can be established, which will help in the development of novel biomarker(s) for assessing individual susceptibility to LILI in future studies.

PI: Varma, Vijayalakshmi, Ph.D.
Epigenetics, DNA Methylation, and Obesity (E0733101)

Responsible Division: Systems Biology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Objective(s):
Evaluate the effect of differences in DNA methylation and agouti signaling protein in the offspring of Avy/a dams x a/a sires as a result of nutrient x gene interactions. These preliminary data will be used to select the appropriate diets for further studies on obesity and type 2 diabetes.

PI: Varma, Vijayalakshmi, Ph.D.
Viable Yellow Agouti Mouse Breeding Colony (S00763)

Responsible Division: Systems Biology
Objective(s):
Maintain the Viable Yellow Agouti Mouse breeding colony at NCTR, to provide animals needed to support studies addressing research

questions on:

a. Understanding the role of epigenetic and genetic mechanisms in health and disease states.
b. Understanding the developmental origin of adult diseases in response to toxicants, drugs, or nutrients.
c. Understanding the differences in disease susceptibilities of lean and obese phenotypes/non-diabetic and diabetic phenotypes or non-neoplastic and neoplastic phenotypes having similar genotype.
d. Investigating potential differences in drug toxicities between lean and obese/non-diabetic and diabetic/non-neoplastic and neoplastic states.

PI: Wagner, Robert, Ph.D.

Immunological Effects of Nanoparticles on Induction of Pro-inflammatory Responses to *Candida albicans* by Vaginal Epithelial Cells (VEC) (E0752001)

Responsible Division: Microbiology

Collaborating FDA Office: ORA

Objective(s):

1) Measure the effects of graphene and PLGA nanoparticles on mRNA and protein expression of inflammatory cytokines and signal transduction proteins by VEC stimulated with *C. albicans*.
2) Measure oxidative effects and DNA damage in VEC by nanoparticles.
3) Evaluate effects of nanoparticles on estrogen receptor-mediated signal transduction and suppressed cytokine responses using estrogen receptor inhibitor ICI182780 and an estrogen receptor reporter assay.

PI: Wang, Yuping, Ph.D.

Assessment of the Disparities on Drug-Host Interaction of Drug-Induced Liver Injury (DILI) Reported in Large Electronic Medical Record (EMR) System Using Advanced Methodologies for Minority Populations (E0760001)

Responsible Division: Bioinformatics and Biostatistics

Collaborating Office: Office of Scientific Coordination

Collaborating FDA Center: CDER

Objective(s):

Systematically assess risks of DILI among the minority population (black, Hispanic, and others) with white population as a control. Also, we will assess potential drug property/class-race interaction in contributing DILI in humans.

FY 2015 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 2015, and book chapters that were accepted in FY 2015**.

- Alusta, P., Buzatu, D., Williams, A., Cooper, W., Tarasenko, O., Dorey, C., Hall, R., Wilkes, J. (2015). Instrumental improvements and sample preparations that enable reproducible, reliable acquisition of mass spectra from whole bacterial cells. *Rapid Communications in Mass Spectrometry*, 29:1961-1968.

  Responsible NCTR Division: Systems Biology

- Ayyadevara, S., Balasubramaniam, M., Gao, Y., Yu, L., Zybailov, B., Alla, R., Shmookler, R. (2015). Proteins in aggregates functionally impact multiple neurodegenerative disease models by forming proteasome-blocking complexes. *Aging Cell*, 14:35-48.

  Responsible NCTR Division: Systems Biology

- Bae, D., Cheng, C., Khan, A. (2015). Characterization of extended-spectrum Beta-Lactamase (ESBL) producing non-typhoidal *Salmonella* (NTS) from imported food products. *International Journal of Food Microbiology*, 214:12-17.

  Responsible NCTR Division: Microbiology

- Banda, M. (2015). Detection of mutant RAS subpopulations in colorectal cancer patients. *EBioMedicine Journal*, 2(4): 280–281.

  Responsible NCTR Division: Genetic and Molecular Toxicology

- Banda, M., McKim, K., Haber, L., Macgregor, J., Gollapudi, B., Parsons, B. (2015). Quantification of Kras mutant fraction in the lung DNA of mice exposed to aerosolized particulate vanadium pentoxide by inhalation.

Mutation Research Genetic Toxicology and Environmental Mutagensis, 789-790:53-60. doi: 10.1016/j.mrgentox.2015.07.003.

Responsible NCTR Division: Genetic and Molecular Toxicology

- Beger, R., Bhattacharyya, S., Yang, X., Gill, P., Schnackenberg, L., Sun, J., James, L. (2015). Translational biomarkers of acetaminophen-induced acute liver injury. *Archives of Toxicology*, 89:1497-1522.

  Responsible NCTR Division: Systems Biology

- Beland, F., Olson, G., Mendoza, M., Marques, M., Doerge, D. (2015). Carcinogenicity of glycidamide in B6C3F1 mice and F344/N rats from a two-year drinking water exposure. *Food and Chemical Toxicology*, 86:101-115.

  Responsible NCTR Division: Biochemical Toxicology

- Bowyer, J., Tranter, K., Hanig, J., Crabtree, N., George, N., Schleimer, R. (2015). Evaluating RNA-Seq transcriptome profile fidelity in whole blood and the feasibility of pooling expressions from replicate experiments when determining immune-related expression changes. *Plos One*, 1-22.

  Responsible NCTR Division: Neurotoxicology

- Bozza, W., Tolleson, W., Di, X., Rivera Rosado, L., Zhang, B. (2014). Ricin detection: tracking active toxin. *Nature Methods*, S0734-9750(14).

  Responsible NCTR Division: Biochemical Toxicology

- Chang, C., Ning, B. (2015). A reachable goal: application of pharmacogenomics biomarkers to improve drug efficacy and safety. *Advances in Pharmacoepidemiology and Drug Safety*, http://dx.doi.org/10.4172/2167-1052.1000e129.

  Responsible NCTR Division: Systems Biology

- Chen, G., Yu, D., Chen, J., Cao, R., Yang, J., Wang, H., Ji, X., Ning, B., Shi, T. (2015). Functional assessments of previously presumed noncoding disease/trait associated genetic variants by integrative analyses. *Scientific Reports*, 5: 9453 | DOI: 10.1038/srep09453.

Responsible NCTR Division: Bioinformatics and Biostatistics

- Chen, H., Chen, J. (2015). Hybrid mixture model for subpopulation identification. *Statistics in Biosciences,* pp 1-15.

  Responsible NCTR Division: Bioinformatics and Biostatistics

- Chen, H., Pan, H., Xu, J., Kweon, O., Zou, W., Feng, J., He, G., Cerniglia, C. (2015). Differential gene expression in *Staphylococcus aureus* exposed to Orange II and Sudan III azo dyes. *Journal of Industrial Microbiology and Biotechnology*, 42 (5):745-757.

  Responsible NCTR Division: Microbiology

- Chen, J., Chen D., Zhao W. (2015). Statistics in big data. *Journal of Chinese Statistical Association*, 53:1-17.

  Responsible NCTR Division: Bioinformatics and Biostatistics

- Chen, J., George, E. (2015). Dose-response modeling for clustered data. *Encyclopedia of Environmetrics.* 3rd Edition, 1:569-573.

  Responsible NCTR Division: Bioinformatics and Biostatistics

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  Responsible NCTR Division: Bioinformatics and Biostatistics

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Glossary of Acronyms and Abbreviations

This glossary is provided to assist you in interpreting acronyms, abbreviations, and phrases you encounter while reading this publication. This is not meant to take the place of standard language or scientific dictionaries, which should be referred to if any short form of a scientific term does not appear in this glossary. Also, you may refer to the Index of Key Terms, located at the end of this publication, as a quick reference to locate other occurrences of a specific term.

Acronym/ Abbreviation	Name
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care, International
AALAS	American Association for Laboratory Animal Science
ABPM	Ambulatory blood pressure monitoring
ACB-PCR	Allele-specific competitive blocker-polymerase chain reaction
ACLAM	American College of Laboratory Animal Medicine
AD	Alzheimer's Disease
AgNP	Silver nanoparticles
APAP	Acetaminophen
AR-BIC	Arkansas Bioinformatics Consortium
ARL	Arkansas Regional Laboratory
BBB	Blood-brain barrier
BBDR	Biologically based dose-response
BP	Blood pressure
BPA	Bisphenol A
BSC	NIEHS, National Toxicology Program Board of Scientific Counselors
BVO	Brominated vegetable oil
CBER	Center for Biologics Evaluation and Research, FDA
CARB	Combating Antibiotic-Resistant Bacteria
CDER	Center for Drug Evaluation and Research, FDA
CDRH	Center for Devices and Radiological Health, FDA
cdtB	Detection of Cytolethal Distending toxin
CF	Cystic fibrosis
CFSAN	Center for Food Safety and Applied Nutrition, FDA
CORES	Collaborative Opportunities for Research Excellence in Science

Acronym/ Abbreviation	Name
CRADA	Cooperative Research and Development Agreement
CSC	Cigarette smoke condensate
CTP	Center for Tobacco Products, FDA
CVM	Center for Veterinary Medicine, FDA
DA	Dopamine
DEHP	Di-(2-ethylhexyl)phthalate
DGMT	Division of Genetic and Molecular Toxicology
DILI	Drug-induced liver injury
DQTG	Data Quality Task Group
ED	Endocrine disruptor
EDKB	Estrogen Disruptor Knowledge Base
EHEC	Enterohemorrhagic *E. coli*
EMS	Ethylmethane sulfonate
ENM	Engineered nanomaterials
ENU	N-ethyl-N-nitrosourea
ES	Embryonic stem cells
ESBL	Extended-spectrum β-lactamase (ESBL)
FPDI	Food Protection and Defense Institute
GABA	Gamma-aminobutyric acid
GI	Gastrointestinal
GSRS	Global Summit on Regulatory Science
GTTC	Genetic Toxicology Technical Committee
HESI	Health and Environmental Sciences Institute
HHS	Department of Health and Human Services
HLA	Human leukocyte antigens
IACUC	Institutional Animal Care and Use Committee
IAG	Interagency agreement
ICAW	International Workshop for Comet Assay
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICP-MS	Inductively coupled plasma mass spectroscopy
IDR	Idiosyncratic drug reactions
ILSI	International Life Sciences Institute
InhaleCore	CTP/NCTR Inhalation Toxicology Core Facility
in silico	Modeled on a computer
in situ	In place; localized and confined to one area
In vitro	In animal models
In vivo	In cell cultures

Acronym/ Abbreviation	Name
IPS	Induced pluripotent stem cell line
iPSC-CM	Induced pluripotent stem cell-cardiomyocyte
IWGT	International Workshop for Genotoxicity Testing
LILI	Leflunomide-Induced Liver Injury
LMA	Locomotor activity
LTKB	Liver Toxicity Knowledge Base
MAQC	MicroArray Quality Control
MCBIOS	MidSouth Computational Biology and Bioinformatics Society
miRNA	MicroRNA
MOA	Mode-of-action
MOU	Memorandum of Understanding
MPH	Methylphenidate hydrochloride
MPP+	(1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MRSA	Methicillin-resistant *Staphylococcus aureus*
NAEHS	National Advisory Environmental Health Services Council
Nano-PEAS	NanoCore, Particle Evaluation and Analytical Spectroscopy team
NASH	Nonalcoholic steatohepatitis
NEHI	National Nanotechnology Initiative: Nanotechnology Environment Health Implications
NHEK	Normal human epidermal keratinocytes
NIEHS	National Institute of Environmental Health Sciences
NGS	Next-generation sequencing
NIOSH	Centers for Disease Control and Prevention's National Institute for Occupational Safety and Health
NIH	National Institutes of Health
NMDA	N-methyl-d-aspartate
NM	Nanomaterial
NNK	Nicotine-derived nitrosamine ketone
NNN	N-Nitrosonornicotine
NP	Nanoparticle
NSET	National Nanotechnology Initiative: Nanoscale Science, Engineering, Technology
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
OMH	Office of Minority Health, FDA

Acronym/ Abbreviation	Name
ORA	Office of Regulatory Affairs, FDA
ORISE	Oak Ridge Institute for Science and Education
OSC	Office of Scientific Coordination, FDA/NCTR
OTB	Operant Test Battery
OWH	Office of Women's Health, FDA
PAH	Polycyclic aromatic hydrocarbon
PBPK	Physiologically based pharmacokinetic
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PD	Parkinson's Disease
PDB	Protein Data Bankqsdar
PET	Positive emission tomography
PI	Principal Investigator
PIG-A	Phosphatidylinositol glycan anchor biosynthesis, class A
PND	Post-natal day
PoD	Point of Departure
PPF	Glial Modulator
QAW	Quantitative Analysis Workgroup
QSDAR	Quantitative Spectrometric Data-Activity Relationship
R2R	Review-to-research and return
RAPID-B™	Rapid Identification of Bacterial Pathogens
ROS	Reactive-oxygen species
SAB	Science Advisory Board
SBP	Systolic blood pressure
SEM	Scanning electron microscopy
SEQC	Sequencing Quality Control
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SOT	Society of Toxicology
SSL	Simulated solar light
STEC	Shiga-toxin producing *Escherichia coli*
TdP	Torsades de Pointes
TERA	Toxicology Excellence for Risk Assessment
TiO2	Titanium dioxide
TKI	Tyrosine kinase inhibitors
TNBC	Triple-negative breast cancer
TSNA	Tobacco-specific nitrosamines
TSST-1	Toxic-Shock Syndrome Toxin-1

Acronym/ Abbreviation	Name
UALR	University of Arkansas at Little Rock
UV	Ultraviolet
VGDS	Voluntary Genomic Data Submission
VRE	Vancomycin-resistant Enterococcus
VSS	Veterinary Services Staff
VXDS	Voluntary eXploratory Data Submission

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