National Center for Toxicological Research

2018 Annual Report

www.fda.gov/nctr

Vision

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

Mission

NCTR conducts scientific research to generate data for FDA decision making, and develops and supports innovative tools and approaches that FDA uses to protect and promote individual and public health.

Strategic Plan

NCTR's Strategic Plan sets forth our long-term strategic goals and objectives. The plan also details specific actions we are committed to taking as we carry out our mission 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 strategic goals.

NCTR Research Goals

- Advance scientific knowledge and tools required to support personal, animal, and public health
- Enhance collaborations with other FDA Centers
- Promote global interactions in regulatory science

Contents

Preface

Message from the Director

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 sup- port 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.

NCTR has long been recognized as a valuable resource in FDA's commitment to regulatory science and science-based decision making. We invite you to learn more about NCTR's wide array of re- search expertise and scientific capabilities in the areas of drugs, foods, devices, biologics, veterinary medicine, and tobacco products.

/s/

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

About the Annual Report

We are excited to showcase NCTR's 2018 Annual Report. This document is intended to provide a synopsis of NCTR's accomplishments–it is highly interactive and best viewed electronically. As you move through the document you will notice many links to NCTR's FDA.gov pages as well as links to many scientific publications. If you would like to delve deeper into a topic, please visit the links for more information.

Thank you for your interest in the NCTR!

NCTR Organization Structure – NCTR Leadership

William Slikker, Jr., Ph.D.
Center Director
FDA/NCTR

Tucker Patterson, Ph.D.
Associate Director
Science and Policy, Office of Research

Winona Cason
Executive Officer
Office of Management

Bradley Schnackenberg, Ph.D.
Associate Director
Office of Scientific Coordination

Rajesh Nayak, Ph.D.
Associate Director
Office of Regulatory Compliance and Risk Management

Donna Mendrick, Ph.D.
Associate Director
Regulatory Activities

NCTR Organization Structure – Research Division Directors

Frederick Beland, Ph.D.
Division Director
Biochemical Toxicology

Carl Cerniglia, Ph.D.
Division Director
Microbiology

Robert Heflich, Ph.D.
Division Director
Genetic and Molecular Toxicology

Sherry Ferguson, Ph.D.
Division Director
Neurotoxicology

William Mattes, Ph.D., DABT
Division Director
Systems Biology

Weida Tong, Ph.D.
Division Director
Bioinformatics and Biostatistics

Click here for more information about NCTR Research Divisions

NCTR at a Glance

Key Priorities

1. Develop and validate innovative approaches to assess FDA-regulated products
2. Accelerate FDA's capability to manage, analyze, and interpret biological research data generated from new technologies
3. Share research knowledge, advice, and training, such as the Global Summit on Regulatory Science, with global partners
4. Understand the risks and benefits of nanoscale materials in FDA-regulated products
5. Understand a compound's toxicity and provide data for improved FDA safety guidelines

Accomplishments

- **Protect Children** – Conducted safety studies on pediatric anesthetics and identified a compound that may protect a child's brain during anesthesia.
- **Promote Product Safety** - Generated data to determine the safety of several FDA-regulated compounds, such as Bisphenol A, found in common food and drug products.
- **Advance Science** - Collaborated with state and private entities to research nanotechnology and gene expression changes to improve understanding of public risk and potential treatment.
- **Save Lives** - Treated mice with an anti-cancer drug and discovered genetic changes that may be used in the early detection of heart injury.
- **Foster Precision Medicine** - Demonstrated that FDA's adverse events reporting system could be used to better understand disease risk factors such as age, sex, or ethnicity.
- **Save Money on Drug Development** - Developed a way to use FDA-approved drug labels to predict which compounds are most likely to cause liver injury in patients.

By the Numbers

- 123 experimental laboratories operate on NCTR's research campus
- 277 research projects ongoing at NCTR in 2018
- >100k searchable labeling document in the publicly available FDALabel database

NCTR – Numbers at a Glance for 2018

Manuscript Submissions and Newsletter Subscriptions

Research performance is typically difficult to measure and quantify. One way NCTR measures research performance is by tracking how much NCTR research is being published in reputable scientific journals. Below are the publication numbers for 2018.

Number of Publications, NCTR (2016-2018)

2018 – 170
2017 – 155
2016 – 150

Another way NCTR measures research performance is by tracking subscriptions to NCTR Research Highlights and NCTR Science Insights. NCTR offers email subscriptions to these two resources. If you are interested in subscribing to NCTR Research Highlights and/or Science Insights, please visit our website and subscribe with your email address.

2018 NCTR Research Highlights & Science Insights Subscriptions

- Total Subscriptions (Research Highlights and Science Insights) – 86,311
- Research Highlights Subscriptions – 46,210
- Science Insights Subscriptions – 40,101

NCTR Office of Management – Human Capital

Peer review within FDA pertains to the position classification of scientific positions. FDA's peer review committees provide advisory recommendations to the Peer Review Office concerning the classification (i.e., title, series and grade level) of scientific research positions. All permanent research scientists (GS-13 and above) at NCTR are required to undergo peer review every 5 years. NCTR Research Scientists may request to be reviewed earlier than the mandatory 5-year cyclical review. NCTR Staff Fellows and Visiting Scientists are also eligible to undergo peer review in order to be promoted. Overall, the peer review program is a critical component of NCTR's ability to recruit, promote, and retain a high-performing scientific workforce, and ensures NCTR has the best scientific workforce to conduct its research mission.

The Office of Management's Human Capital team coordinates NCTR's Research Peer Review Program in collaboration with the FDA's Office of Human Resources. In this role, the Human Capital team helps train candidates on how to prepare their peer review packages to increase their promotability, review the criteria used to make grade level determinations, and provide input to the Peer Review Committee Chair and Co-Chair for each scientists' career evaluation report.

As a result, highly productive scientists are rewarded, employee morale is boosted, and new scientists see the potential for career growth and advancement at NCTR. In 2018, NCTR achieved its highest peer review promotion rate ever (89%).

NCTR Research Peer Review Promotion Rate (by Year)

2015 – 70%

2016 – 79%

2017 – 67%

2018* – 89%

*In 2018, NCTR achieved its highest peer review promotion rate

FDA-TRACK NCTR

FDA-TRACK is FDA's agency-wide performance management system that monitors FDA Centers and Offices through key performance measures and projects. NCTR has several key research projects and other related metrics that are tracked and published to the public facing FDA.gov site. Explore the progress NCTR is making towards its strategic priorities here.

2018 Research Collaborations

A critical component of NCTR's science portfolio is collaborations with other FDA Centers to leverage knowledge and to establish partnerships. Expertise from each Center has contributed to the development of critical scientific projects that have focused on the regulatory needs of the agency. A strong in-house science base and a network of collaborations are necessary to support FDA's success in addressing public health challenges. Scientific advancements are enhanced by participation in meetings and conferences where experts present their current research. Collaborations and relationships built at these meetings provide FDA with access to cutting-edge science. Support of this important strategic priority is reflected in the following highlighted collaborations.

110 of 277 (40%) NCTR ongoing research projects are FDA collaborations. 55 of 277 (20%) NCTR ongoing research projects are collaborative with organizations outside of FDA. These collaborations include, but are not limited to:

- *National Institute of Environmental Health Sciences/ National Toxicology Program (NIEHS/NTP)*
- *National Institutes of Health (NIH)*
- *United States Department of Agriculture (USDA)*
- *University of Arkansas for Medical Sciences (UAMS)*
- *University of Arkansas at Little Rock (UALR)*

2018 Collaborations with FDA Product Centers

CBER 5%

CDER 48%

CDRH 13%

CFSAN 12%

CTP 11%

CVM 9%

ORA 2%

Collaboration – Center for Drug Evaluation and Research (CDER)

NCTR appreciates the opportunity to continue collaborating with the Center for Drug Evaluation and Research (CDER) scientists to help address important and timely questions that influence decision-making for FDA-regulated products. The NCTR-CDER partnership continues the synergy between the Centers and the researchers furthering FDA's regulatory-science knowledge.

Over the Counter (OTC) Drug Review

In 2018, NCTR, in collaboration with CDER, completed thorough literature searches and reviews for the following OTC drugs:

- The six potential Food Handler Antiseptics Active Ingredients
- Impurities/degradants in selected United States Pharmacopeia (USP) Monograph drug product, Tetrahydrozoline Hydrochloride Ophthalmic Solution.

This research effort involved summarizing 2274 relevant references, providing relevant articles, a list of irrelevant references, the search tables, and by late 2018, NCTR scientists had also summarized ~300 relevant references on chlorine safety.

Perinatal Opioid Exposure

The FDA Opioid Action Plan provides comprehensive guidance for reestablishing safe-use standards for these products. In support of the plan, NCTR completed a methods-development protocol that gave FDA hands-on-experience in neural stem-cell growth. This experience will allow scientists to con-duct lab-based in vitro research rather than relying solely on whole animal (in vivo) research. NCTR scientists conducted research to assess perinatal opioid exposure, a concern shared in the perinatal-related FDA Drug Safety Communication. NCTR, in collaboration with CDER, began finalizing the data on opioid exposure to brain cells during perinatal development.

Cancer Drug Toxicity

In FY 2018, NCTR scientists and CDER demonstrated that direct liver cell toxicity may contribute to the mechanism of kinase inhibitor (KI)-induced liver damage. KIs are a relatively new type of drug that has played an increasingly important role in the treatment of cancer and inflammation. NCTR examined the toxicity of 34 FDA-approved KIs in cultured rat and human liver cells (hepatocytes). The hepatocytes

were treated with KIs at 10 concentrations that reflect maximum therapeutic clinical blood levels. The data from this study help FDA develop a better understanding of why some KIs result in liver damage. Furthermore, the results suggest that in vitro models may be useful in predicting clinical liver toxicity. A manuscript describing the study is available in *Toxicology Letters*.

Collaboration – Center for Tobacco Products (CTP)

The collaboration between Center for Tobacco Products and NCTR provides research within FDA to support the regulatory authorities within the Family Smoking Prevention and Tobacco Control Act to protect public health. The tobacco regulatory science conducted at NCTR can be summarized in the following research areas.

Inhalation Toxicology

Inhalation toxicology studies are necessary to evaluate the dose-response toxicity of inhaled chemicals that are found in tobacco products or that form during the combustion process. The Center for Tobacco Products/NCTR Inhalation Toxicology Core Facility (InhaleCore) provides the technical expertise to conduct these inhalation studies in compliance with international test guidelines (e.g., Organization for Economic Co-operation and Development, OECD). In collaboration with CTP, the InhaleCore researchers study animal biological responses using various toxicological endpoints after they are exposed in a well-defined environment via nose-only inhalation. The re-search outcomes provide data to better the understanding and quantification of the adverse health risks associated with humans using tobacco products, thereby supporting the FDA mission of regulating tobacco products.

Predictive Modeling

While it has been established that nicotine is the main constituent responsible for addiction to tobacco products, it is unknown if the other thousands of constituents present in tobacco products also have addictive potential. Utilizing in silico techniques, CTP and NCTR collaborators developed a prediction model for the potential binding of tobacco constituents to the nicotinic acetylcholine receptor α7 subtype, which plays an important role in addiction. The developed model could be a useful tool for high-throughput screening of potential addictive tobacco constituents. A manuscript describing the study can be found in *Oncotarget*.

The collaboration between the NCTR and CTP modeling groups continued to develop a human physiologically-based pharmacokinetic (PBPK) model for nicotine. Using biomarker and dose-response data from existing studies and scientific literature, this model will describe the internal dose metrics of nicotine for multiple exposure pathways and assess the nicotine exposure-response relationship across different tobacco product types.

Alternative Models: Air-Liquid Interface (ALI) Cultures

Scientists from NCTR, in collaboration with CTP, have developed a hu-man air-liquid interface (ALI) airway tissue model to assess the toxicological and inflammatory effects of whole tobacco smoke (or tobacco smoke constituents) in an in vitro system. The metabolic activities of undifferentiated normal human primary bronchial epithelial (NHBE) cells were compared to those of the human ALI airway tissue models using acrolein, a known toxicant found in tobacco smoke. Their findings support using ALI cultures as an alternative in vitro model to evaluate inhaled toxicants requiring metabolic transformation. A manuscript describing the study can be found in *Toxicology In Vitro*.

Collaboration – Center for Food Safety and Nutrition (CFSAN)

Rapid Detection of Bacterial and Microbial Contamination

NCTR scientists, in collaboration with the Center for Food Safety and Nutrition (CFSAN), significantly improved a method for rapidly detecting low levels of harmful bacteria such as E. coli O157:H7 in various foods. This method measures single bacterial cells without requiring a time-consuming growth period in a Petri dish. This method is proven to be superior to the current FDA regulatory method. A publication describing the application of this method in raw spinach was published in Frontiers in Microbiology.

In 2018, NCTR and CFSAN scientists demonstrated RAPID-B—a field portable, ultrasensitive, and selective real-time detector of bacteria in food, such as *E. coli* O157, *Salmonella*, and *Listeria monocytogenes*—at a national government conference. The scientists demonstrated its portability by transporting the instrument by car from Arkansas to the 2018 USDA Food Safety Inspection Service and Agriculture Research Service Annual Conference in West Virginia. As part of the conference, the NCTR scientists demonstrated RAPID-B and described how it can detect different bacterial pathogens and the "mad cow" disease-causing agent. Collaborations between NCTR and USDA are planned in this area. USDA has identified portable, real-time detection of Listeria and Salmonella in food as a priority. Ongoing collaborative research efforts by NCTR and CFSAN scientists include:

- detecting *Listeria monocytogenes* faster using genetic tags
- improving the ability to detect low levels of *Listeria* cells in foods, such as cantaloupe, avocado, or carrots
- expanding the scalability to identify the source of a contamination
- detecting microbial contaminants—including mycobacteria—in tattoo inks.

Contaminated Tattoo Inks

The dramatic increase in tattooing and the use of permanent makeup have made this study relevant to the mission of FDA. A manuscript describing the progress of this study was published in the ***Journal of Ap-plied Microbiology***. This study continues with the goals of:

- importance of monitoring the microbial contamination of tattoo inks for potentially pathogenic microorganisms
- increasing understanding of tattoo-related infectious diseases and their impact on public health
- providing FDA and the public with data and methods for determining the safety of tattoo inks from a microbiological-risk perspective.

Collaboration – Center for Veterinary Medicine (CVM)

The Centers for Disease Control and Prevention estimates that each year roughly one in six Americans get sick from eating contaminated food. NCTR scientists conduct projects to limit the emergence and spread of drug resistance in bacterial pathogens that compromise our ability to treat foodborne illnesses. These projects support FDA's regulatory needs related to the pool of Anti-microbial Resistance (AMR) genes and bacterial pathogens in feed, foods, clinical and environmental samples; and the potential effects of transmission of resistant bacteria on human health.

In 2018, NCTR scientists demonstrated that when certain Salmonella strains were exposed to different concentrations of specific antibiotics, there was an increase in the rate of resistance. In collaboration with FDA's Center for Veterinary Medicine (CVM), NCTR scientists used techniques to better understand the diversity of organisms. NCTR scientists also studied the presence of plasmids—independent DNA molecules commonly found in cells—that can contribute to AMR and enhanced disease-causing ability. NCTR and CVM continue their efforts in this vastly understudied area of research and are developing a database and analysis tool to better understand and control Salmonella enterica in foods and feed. A publication describing NCTR's and CVM's research in this area can be found in the *International Journal of Food Microbiology*.

Also in 2018, NCTR, in collaboration with CVM, published an article related to antimicrobial drug residues and the human intestinal microbiome permeability in Anaerobe. This research involved:

- Studying the effects of tetracycline (a common antimicrobial drug) on the human intestinal microbiome.
- Analyzing the slight differences in these effects between individuals.
- Accumulating more data to contribute to the knowledge-base on the impact of tetracycline.

The study was extended to assess if the gut bacteria degrades or inactivates the antibiotic. This data was presented at the 2018 Annual Meeting of American Society of Microbiology and a manuscript was submitted to *Regulatory Toxicology and Pharmacology*.

The Human Microbiome

Microorganisms associated with the human gut are known collectively as the "human microbiome" or "microbiota" and play an important role in health and disease. The use of veterinary antimicrobial agents in food-producing animals may result in continual hu-man exposure to low levels of antimicrobial residues in food as part of their daily diet. There is concern that antimicrobial agents at residue-level concentrations could potentially disrupt the microbial colonization that serves as a protective barrier in the gastrointestinal tract—important in combating certain diseases. These issues as well as other drug, bacterial, and food interactions associated with the human microbiome are becoming an increasingly important research area for FDA.

Collaboration – NIEHS/National Toxicology Program (NTP)

The National Toxicology Program (NTP) was 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.

Notable chemicals studied as a result of the NCTR/ collaborative agreement:

- Bisphenol A (BPA)
- Arsenic
- Triclosan
- Acrylamide

NTP/NCTR Quick Facts

- **I**n 2018 NTP and NCTR had over **15 active collaborative projects with NTP**.
- The success of this IAG has led to **over 25 years of collaborative toxicity testing** on compounds of interest to FDA and NTP.
- The IAG program has led to the toxicity assessment and mechanism-of-action studies of many classes of chemicals including: **food contaminants, cosmetics, endocrine-disruptor compounds, food cooking by-products, dietary supplements, drugs, and anesthetics.**
- To test the effect of sunlight on the toxicological risk of chemicals, the IAG supported the **development of the NIEHS/FDA Phototoxicology Research and Testing Laboratory.** 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.

Science Advisory Board to NCTR

Toxicological Research, Science Advisory Board to NCTR

NCTR—in partnership with researchers from FDA Centers, other government agencies, academia, and industry—provides innovative technology, methods development, vital scientific training, and technical expertise. The unique scientific expertise of NCTR is critical in supporting FDA product centers and their regulatory roles.

Purpose

The Science Advisory Board (SAB) to NCTR advises the NCTR Director in establishing, implementing, and evaluating the research programs that assist the FDA Commissioner in fulfilling his regulatory

responsibilities. The Board provides an extra-agency review in ensuring that the research programs at NCTR are scientifically sound and pertinent.

Board Membership

The Committee shall consist of a core of nine voting members including the Chair. Members and the Chair are selected by the Commissioner or designee from among authorities knowledgeable in the fields of toxicological research. Members will be invited to serve for overlapping terms of up to four years. Almost all non-Federal members of this committee serve as Special Government Employees. The core of voting members may include one technically qualified member, selected by the Commissioner or designee, who is identified with consumer interests and is recommended by either a consortium of consumer-oriented organizations or other interested persons.

The SAB to NCTR advises the Commissioner or designee in discharging responsibilities as they relate to helping to ensure safe and effective drugs for human use and, as required, any other product for which the FDA has regulatory responsibility.

Click here for more information about the SAB to NCTR and its members.

2018 SAB Meeting

The 2018 meeting of the NCTR SAB took place in March 2019 in Little Rock, Arkansas. The meeting lasted two days and covered a variety of topics such as:

- Overview of NCTR
- Overview of NCTR Research Divisions
- Imaging and Nanotechnology Review
- NCTR Collaborations with FDA Centers

For more information regarding the 2018 SAB meeting please click here.

Perinatal Health Center of Excellence

2018 PHCE Highlights

- Initial funding for the PHCE has been placed in the FY19 budget request.
- FDA Centers/ORA have identified liaisons to manage the development and review of proposals.
- A review process has been jointly established by the Center liaisons.
- 14 proposals have been developed for review and consideration for funding.

FDA scientists working independently have recognized the need to come together to address a special public health need. NCTR, an FDA center focused on research, is spearheading the formation of an FDA virtual Perinatal Health Center of Excellence (PHCE) that is focused on the perinatal period as defined to include: maternal, premature, neonatal periods, and development throughout childhood.

The PHCE is a model that can uniquely accomplish the needs for these understudied populations. With the support of collaborating product centers and ORA, the NCTR staff will coordinate the activities of the PHCE. Through coordinated efforts across the centers/ORA, studies will be planned and conducted to address important regulatory-science needs facing FDA. Broadly speaking, the PHCE-funded research falls into the categories of in silico models, stem cell systems and other in vitro models, laboratory animal studies, translational and clinical studies, mathematical modeling, bioanalytical

chemistry, exposure science, and bioinformatics targeting the perinatal period.

Drugs and other medical products are sometimes used off-label because of the barriers to perform clinical trials needed for drug, device, and other product approval in these populations. FDA incentivizes registration of drugs for these populations and encourages the use of computational tools, such as physiologically based pharmacokinetic (PBPK) modeling, to assist with first-in dose selection for clinical trials. Even with the use of these approaches, more must be done across the entire product development pipeline to better characterize safety, efficacy, or potential toxicity.

Global Summit on Regulatory Science (GSRS)

The Global Summit on Regulatory Science (GSRS) is an international conference for discussion of innovative technologies and partnership to enhance translation of the 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, omics for translational science and personalized medicine) for addressing regulatory-research questions and to discuss the best way to translate these technologies into real-world applications. This is a unique conference where the regulators, policy makers, and bench scientists from various countries can exchange their views on how to develop, apply, and implement the innovative methodologies into regulatory assessments in their respective countries as well as harmonize the strategy between countries via collaboration. To engage the global community to address regulatory science research and training needs, GSRS will be held in different countries on an annual basis.

NCTR's Director serves as the co-chair of the Coalition's executive committee and works with the Coalition to promote global interaction. The Global Summit is led by the Global Coalition which is comprised of regulatory science leaders from around the world including Australia, Belgium, Brazil, Canada, China, Italy, Japan, Nigeria, Singapore, India, Korea, Thailand, and the U.S. The 9th Global Summit will take place September 2019 in Ispra, Italy, at the Joint Research Center. The meeting will focus on nanotechnology and nanoplastics.

Summary of GSRS18 – Beijing, China

The topics that were discussed and presented covered risk/benefit of dietary supplements and herbal medicine in the era of data science. Over 200 scientists from 13 countries attended GSRS18: the presenters represented Asia (China, Korea, Japan and India), EU (Belgium and Italy), USA and Canada from North America, and Australia.

The conference started with a discussion on the global regulatory structure for dietary supplements and herbal medicine. Given over 80% of the world's population uses dietary supplements and herbal medicine, the follow-up discussion was focused on their safe use. The conference ended with discussing the challenges and opportunities of using new tools and methodologies in this area.

For additional details, updates, abstract guidelines, agenda, and more visit http://www.fda.gov/globalsummit.

2018 NCTR Research Divisions and Important Accomplishments

The NCTR Research Divisions work closely in a seamless effort to support FDA's mission to bring safe and efficacious products to the market rapidly and to reduce the risk of adverse health effects from products on the market.

(Click on the links below to navigate to the division pages on FDA.gov.)

NCTR Research Divisions

- Biochemical Toxicology – Frederick A. Beland, Ph.D., Division Director
- Bioinformatics and Biostatistics – Weida Tong, Ph.D., Division Director
- Genetic and Molecular Toxicology – Robert Heflich, Ph.D., Division Director
- Microbiology – Carl Cerniglia, Ph.D., Division Director
- Neurotoxicology – Sherry Ferguson, Ph.D., Division Director
- Systems Biology – William B. Mattes, Ph.D., DABT, Division Director

DBT – Division of Biochemical Toxicology

About the Division:

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.

Research within the Division is centered on quantifying the toxicities and carcinogenic risks associated with specific chemicals and introducing new risk-assessment techniques to enable regulatory agencies to evaluate the risks associated with exposure to chemicals. The Division of Biochemical Toxicology capitalizes on scientific knowledge in the areas of biochemistry, organic and analytical chemistry, cellular and molecular biology, nutritional biochemistry, toxicology, phototoxicology, computational modeling and simulation-based risk assessment methods, and pharmacology.

2018 Select Accomplishments

NTP Report on the CLARITY-BPA Core Chronic Study

A "Draft NTP Research Report on the CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats" was publicly released by the NTP in February 2018 and was reviewed by an external panel of experts in April 2018. The final, peer-reviewed NTP Research Report was publicly re-leased in September 2018. The NCTR-conducted two-year study was part of an NTP-led effort known as CLARITY-BPA — short for Consortium Linking Academic and Regulatory Insights on BPA Toxicity. As stated in NTP's Update Newsletter, "NIEHS and FDA convened CLARITY-BPA to study the full range of potential health effects from exposure to BPA in rats and to provide data that could be used for regulatory decisions. CLARITY-BPA united standard research practices used by regulators, called federal guideline studies, with innovative research conducted at universities through grants from NIEHS."

More information about the CLARITY-BPA program can be found at https://ntp.niehs.nih.gov/whatwestudy/topics/bpa/index.html.

Metabolism and Disposition of Arsenic Species Across Life-Stages in a Rodent Model

Results from a Division study provide links between administered dose, metabolism, and internal exposures to inorganic arsenic across life-stages in a rodent model. Metabolic methylation of inorganic arsenic decreases acute toxicity; however, tissue binding of arsenic metabolites is evidence for

simultaneous formation of toxic species. Pregnant and fetal CD-1 mice represent a key animal model for arsenic carcinogenesis, since adult-only exposures have minimal effects. The study evaluated inorganic arsenic and its metabolites in blood and tissues from maternal and fetal CD-1 mice, as well as neonatal and adult CD-1 mice. Because arsenic is widely distributed in the earth's soil and water, and human diseases are linked with arsenic exposures similar to dietary-intake estimates, it is important for FDA to better understand the cancer risks from human exposure to environmental arsenic. Results from this study have been published in a series of papers in *Food and Chemical Toxicology*.

Effect of Carcinogens on Transcriptomic and Epigenetic Alterations in Liver Cells
NCTR scientists, in collaboration with the University of New Mexico Comprehensive Cancer Center, investigated the utility of high-throughput microarray gene expression and next-generation sequencing for the in vitro identification of genotoxic and non-genotoxic carcinogens. This approach may substantially enhance the identification and assessment of potential liver carcinogens. The increasing number of man-made chemicals in the environment that may pose a carcinogenic risk highlights the need for developing reliable time- and cost-effective approaches for carcinogen detection and identification.

Transcriptomic analysis of human-liver HepaRG cells treated at minimally toxic concentrations with three different carcinogens generated distinct gene-expression profiles. In contrast to transcriptomic alterations, treatment of liver cells with the carcinogenic and non-carcinogenic chemicals resulted in profound changes in the DNA methylation footprint; however, the correlation between gene-specific DNA methylation and gene expression changes was minimal. Among the carcinogen-altered genes, transferrin (TF) emerged as a sensitive marker for an initial screening of chemicals for their potential liver carcinogenicity. Potential liver carcinogens (i.e., chemicals causing altered TF gene expression) could then be subjected to gene-expression analyses to differentiate genotoxic from non-genotoxic liver carcinogens. Information about this study can be found in *Food and Chemical Toxicology*.

DBB – Division of Bioinformatics and Biostatistics

About the Division:

The Division of Bioinformatics and Biostatistics develops integrated bioinformatics and bio-statistics capability to address increasing needs in biomarker development, drug safety, drug repositioning, precision medicine, and risk assessment.

2018 Select Accomplishments

Scientists from NCTR's Division of Bioinformatics and Biostatistics and Immuneering Corporation have identified the structural changes of the androgen receptor (AR) caused by antiandrogens (chemicals that inhibit male hormones) using simulations. The identified structural changes could facilitate AR targeting-drug discovery. More information can be found in the May 2018 issue of *Frontiers in Pharmacology*.

The inaugural meeting of the Little Rock Chapter of the Massive Analysis Quality Control Society — organized by NCTR — was held September 7-8, 2018, in Little Rock, Arkansas. The participants were from FDA, the National Institutes of Health, biotechnology companies, and academia, with roughly 40 participants onsite and 10 online. Participants examined the progress of the FDA-led Sequencing Quality Control Project/Phase 2 (SEQC-2) in comprehensively assessing next-generation sequencing oncology-panel technologies. The panels examined the similarities and differences among the genomic and

cellular alterations found across diverse tumor types. They also examined four liquid biopsy-focused panels that can detect rare mutations of circulating tumor DNA.

Division scientists developed novel data mining and visualization methods which resulted in a total of 63,082 drug adverse-event pairs that were identified from the FDA Adverse Event Reporting System as the significant association between 936 drugs and 10,316 adverse events. New safety signals were identified when compared with the currently available information in various sources. Results were presented in the Society of Toxicology 2018 Annual Meeting.

NCTR Bioinformatics Tools

(See links below for more detailed information on Bioinformatics Tools.)

ArrayTrack™ HCA-PCA Standolone Package — Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) – powerful data-exploring tools extracted from ArrayTrack™.

The *de novo* Assembly Quality Evaluation Tool (dnAQET) — Framework designed to evaluate the contigs of a de novo assembly against a trusted reference genome.

Decision Forest — Novel pattern-recognition method for analysis of data from microarray experiments, proteomics research, and predictive toxicology.

Drug-Induced Liver Injury Rank (DILIrank) Dataset — A large reference list of drugs ranked by their risk for developing DILI in humans. This is an updated list from the LTKB Benchmark dataset.

Estrogenic Activity Database (EADB) — Comprehensive set of estrogenic activity data from a variety of data sources and a component of the enhanced Endocrine Disruptors Knowledge Base (EDKB).

Endocrine Disruptor Knowledge Base (EDKB) — Scientific resources for estrogen and androgen activity of potential endocrine disruptor chemicals.

FDALabel — Tool to conduct full-text search of drug labeling.

Liver Toxicity Knowledge Base (LTKB) — Collection of diverse drug-induced liver injury data associated with individual drugs and the use of systems biology analysis.

MicroArray/Sequencing Quality Control (MAQC/SEQC) Project — Project to develop microarray quality control metrics and thresholds.

Mold2 — Software that generates molecular descriptors from two-dimensional structures.
NCTR Liver Cancer Database (NCTRlcdb) — Database of 999 chemicals with assigned liver-toxicity classifications to facilitate the construction of better carcinogenicity models by FDA and other organizations.

For more information please visit: www.fda.gov/nctrbioinformatics or subscribe to the NCTR Bioinformatics Tools newsletter.

DGMT – Division of Genetic and Molecular Toxicology

About the Division:

The Division of Genetic and Molecular Toxicology is internationally recognized for its expertise in developing and validating genetic toxicity assays and in interpreting genetic toxicity findings for regulatory decision-making.

Research Themes

1. Develop and validate regulatory genetic-toxicology assays
2. Conduct chemical-specific research
3. Develop new paradigms for regulatory decision-making that integrate measures of genetic risk with biomarkers of toxicity by conducting research to develop:
   - Relevant biological models.
   - Comprehensive approaches to monitor genetic variation using technologies such as Next Generation Sequencing.
   - Better ways of evaluating data to determine human risk.

2018 Select Accomplishments

Minimally Invasive Gene-Mutation Assay May Identify Mutagens and Carcinogens

Division scientists developed a novel version of the *Pig-a* assay for rat bone-marrow erythroid cells. These cells are the direct precursors of red blood cells found in circulating blood that are routinely used for the *Pig-a* assay. Mammalian erythrocytes lack genomic DNA; therefore, confirmation of mutation induction — a necessary step for assay validation — is not possible in erythrocytes. The data from an NCTR study conclusively demonstrated that the *Pig-a* mutant erythrocytes (red blood cells) measured in the circulating blood of mutagenized rats were descendent from cells containing *Pig-a* mutations. Bone marrow erythroid precursor cells have nuclei and DNA, and thus are suitable for sequencing *Pig-a* mutations. Two publications resulted from this research and can be found in the October 2018 issue of *Environmental Molecular Mutagenesis.*

Detection of Rare Genomic Mutations Using Next-Generation Sequencing (NGS)

FDA scientists from NCTR and the Center for Drug Evaluation and Research are developing a sensitive method to detect mutations induced by chemicals. Mutations are changes in the DNA sequence of an organism, ranging from small point mutations to large chromosome alterations that can cause adverse health effects, such as cancer and genetic disease. The goal of the ongoing study is to establish a new NGS assay that may become a powerful, rapid, and practical tool to routinely evaluate the mutagenicity of FDA-regulated products. Data from this study were interpreted and reported in the December 2018 issue of *Archives of Toxicology*.

Lung Tumor Model Improves Understanding of Cancer-Drug Resistance

Division scientists detected the outgrowth and enrichment of mutant tumor cells clinically associated with the development of drug resistance. They cultured primary lung tumor organoids—tiny, self-organized three-dimensional tissue cultures—in the presence of varying concentrations of erlotinib (Tarceva®), a drug used to treat lung cancer. Using this novel model and a sensitive method for mutation detection (ACB-PCR) they detected increases in mutant tumor cells after culture. Better patient outcomes are being achieved using personalized cancer treatments by selecting therapies based on tumor genetics. Unfortunately, resistance to the therapy occurs frequently and limits drug efficacy. Because this lung tumor-organoid model reproduces the cellular and mutational diversity of human lung

adenocarcinomas, it has the potential to identify treatment strategies and drug combinations that reduce or eliminate drug resistance.

DM – Division of Microbiology

About the Division:

The Division of Microbiology's goals are to perform fundamental and applied research to address critical issues in support of the FDA mission. The Division's research projects are based on expertise of division staff and consultation with scientists from other FDA Centers, regulatory agencies, academia, and industry.

Research within the Division

The Division of Microbiology scientists engage in research addressing FDA issues with special emphasis on:

1. Improving methods to detect, identify, and characterize foodborne pathogens.
2. Determining antimicrobial resistance and virulence mechanisms of microbial pathogens.
3. Using state-of-the-art molecular biological approaches to monitor interactions between the human microbiome and antimicrobial agents, nanomaterials, food contaminants, and FDA-regulated products.
4. Conducting studies related to women's health.
5. Improving environmental risk assessments of priority pollutants, including polycyclic aromatic hydrocarbons and drugs, by integrating systems biology approaches.
6. Conducting research involving nanotechnology.
7. Evaluating smokeless-tobacco products for toxicity from a microbiology perspective.

2018 Select Accomplishments

Development and utilization of approaches for the evaluation of the plasmid-associated antimicrobial resistance and virulence in *Sal*monella

This study demonstrated that certain antimicrobial exposures impact plasmid transfer dynamics in a dose-dependent fashion. It also showed that plasmid-encoded factors likely contribute to infection under low-iron conditions as seen by the fact that iron acquisition systems were up-regulated during infection.

Detection of microbial contaminants including pathogenic mycobacteria in tattoo inks

Investigators completed a survey of 85 unopened, sealed tattoo and permanent makeup inks purchased from 13 companies available in the US, for microbial contamination and found that 42 inks (49%) were contaminated with microorganisms, often with relatively high levels that includes *Derma-coccus barathri* and *Roseomonas mucosa*, which have been associated with skin infections.

Conducted host-microbiome assessments to evaluate the effects of FDA-regulated products on the microbiome

One study analyzed the metabolic alterations in oral bacteria as a result of smokeless tobacco exposure and demonstrated with in vitro studies that STPs affected the growth and viability of some oral bacterial species in a concentration-dependent manner. Similarly, the investigators evaluated the effects of STPs on oral microbiota in a Syrian Golden hamster cheek pouch carcinogenesis model and found that the use of STPs tobacco significantly disrupted the oral microbiota. Additionally, capability-building efforts to standardize sample collection and data analysis methodologies for gut microbiome and gut mucosa

associated immune responses were undertaken. As part of these efforts, investigators established 16s rRNA gene sequencing approaches to facilitate assessment of: animal model species, sample anatomical collection sites, exposure vehicle, and toxicological relevance to human disease.

Also the effects of residue levels of antimicrobial agents on the intestinal microbiome were evaluated and it was found that higher concentration levels above established acceptable daily intake values impacted intestinal microbiome composition and intestinal barrier functions.

DNT – Division of Neurotoxicology

Research Themes

The Division of Neurotoxicology focuses on increasing FDA's understanding of the processes associated with neurotoxic outcomes—harmful effects associated with the brain and nervous system. This increased understanding may provide opportunities for improved risk assessments and identification of new approaches to diagnosis. The Division's strategy has been to use a broad range of research approaches that capitalize on the expertise of personnel in diverse areas of neuroscience and other scientific disciplines.

The Division is continually expanding its capabilities in the area of bioimaging by adding both microPET (positron emission tomography) and MRI (magnetic resonance imaging) instruments along with trained personnel. These innovative imaging technologies give researchers a unique way to monitor brain and nervous-system activity with minimal discomfort to the study subject.

2018 Select Accomplishments

Division scientists — in collaboration with scientists from the University of Arkansas at Fayetteville and Universidad Autonoma de San Luis Potosi and Universidad Juarez Autonoma de Tabasco in Mexico — have developed an in vitro Blood-Brain Barrier (BBB) model that simulates the BBB damage resulting from traumatic brain injury (TBI) observed in vivo. This study demonstrates the utility of the in vitro BBB model in simulating TBI and supports the use of biaxial stretch as a valuable tool to study the mechanisms of TBI and potential therapies. A manuscript describing the newly developed model was published in the January 2018 issue of *Molecular Neurobiology*.

NCTR scientists have successfully set up a high-throughput Mulit-Electrode Array (MEA) system to record electrical functions of brain cells as a read-out for neurotoxicity, and have demonstrated that certain neurotoxic agents can alter electrical functions of cultured human brain cells, such as neuronal firing rates, neuronal spikes, and electrical bursts. The MEA system is a unique emerging technology in the drug-development industry to evaluate the neurotoxicity of a drug or other compound and to predict their safety and efficacy. These NCTR studies can help support agency reviewers with validation or interpretation of MEA data submitted by industry and inform decisions about the safety and efficacy of such drug candidates.

Scientists from NCTR and the University of Arkansas for Medical Sciences have demonstrated that two commonly used chemotherapeutics (cyclophosphamide and doxorubicin), administered alone or in combination, did not induce behavioral alterations in an animal model reflective of human breast cancer patients. The study was designed to investigate the memory and attention problems that some female breast-cancer patients experience after chemotherapy — sometimes known as "chemo" brain. The lead author of the article — Timothy Flanigan, Ph.D. — was awarded the Developmental Neurotoxicology

Society's "2018 Richard Butcher New Investigator Award" for this publication. The article is available in the April issue of *Toxicological Sciences*.

DSB – Division of Systems Biology

About the Division:

The Division of Systems Biology strives to address problems of food, drug and medical product safety using systems biology approaches and innovative technology.

Research Themes

The Division is divided into three branches:

Biomarkers and Alternative Models Branch
Finds new translational biomarkers to a) improve detection of safety concerns with drugs and other FDA-regulated products and b) improve the identification of disease onset and its progression to enable better medical intervention.

Innovative Safety and Technologies Branch
Develop and evaluate innovative in vivo and in vitro methods to evaluate drug toxicity, develop analytical methodologies to advance the identification of foodborne pathogens and chemical adulteration, and develop models to enhance diagnostic procedures.

Personalized Medicine Branch
Determines the impact of differences in the responses of species and human sub-populations on current assessments of drug safety and efficacy.

2018 Select Accomplishments

Biomarkers of Doxorubicin-Induced Heart Injury
The use of a potent chemotherapeutic drug, doxorubicin (DOX), is restricted because of the risk of heart damage in cancer patients and survivors. In one study, a mouse model of DOX-induced heart injury developed at NCTR was utilized and transcriptomics analyses identified two proteins (NOTCH1 and vWF) that were elevated in plasma prior to the release of cardiac-specific injury marker, troponin T, and development of pathology in the heart. Increased level of both proteins was mitigated when toxic effects of DOX were diminished in the heart in mice that received a cardioprotective drug, dexrazoxane, suggesting these proteins as candidate early markers of DOX cardiotoxicity. These early protein markers of DOX-induced heart injury with potential applications in the clinic for monitoring and/or predicting cardiotoxicity induced by DOX will help design more effective treatment regimens. These results have been published in *Toxicology and Applied Pharmacology*.

Inter-Individual Heterogeneity Among hiPSC-CMs Reponses to Kinase Inhibitors
While human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide unprecedented opportunities for examining the cardiac effects of drugs on heart cells in vitro, commercially-available hiPSC-CMs are typically derived from single donors. A team of scientists from NCTR, the Arkansas College of Osteopathic Medicine, and the Medical College of Wisconsin hypothesized that hiPSC-CMs derived from different individuals would display heterogeneous sensitivities to kinase inhibitors, drugs used in cancer therapy that also can cause heart problems. The team reported at the annual meeting of the American Heart Association that following drug exposure,

they observed cell-line and drug- dependent differences in cell beating rate and toxicity. Their results strongly suggest that inter-individual differences impact hiPSC-CM cardiotoxicity assessments and support the need to test multiple cell lines during in vitro toxicity screens. The results of the study were presented at the 2018 annual meeting of the American Heart Association.

Effect of Diet on Drug Prevention of Mammary Cancer

Efforts have been underway to explore if various drug treatments might prevent breast cancer. In one of these studies, using rats as a model, the effect of diet on these treatments was examined. Scientists from NCTR collaborated with those from the National Cancer Institute to analyze the metabolic changes induced by a standard diet and a high fat diet (HFD) with and without treatments. The HFD significantly increased the number and size of tumors and had significant impact on the serum metabolites. These results have been published in *Cancer Prevention Research*.

Development of a Mouse Testis Organ System

The potential for medicines to have adverse effects on male reproductive capacity remains a concern in drug development. While animal tests have been useful in assessing the risk new drugs might have, faster methods would be desirable. At the meeting titled "FutureTox IV Progress to Maturity: Predictive Developmental and Reproductive Toxicology for Healthy Children," scientists from NCTR and CDER presented results of a new, in vitro assay where a mouse testis organ system is used to examine the toxicity of chemicals. Further work is planned to refine this system.