NCTR 2020 ANNUAL REPORT

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH (NCTR)

To learn more about NCTR, visit

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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 protect and promote the health of the American public.

NCTR holds a unique and foundational position at FDA because it is the only center that supports all FDA offices and product centers with the essential toxicological research and scientific data they need to conduct their regulatory activities. NCTR's work has been critical to the development and evaluation of emerging toxicological methods and other new technologies that play such a large role in FDA's regulatory decision-making. Over the past year, NCTR staff have continued to generate and evaluate research data in the Nation's fight against COVID-19. More than 20 research projects have been developed and initial progress has been accomplished to describe rapid testing approaches for SARS-COV-2 and related viruses, evaluate the developmental effects and ethnic disparities of viruses, prioritize antiviral therapeutic repurposing, and understand how new variants will affect vaccine efficacy.

We invite you to learn how NCTR scientists are supporting the agency in generating essential data and advancing the innovative tools and approaches that are vital to FDA's predictive capability and our ability to predict risk and efficacy.

/s/

William Slikker, Jr., Ph.D.

Director, National Center for Toxicological Research

Click to listen to Dr. Slikker introduce the Global Summit on Regulatory Science 2020.

NCTR's Vision, Mission, and Research Goals

Thank you for your interest in NCTR! The report highlights NCTR's accomplishments for the 2020 calendar year. Throughout the document are links to other sections of the report denoted with a page number, links to NCTR pages on FDA.gov, and various scientific publications referenced.

Vision, Mission, and Goals

Vision

Solving FDA's needs with high-quality research and by serving as a global resource for collaboration, training, and innovative scientific solutions.

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.

Research Goals

1. Advance the scientific knowledge and research data required to support public health
2. Develop and evaluate the next generation of science and emerging technologies
3. Address emerging public health challenges, such as COVID-19
4. Collaborate with FDA product centers and ORA to address issues of regulatory concern
5. Promote global outreach and collaborative research

NCTR at a Glance

Evolving Scientific Areas

- Artificial intelligence (e.g., machine learning, text mining, in silico modeling)
- Nanotechnology
- Microbiome and host interactions
- Microorganism detection in food, tobacco, and biological products
- Microphysiological systems, organotypic models, and stem cells
- Omics (genomics, metabolomics, proteomics, epigenetics)
- Perinatal and maternal health
- Translational and precision medicine

NCTR Expertise

- Analytical chemistry
- Antimicrobial resistance and pathogenicity
- Behavioral assessments
- Bio-Imaging (MALDI, MicroPET, MRI, MRS, CT)
- Bioinformatics and biostatistics (data mining)
- Biomarker development
- Genetic toxicology assay development
- Neurochemistry
- Neuropathology
- Physiologically-Based Pharmacokinetic (PBPK) modeling
- Reproductive and developmental toxicology
- Virology

NCTR identifies new biomarkers of toxicity using traditional approaches and innovative genomics, metabolomics, proteomics, epigenetics, and imaging technologies and approaches.

Facilities

>1 million square feet across 30 buildings

- Nanotechnology Core Facility
- Inhalation Core Facility
- Bio-Imaging Facility
- Animal Facilities

>100 experimental laboratories operate on NCTR's research campus

>75 Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) laboratories

NCTR Leadership and Organization Structure

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

Tucker Patterson, Ph.D.
Deputy Director for 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 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

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

John Talpos, Ph.D.
Acting Division Director, Neurotoxicology

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

Diversity, Equity, and Inclusion at NCTR

NCTR considers diversity integral to the success of our research mission and one of our greatest strengths. A diverse and inclusive workforce positively affects operational performance and employee engagement, and is critical to maintaining a strong, effective, and high-performing organization. In 2020, NCTR made significant strides in this effort by strengthening engagement with supervisors and employees through HQ-led training sessions that promote equal opportunity, raise awareness of unconscious bias, and prevent discrimination and harassment in the workplace. Our other successful initiatives included:

- ***Recruitment and outreach materials*** revised to more effectively communicate NCTR's inclusive work environment with the goal of attracting more diverse talent.
- ***"Meet and Greet" sessions with senior leadership*** added to the onboarding process for a more inclusive experience.
- ***Diversity and Inclusion Committee*** sponsored several diversity events and commemorative programs designed to support and cultivate an inclusive culture.
    - **Dr. Martin Luther King, Jr. Luncheon** hosted in collaboration with Blacks in Government.
    - **Lead with Pride campaign** held virtually to recognize NCTR's growing LGBTQ+ community.
    - **Faces of NCTR** launched during Hispanic heritage month – a virtual newsletter highlighting minority NCTR staff.
- ***Partnership with Florida A&M University's (FAMU) HBCU College of Pharmacy and Pharmaceutical Sciences*** strengthened our outreach to historically black colleges and universities (HBCUs) and allowed students and recent graduates working at NCTR to share research opportunities with FAMU faculty members and deans.
- ***Participation in National HBCU Week and Virtual Conference*** strategically positioned NCTR to recruit at the nation's premier gathering of students and stakeholders of America's HBCUs where 1800+ participants from 100+ HBCUs were represented.

Safety at NCTR

NCTR Regulatory Compliance and Risk Management (RCRM) staff report directly to the NCTR Director and are responsible for the overall safety and security of the Jefferson Laboratories. RCRM's mission is to ensure that the research conducted at NCTR is compliant with state and federal regulations and to assist in the assurance of quality and integrity of the research data.

The RCRM staff are responsible for the following:

- Conduct comprehensive risk assessments of research protocols to ensure regulatory compliance in chemical, biological, radiological, and environmental safety.
- Maintain inventories of hazardous chemicals and biological materials.
- Monitor employees under the medical and animal surveillance programs.
- Provide necessary job-related training to employees.
- Oversee campus security, records management, archiving, and quality assurance.
- Support FDA Office of Laboratory Safety (OLS) providing consistent and standardized guidelines, policies, procedures, and training across FDA.
- Support the FDA Employee Safety and Occupational Health program organized under the Office of Facilities Engineering and Mission Support Services by providing occupational health and medical services to all government and contract employees as well as research training program participants.
- Provide real-time data to OLS on lab safety inspections, safety training, employee occupational health and accident investigations/reports, and environmental safety programs.
- Review and test a variety of policy guidance documents for OLS, such as safety training modules, lab moving guide, animal biosafety manual, lab-safety data initiative, guidelines for working in lab/vivarium during the COVID-19 pandemic, and FDA's Contact Tracing and Return-to-Facilities programs.

RCRM agency committee/working group membership includes:

- Environmental safety and health council
- COVID-19 contact tracing program
- Institutional biosafety committee
- Institutional scientific collections working group
- Lab safety and security council
- Security operations and emergency management continuity of operations
- Good laboratory practices, quality resource and guidance, and human subject protection/bioresearch monitoring
- Records liaison and reports clearances

Manuscript Submissions and Newsletter Subscriptions

Number of NCTR Publications (2019–2020)

2020 – 132

2019 – 134

One of the ways NCTR measures research performance is by tracking how much NCTR research is being published in reputable scientific journals. Above are the publication numbers for 2019–2020.

NCTR Research Highlights and Science Insights

NCTR also measures its research performance by tracking the number of stakeholders who subscribe to:

- **NCTR Research Highlights** — brief summaries of NCTR research accomplishments and activities, research publications, and special events.
- **NCTR Science Insights** — a newsletter sent periodically with a more comprehensive summary of NCTR research activities, accomplishments, collaborations, and special events.

Total Subscriptions

*December 2020***

Total Subscriptions – 89,704

Research Highlights – 47,416

Science Insights – 42,288

December 2019

Total Subscriptions – 91,918

Research Highlights – 48,807

Science Insights – 43,111

December 2018

Total Subscriptions – 86,311

Research Highlights – 46,210

Science Insights – 40,101

***In May 2020, many unreachable email addresses were removed from the contact lists to prepare for the platform switch from Eloqua to govDelivery in September 2020. Periodic cleaning of the lists helps to improve email deliverability and overall reachability.*

If you are interested in subscribing to NCTR Research Highlights and/or Science Insights, please visit our website and subscribe with your email address.

FDA-TRACK

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 in FDA-TRACK.

Explore the progress NCTR is making towards its strategic priorities.

Science Advisory Board to NCTR

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 or her 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.

2020 SAB Meeting

NCTR's 2020 SAB meeting was held virtually in August 2020 over two days and covered a variety of topics including:

- Overview of NCTR
- Overview of NCTR Research Divisions
- NCTR Division of Neurotoxicology Review
- NCTR Collaborations with FDA Centers

Click to listen to SAB Day 1.

Click to listen to SAB Day 2.

More information on the 2020 SAB meeting can be found on our website.

NCTR Science Day at White Oak

The first annual NCTR Science Day was held on March 6, 2020, where over 30 NCTR scientists traveled from Arkansas to FDA's White Oak campus for the event. The goals for NCTR Science Day were to give product-center leadership and scientists the opportunity to:

- Discover the range and scope of NCTR's cutting-edge research.
- Interact and network with NCTR leadership and research staff.
- Explore potential avenues for collaboration and greater alignment with center scientific priorities.

Dr. William Slikker, NCTR Director, provided a brief overview of NCTR's capabilities and approaches to enhance collaborative research across all of FDA to the 200+ attendees. He then introduced FDA Chief Scientist, RADM Denise Hinton, who provided examples of ongoing collaborations between NCTR and other FDA centers and offices. The directors of NCTR's six research divisions and other research units provided examples of emerging technologies that are being developed and/or evaluated for use by FDA.

An award ceremony was conducted honoring collaborators on NCTR studies from across FDA, as well as reviewers of NCTR research proposals. Over 100 certificates were awarded to FDA scientists for their support of NCTR collaborative research efforts. During the poster sessions, NCTR principal investigators shared how they are advancing the tools and approaches that are vital to FDA's ability to predict risk and efficacy.

NCTR Collaborations

NCTR Collaborative Projects – 2020

CBER – 8%

CDER – 42%

CDRH – 10%

CFSAN – 9%

CTP – 4%

CVM – 5%

ORA – 1%

Other (Academia, Industry, Other Government Organizations, etc.) – 21%

***Collaboration data collected for January - December 2020 as of February 5, 2021.**

211 of 270 (78%) of NCTR ongoing projects are collaborative.

168 of 270 (62%) are collaborative with other FDA Centers/Offices.

45 of 270 (16%) are collaborative with organizations outside of FDA.

NCTR encourages the research community to reach out to NCTR Principal Investigators to explore collaborative research opportunities. To find potential collaborators by research area, Search All Bio-Sketches Using "Find."

The coming pages will discuss collaborative projects between NCTR and other centers or organizations. There are many acronyms and abbreviations, so we have provided a key below.

FDA Product Centers and Offices that Collaborate with NCTR

- Center for Biologics Evaluation and Research (CBER)
- Center for Devices and Radiological Health (CDRH)
- Center for Drug Evaluation and Research (CDER)

- Center for Food Safety and Applied Nutrition (CFSAN)
- Center for Tobacco Products (CTP)
- Center for Veterinary Medicine (CVM)
- Office of the Commissioner (OC)
- Office of the Chief Scientist (OCS)
- Office of Regulatory Affairs/Arkansas Labs (ORA/ARKL)
- Office of Women's Health (OWH)

Organizations Outside of FDA

- Organization for Economic Co-operation and Development (OECD)
- National Institutes of Health (NIH)
  - National Center for Advancing Translational Sciences (NCATS)
  - National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/ NTP)
- National Institute for Occupational Safety and Health (NIOSH)
- University of Arkansas for Medical Sciences (UAMS)

The heart of NCTR is its research staff who are dedicated to advancing regulatory science for FDA. NCTR scientists work independently and collaboratively across NCTR, across FDA, with academia, and with industry.

Global Summit on Regulatory Science

The **10th Global Summit on Regulatory Science (GSRS20)** was held virtually September 28-30, 2020, co-hosted by NCTR and the National Center for Advancing Translational Sciences. The theme for the 10th anniversary Global Summit was “Emerging Technologies and Their Application to Regulatory Science—A Global Perspective.”

It included over 60 presentations and 2 workshops, speakers from 14 different countries, and scientific leadership from FDA, National Institutes of Health, European Food Safety Agency, Joint Research Centre, Canadian Food Inspection Agency, National Institutes for Food and Drug Control, and National Institute of Health Sciences—to name just a few organizations. You can click on the links throughout the **GSRS20 program** to listen to the recorded scientific presentations and workshops on the following topics:

- Plenary Presentations from World Health Leaders
- Artificial Intelligence for Drug Development and Risk Assessment
- OMICS, Biomarkers, and Precision Medicine
- Microphysiological Systems and Stem Cells
- Bio-Imaging
- Microbiome
- Artificial Intelligence/Machine Learning
- Nanotechnology/Nanoplastics

The annual Global Summit is sponsored 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 co-chair of the Coalition’s executive committee and works with the Coalition to promote global interaction. The GSRS20 drew over 1,100 registrants from 51 countries representing regulatory and research institutes along with academic and industry participants.

Despite the worldwide challenges from the COVID-19 pandemic, the virtual GSRS20 was a huge success with in-depth scientific presentations on emerging technologies for regulatory application. A limited number of academic and industry presenters were invited to showcase cutting-edge research, successes, ongoing clinical trials, and challenges. A recording of the Q & A session where research presenters answered participants’ questions in real-time via chat is **available.**

Maternal and Perinatal Health

For more than 30 years, maternal and perinatal research has been a cornerstone of NCTR research. In this area, NCTR has worked collaboratively with product centers like CDER, external partners like the Mayo Clinic, and academic institutions like the University of Arkansas for Medical Sciences. NCTR research on pediatric anesthetics led to the FDA Drug Safety Communication that alerted the public to the possible negative effects of general anesthetics and sedation drugs on brain development in children younger than 3 years.

NCTR provides the additional infrastructure to stimulate robust research efforts through faster, less expensive, and more predictive approaches and models, leading the way to improved safety and/or efficacy of FDA-regulated products in these susceptible perinatal populations. Many drugs and other medical products provided to pregnant women, neonates, and infants are used off-label because of the difficulties with performing clinical trials required needed for drug approval in these populations.

The **FDA Perinatal Health Center of Excellence (PHCE)**, launched and managed by NCTR, focuses on the understudied perinatal period, spanning pregnancy, childbirth, and infant/child development. PHCE funds FDA research to close knowledge gaps about safety, efficacy, or potential toxicity that could exist during the perinatal period.

In 2020 the PHCE funded three new 2-year research projects. A total of 15 ongoing PHCE projects include principal investigators representing CBER, CDER, CFSAN, NCTR, ORA, CVM, and several universities/clinical institutions.

Current PHCE Research Topics:

- Neonatal immune responses to vaccines
- Computer-based pregnancy models
- COVID-19 effects on pregnancy, prenatal, and postnatal development
- Drug labeling associated with pregnancy
- Using Physiologically-Based Pharmacokinetic (PBPK) model to study depression treatment during pregnancy. p. 26
- Abuse-related effects of opioids. p. 37
- Effects of cannabidiol (CBD) exposure during development. p. 22
- Using the zebrafish model to explore how arsenic causes neurotoxicity. p. 35
- Evaluation of drug toxicity on placenta immunity. p. 38

A PHCE-funded project — focused on vaccines given to perinatal patients — that resulted in a review article in 2020 can be found in JBI Evidence Synthesis.

COVID-19 Response

FDA continues to be at the forefront of the United States' response to the challenges associated with COVID-19. ***In March 2020, NCTR was among the first within FDA to initiate COVID-19 research to develop an approach to rapidly determine the effectiveness of COVID-19 therapeutic treatments.*** Since the epidemic began, NCTR has taken many additional actions to support FDA's regulatory role. Below are a few examples of NCTR's COVID-19 response:

- NCTR leadership organized weekly/bi-weekly conference calls to conduct and complete research to address the COVID-19 pandemic. Over 100 participants met routinely to maximize collaborative efforts between FDA/NCTR researchers and those from regional university/clinical facilities with critical BSL-3 capability (p. 37).
- NCTR's **Division of Biochemical Toxicology** developed a method to detect SARS-CoV-2 RNA in wastewater and are applying it to selected metropolitan areas in Arkansas to monitor the presence and the extent of the COVID-19 epidemic. This method can help estimate viral spread at the community level without individual testing and may serve as an early warning tool for increased circulation of the virus.
- NCTR's **Division of Bioinformatics and Biostatistics** developed two AI-related projects:
    1. Using computational drug-repositioning principles with AI to systematically survey and prioritize approved or investigational drugs for their potential use to treat COVID-19.
    2. Developing AI-powered network pharmacology approach to comprehensively explore the opportunity of existing drugs for their potential to treat COVID-19 with over 3 million data points.
- Researchers in NCTR's **Division of Microbiology** are developing an approach to rapidly indicate effectiveness of COVID-19 therapeutic treatments.

Alternative Methods

FDA Alternative Methodologies Report – NCTR Achievements

FDA's Alternative Methodologies Working Group—co-chaired by Dr. Donna Mendrick, NCTR's Associate Director of Regulatory Activities—released the Advancing Alternative Methodologies report that focuses on Agency activities to improve predictivity of human and animal response to FDA-regulated products. FDA developed the Advancing Alternative Methods webpage, which hosts related publications, presentations, and a webinar series that enables developers to showcase their pioneering technologies to FDA scientists.

NCTR Alternative Methods Activities and Publications in 2020

Publications

- *Translational Toxicology*, a special edition for *Current Opinion in Toxicology* – Co-edited by Dr. Donna Mendrick and Dr. William Mattes (DSB); co-authored "Translational Toxicology: An Overview."
- "Biology-Inspired Microphysiological Systems to Advance Patient Benefit and Animal Welfare in Drug Development." *ALTEX*.
- "An FDA/CDER Perspective on Nonclinical Testing Strategies: Classical Toxicology Approaches and New Approach Methodologies (NAMs)." *Regulatory Toxicology and Pharmacology*.

FDA Alternative Intelligence Working Group (AIWG) Monthly Meetings

- Internal and external speakers — including the US Department of State, Columbia, MIT, and Stanford—discussed their work in AI.
- Established relationship with the Harvard-MIT Regulatory Center; basic AI course attracted >250 attendees.
- Chaired by Dr. Donna Mendrick, NCTR's Associate Director of Regulatory Activities.

Office of the Chief Scientist – Committee for the Advancement of Clinical and Scientific Education (CACSE)

- Developed lectures and workshops for FDA employees.
- Hosted speakers from University of Liverpool, University of California-San Diego, University of Connecticut, and Harvard Medical School.
- Hosted a joint educational initiative with the American Society of Clinical Pharmacology and Therapeutics—the William B. Abrams Lecture.
- Chaired by Dr. Donna Mendrick, NCTR's Associate Director of Regulatory Activities.

Nanotechnology

NCTR/ORA Nanotechnology Core Facility (Nanocore)

Anil Patri, Ph.D., Director, NCTR Nanocore

The Nanocore supports collaborative research efforts within FDA and with other government agencies and universities. This work provides information on issues related to nanomaterial characterization and safety of products containing nanomaterials in FDA-regulated products. This research data is also used in staff and reviewer training and standards development through stakeholder involvement for responsible development of nanotechnology products.

Current Nanotechnology Research Topics:

- Safety and efficacy of nanomaterials in drugs (with CDER)
- Toxicity of silver nanoparticles used in feminine-hygiene products (with OWH)
- Effect of silver nanoparticles on intestinal microbiota (with NTP)
- Epigenetic effects of titanium dioxide nanoparticles
- More nanotechnology information can be found in NCTR Research Highlights.

2020 NCTR Accomplishments

- **Two work items became ASTM standards**.

  Standard Practice for Performing Cryo-Transmission Electron Microscopy of Liposomes

  Standard Test Method for Quantitative Measurement of the Chemoattractant Capacity of a Nanoparticulate Material in vitro

- **Report on the current state of science in nanotechnology and progress at FDA** was published by the **Nanotechnology Task Force (NTF)**, chaired by Dr. Anil Patri.

  Click to read the NTF report.

- **FDA Grand Rounds seminar** highlighted the 2020 Nanotechnology Task Force Report.

  ***Click to listen to the Grand Rounds Nano seminar*.**

- **Nano Day Virtual Research Symposium: "A Decade of Progress and Innovation in Nanotechnology at U.S. FDA"** was organized by NCTR and hosted by the NTF and drew over 300 participants from around the globe. A list of the 20+ speakers is available in the **agenda.**

  Click to listen to the Nano Day seminar.

Bioinformatics

Artificial Intelligence (AI) advances bioinformatics tools, computer software, and data science, which provide FDA tremendous opportunities to modernize tools and technologies that assist in fulfilling the FDA mission.

NCTR has developed a variety of bioinformatics datasets and tools for public use. Among them, DILIrank is the largest publicly available annotated dataset of FDA-approved drugs for the study of drug-induced liver-injury (DILI) potential. Both the Endocrine Disruptor Knowledge Base (EDKB)—a database of about 3,000 chemicals that interfere with endocrine systems—and the Estrogenic Activity Database (part of EDKB) have been widely used by the research community and incorporated into larger government projects.

FDALabel

FDALabel is a publicly-available web-based application used to perform full-text and customizable searches of over 130,000 human prescription, biological, over-the-counter (OTC), and animal-drug labeling documents. In 2020, over 19,000 unique users accessed FDALabel 41,000 times. *Nature Biotechnology* and *Drug Discovery Today*.

Database of Pharmacogenomic Information in Ethnic Minority Populations

NCTR and CDER scientists, in collaboration with Virginia Commonwealth University, developed and published the Database of Pharmacogenomic Information in Ethnic Minority Populations (dbPGxEMP) and related research methods. The publicly available database includes information on nonsynonymous single nucleotide polymorphisms (SNPs) that cause frameshift, missense, or nonsense only. In the current database version (version 1.0), 42 drugs, 16 PGx biomarkers, and 67 SNPs with allele frequency information are included. Oncology drugs and their related biomarkers are excluded from this database. The database will be updated quarterly.

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. Throughout 2020 and continuing, NCTR has used the latest genomic and bioinformatic approaches to determine the interactions between the human microbiome and antimicrobial agents, nanomaterials, food contaminants, and FDA-regulated products.

Microbiome research spans microbiology, toxicology, nutrition, immunology, and antimicrobial resistance (AMR) because animal and environmental microbiota play important roles in states of health and disease.

Human Microbiome and Antimicrobial Resistance Research at NCTR

- The human gastrointestinal tract is colonized by beneficial microorganisms — also known as the microbiome — that help prevent infection. Because antimicrobials change the population of the microbiome, they may worsen disease and promote the emergence of antimicrobial-resistant bacteria. Data from NCTR-Center for Veterinary Medicine (CVM) research **(**p. 33**)** in this area supported CVM-issued FDA guidance for industry that determined what fraction of veterinary-drug residue in the human colon remains biologically active.
- NCTR and CDER scientists are assessing how nanoscale-size titanium oxide and zinc oxide in sunscreens interacts with the skin microbiome. Cosmetics are used by over 95 million U.S. women who are 18+ years old and the use of nanomaterials in cosmetic products is rapidly increasing. There are potential risks and a lack of knowledge about the effects of nanoscale materials on human-skin microbiota, making this a critical area of research. This study will enhance FDA’s scientific understanding of the safety and toxicity of nanomaterials in cosmetics and provide data for safety assessments.
- NCTR scientists reported sex-dependent effects of silver nanoparticles on the gut-associated immune status and intestinal-barrier function using ex vivo human-intestinal tissues. The same ex-vivo model was also used to perform a safety assessment of carbon-based nanomaterials; a project initially funded by a Broad Agency Agreement (BAA) between the Arkansas Research Consortium in Nanotoxicity and FDA.
- NCTR and UAMS researchers studied the sex-dependent effects on liver inflammation and gut microbiome **(**p. 33**)** that is associated with disease (dysbiosis) after continuous developmental exposure to the environmental contaminant trichloroethylene.

Opioids and Cannabinoids

Opioid Addiction

Opioid addiction and related deaths remain a serious crisis in the US, and FDA is particularly concerned with evaluating new pain-relieving medications for addiction potential. NCTR's Division of Systems Biology has developed a set of unique computational models to assess the structure of addictive chemicals.

NCTR research continues to use imaging technologies to reveal the brain mechanisms of the abuse-related effects of opioids. While it has been suggested that multiple neurotransmitters play a role, a comprehensive analysis of these effects in response to opioids has yet to be established. It is hoped that imaging technologies may help explain an opioid's mechanism of action. Preliminary study results were presented at the Society for Birth Defects Research and Prevention's Annual Meeting in June 2020. The study is funded by the PHCE and is being conducted in collaboration with CDER scientists.

Cannabidiol (CBD) Use and Exposure

In collaboration with FDA's Office of the Chief Scientist, researchers in NCTR's Division of Neurotoxicology are studying the developmental neurotoxicity effects of CBD. The scientists will examine the effects of CBD exposure during development. This study focuses on the effects of CBD on the developing brain and immune system. Effects from this early exposure will be evaluated through adolescence and into adulthood, which will create valuable information that currently does not exist or is unavailable publicly. NCTR expects that the information generated by this study will aid in guidances about the safe use of CBD and to help the public make healthy decisions regarding the use of CBD-containing products. This research is projected to be completed in 2023.

NCTR is also performing CBD research in collaboration with CFSAN and CVM via the CBD Policy Workgroup (led by the Office of the Commissioner). These recently initiated projects cover a variety of topics related to CBD, such as:

- Evaluation of male reproductive toxicities induced by CBD.
- Pharmacokinetics (movement of a substance within the body) of CBD via skin exposure.
- Pharmacokinetics of CBD via oral exposure.

Tobacco Products

The collaboration between Center for Tobacco Products (CTP) and NCTR provides the research data needed for 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 CTP/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 research outcomes provide data to better understand and quantify the adverse health risks associated with humans using tobacco products, thereby supporting the FDA mission of regulating tobacco products.

Predictive Modeling

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, including electronic nicotine delivery systems (ENDS).

2020 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 or office pages on FDA.gov.)

NCTR Research Divisions and Offices

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 — John Talpos, Ph.D., Acting Division Director

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

Scientific Coordination — Bradley J. Schnackenberg, Ph.D., Associate Director

Division of Biochemical Toxicology

About the Division

The Division of Biochemical Toxicology (DBT) conducts fundamental and applied research specifically designed to define the biological mechanisms of action underlying the toxicity of products regulated by, or of interest to, the centers of the FDA. This research is focused on measuring the toxicities and risk of cancer related to specific chemicals and the introduction of new 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 studies focused on the understanding of toxicological endpoints. This approach allows greater confidence in the subsequent risk assessments.

Select DBT Accomplishments in 2020

Analyzing Arsenic Toxicity

To address questions regarding arsenic toxicity, NCTR scientists performed mode-of-action analysis, investigating ways that glutathione affects the metabolism and disposition of arsenic, beginning with transport from the intestine and liver to other organs like lung, kidney, and bladder, and then oxidation to the excreted arsenic metabolite. Studies of arsenic metabolism and disposition such as this emphasize the link between A) metabolic activation vs. excretion of arsenic and B) the disruption of critical cellular thiol-based regulatory processes that define the human and animal dose-response characteristics of disease which are the basis for risk assessment. This study links the reductive potential of glutathione with the metabolic activation, transport, and elimination of various forms of arsenic in the body to further define the associations between dietary exposure to various forms of arsenic and the development of important human diseases. Publication is available in *Environment International*.

Examining PFAS-Based Compounds in Various Foods, a 2020 PHCE-Funded Study

Scientists from NCTR and Center for Food Safety and Nutrition (CFSAN) performed Pharmacokinetic (PK) evaluation of 6:2 fluorotelomer alcohol (6:2 FTOH)-based per- and polyfluorinated alkyl substance (PFAS), which is a degradation product of polymers used as stain-, water-, and greaseproof coatings that can migrate to foods, to examine the potential for its metabolites to persist in rat plasma, liver, and fat. Results of this PK analysis are the first to characterize the potential for persistence of the 5:3 acid metabolite in rat tissues after 90 days of repeated oral exposure to the parent compound 6:2 FTOH based on steady state PK parameters, and therefore, may inform future toxicity assay study designs to evaluate these and similar compounds. Results also showed that the time to steady state of the metabolite 5:3 acid in plasma, liver, and fat was approximately 1 year which is significantly longer than the 90-day toxicity studies currently available, suggesting that toxicity studies of at least 1-year duration are needed to evaluate any long-term systemic effects due to persistence of the 5:3 acid metabolite following repeated oral exposure to the 6:2 FTOH parent compound. This PHCE-funded study was published in *Toxicological and Applied Pharmacology* and informed this regulatory action by CFSAN.

PBPK Modeling Used to Study Depression Drug in Pregnant Women

NCTR and CDER scientists developed a comprehensive physiologically-based pharmacokinetic (PBPK) modeling framework with a user-friendly interface using sertraline, a drug commonly used to treat depression in pregnant women. PBPK modeling has been a valuable asset for regulatory science and can become a powerful tool in the hands of clinicians. It is especially helpful for drugs used in pregnancy since these drugs cannot be tested for safety and efficacy during pregnancy due to ethical concerns. The approach that was followed can be adapted to predict the change in plasma concentrations of other drugs across increasing gestational age, allowing dose adjustments as the pregnancy progresses. Although the model has limitations, it lays a foundation to encourage prediction-based approaches to dosing in pregnancy in a user-friendly and real-time manner. The PBPK model was converted to a prototype web-based interactive dosing tool to demonstrate how the output of a PBPK model may translate into optimal sertraline dosing in pregnancy. Quantitative prediction of drug exposure using PBPK modeling in pregnancy will support clinically appropriate dosing and increase the therapeutic benefit for pregnant women. Publication available in *npj Systems Biology and Applications*.

Investigating Role of Precision Medicine in Triple-Negative Breast Cancer

Researchers at NCTR collaborated with CDER, University of Tennessee Health Science Center (Memphis, TN), and University of Port-Harcourt (Port Harcourt, Nigeria) to investigate the role of vorinostat and indole-3-carbinol (I3C) on regulating critical receptors that are not normally expressed in triple negative breast cancer (TNBC), which is one of the most aggressive sub-types of breast cancer often with poor prognosis. Understanding whether ER, PR, or HER2 receptors can be reactivated in specific subtypes will greatly enhance understanding of epigenetic regulation in these highly deadly cancers and can, thus, expand the treatment potential of the currently approved targeted therapies for these subtypes of TNBC. The effect of treatments using vorinostat as an epigenetic drug and indole-3-carbinol as a dietary agent were assessed alone or in combination in cells representing different TNBC subtypes to determine if re-expressing critical receptors could increase the cells' sensitivity to targeted therapies. Precision medicine is extremely important, and molecular profiling of patients' tumors may prove in the future to be one of the most effective ways for handling aggressive cancers, such as TNBC. Paper was published in *Anticancer Research*.

Division of Bioinformatics and Biostatistics

About the Division

The Division of Bioinformatics and Biostatistics (DBB) develops integrated bioinformatics and biostatistics capability such as Artificial Intelligence (AI) and Machine Learning (ML), molecular modeling, real world data analytics, and chemoinformatics to address increasing needs of FDA in the areas of drug safety, drug repositioning, biomarker development, precision medicine, genomics, rare diseases, endocrine disruptors, and risk assessment. Additionally, the division provides support at NCTR relating to 1) IT infrastructure, 2) data analytics for research, 3) managing commercial and in-house software tools, and 4) conducting training sessions on bioinformatics tools.

DBB Research

The division's mission is to ensure that its activities relate to FDA's review process, its work with product centers continue to be strengthened, and its capabilities evolve to meet the current and future needs of FDA. In 2020, approximately 30 peer-reviewed articles were published despite the adversities caused by the pandemic. The division focused on these areas of research in 2020:

1. **COVID-19**—repurposing existing drugs for potential therapeutic options with bioinformatics which is funded by the FDA Medical Counter Measure Initiatives (MCMi).
2. **AI**—developing Natural Language Processing for FDA documents to support FDA review.
3. **Hepatotoxicity**—supporting the FDA review process with in-house computational models for drug-induced liver injury (DILI).

The division is equipping itself to better address emerging technologies such as AI. This directional shift is prudent to prepare FDA for technological advances in this area now and in the future.

Select DBB Accomplishments 2020

Biostatistics Branch

- SS Pfizer Paper of the Year Award in the Computational Toxicology Section, Society of Toxicology, "Integrating Adverse Outcome Pathways (AOPs) and High Throughput In Vitro Assays for Better Risk Evaluations, a Study with Drug-Induced Liver Injury (DILI)."

Scientific Computing Branch

Worked hand-in-hand with the Office of Information Management and Technology (OIMT) to accomplish the following tasks for NCTR:

- Supported and gathered requirements for updates to and replacements for existing applications.
- Collaborated with Division of Microbiology to develop virulence and plasmid databases and analysis tools.

- Reviewed potential image analysis software with the Nanocore and created a prototype image repository.
- Researched ML and AI algorithms for image analysis to enable support for future research protocols.
- Provided support for the CDER training application, *TrainTrack*.
- Provided support and training for users of the Multispecies Behavior System at NCTR, Arkansas Children's Hospital, Mt. Sinai Health System, and in Mexico City.
- Represented NCTR on the Scientific Computing Board, the Technology Council, and nine other working groups and committees.
- Assisted with over 50 ERIC tickets, installed over 120 computers, assisted OIMT with replacing aging laboratory instrument computers, provided remote and onsite IT and scientific computing assistance to customers during the pandemic.
- Ensured completion of the VOIP implementation and training.
- Worked with OFEMS and the OAGS contract specialist on the Jefferson Labs Distributed Antenna System RFI and submitted the requisition package.

R2R

- Designed and implemented a new Data Analysis and Search Host (DASH) consult process
- Released four new smart template modules in the new production version of PharmTox IND Smart Template system.
- Integrated with CDER NEXUS.
- Released a new production version of the Safety Policy and Research Team (SPRT) system.
- Developed a novel deep-learning approach to identify drug labeling through integrated analysis of image and text embedding. This approach has the potential to enable efficient and accurate product recognition through digital visual screening of product packages. *BMC Medical Informatics and Decision Making*.

Select Projects in 2021

- Opioid Agonists/Antagonists Knowledgebase (OAK) to Assist Review and Development of Analgesic Products for Pain Management and Opioid Use Disorder Treatment
- Artificial Intelligence (AI)-Powered Drug Repositioning for Treating COVID-19
- Development of a Comprehensive Open Access Molecules with Androgenic Activity Resource (MAAR) to Facilitate Risk Assessment of Chemicals
- DeepDILI: Benchmarking and Comparing Computational and Genomic Predictive Methods for Toxicity for Drug Induced Liver Injury (DILI) Using AI Based Methods
- Bioinformatics and Computational Chemistry Supports to CDER Reviewers
- An Integrative Model for Drug-Induced Liver Injury with High Throughput Assays and Real-World Evidence

- Development of a Reproducible Workflow to Analyze Real-World Incomplete or Uncertain qPCR Data
- Advanced Semantic Indexing and Searching for Tobacco Applications (ASSIST4Tobacco)
- The Database of Pharmacogenomic Information in Ethnic Minority Populations (dbPGxEMP)

Division of Genetic and Molecular Toxicology

About the Division

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

Select DGMT Accomplishments in 2020

Using TK-6 Cells to Evaluate Pyrrolizidine Alkaloid-Induced Genotoxicity

- Engineered TK-6 cells to express 14 different human cytochrome P450s for screening genotoxicity of xenobiotics. Using these metabolically competent TK-6 cells, scientists were able to evaluate pyrrolizidine alkaloid-induced genotoxicity. Project collaboration with NIEHS and CVM. *Toxicological Sciences* and *Food and Chemical Toxicology*.

Expertise in Common Regulatory Genetic Toxicology Assays

DGMT scientists on the CDER Genetic Toxicology Subcommittee of the Pharmacology/Toxicology Coordinating Committee are consulted on a regular basis for evaluation of genetic toxicology data. Division members have hands-on expertise in conducting all of the common regulatory genetic toxicology assays (e.g., Ames, TGR, Comet and *Pig-a*):

- Performed high-throughput CometChip assays in primary human hepatocytes (PHH) and two in vitro human hepatoma cell lines using 16 chemicals with different genotoxic modes of action. DNA damage responses among the three different cell types were compared and results demonstrated that 1) the CometChip is a viable high-throughput assay for PHHs and 2) PHHs had higher sensitivity than either HepaRG or Hep-G2 cells for detecting genotoxic carcinogens. Collaboration with CDER. *Archives of Toxicology*.
- Provided recommendations for conducting the rodent erythrocyte *Pig-a* assay to measure in vivo somatic cell mutation for regulatory purposes. Recommendations on best practices for designing and conducting rodent *Pig-a* studies in support of evaluating test substance safety are described in a report from the *Pig-a* Workgroup of the Health and Environmental Sciences Institute/Genetic Toxicology Technical Committee. Collaboration with Organization for Economic Co-operation and Development (project number 4.93).
- Demonstrated that procarbazine and N-propyl-N nitrosourea are rodent mutagens and induce *Pig-a* mutant phenotypes in red blood cells (RBCs). To prove the RBC mutant phenotype have *Pig-a* mutations, error-corrected next generation sequencing was performed on the precursors of RBCs—bone marrow erythroid cells (BMEs)—from rats exposed to these mutagens. The agent-specific mutational spectra in BMEs indicated that the rat RBC *Pig-a* assay scoring *Pig-a* mutant-phenotype RBCs in peripheral blood detects *Pig-a* gene mutation. Collaboration with CDER. *Environmental Molecular Mutagenesis*.
- Evaluated cytotoxicity and genotoxicity of cadmium oxide nanoparticles (CdO NPs) in vitro. CdO NPs are among the most studied and industrially used metal oxide NPs. The results showed that CdO NPs induced concentration-dependent cytotoxicity in TK-6 and HepG2 cells. In addition, CdO NPs were genotoxic in several assays, producing positive

responses in micronucleus, Comet, and mouse lymphoma assays. Collaboration with UALR, CVM, and ORA. *Mutatation Research*.

Using Models to Conduct Research on Lung Cancers and Respiratory Toxicants

- Developed methods for the preparation and growth of spheroids from primary human lung adenocarcinomas to evaluate erlotinib-resistant subpopulations in patient-derived lung tumor spheroids. The ex vivo lung tumor spheroid model mimicked the cellular and mutational tumor heterogeneity of human lung adenocarcinomas, indicating that spheroid cultures could be used to investigate the efficacy of lung cancer combination therapies. Collaboration with Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan. *PLoS ONE*.
- Used acrolein as a model toxicant in normal human lung bronchial epithelial cells and human lung cancer cells for evaluating the dosimetry and key adverse responses. The study suggested that the cell susceptibility to acrolein exposure is closely associated with acrolein uptake and formation of GSH-ACR adducts. The study concluded that dosimetry analysis might be useful for computational modeling and risk assessment of acrolein using different test systems. Collaboration with CTP. *Environmental Toxicology and Pharmacology.*
- Reviewed the properties and utility of human air-liquid-interface (ALI) organotypic airway tissue models derived from primary tracheobronchial epithelial cells for performing toxicological studies. The review indicated that ALI tissue models, because of their in vivo-like structure and functions when they fully differentiate, can be used for conducting in vitro exposures that mimic human respiratory exposures. It was suggested that although toxicity evaluations using ALI models require further validation, these models potentially can supplement or replace animal models for conducting research on respiratory toxicants and pathogens. Collaboration with NIOSH and NIEHS. *In Vitro Cellular & Developmental Biology - Animal*.

Cancer Driver Mutations (CDMs) as Biomarkers

- Reviewed the COSMIC database for mutational hotspots within the 20 most mutated genes across the 10 deadliest cancers. This survey was used to identify the most promising targets to investigate CDMs as biomarkers for use in cancer risk assessment. The review provided a roadmap for the development of tissue-specific, CDM-based biomarkers of carcinogenic potential, which could be measured by error-corrected next-generation sequencing. Using this information, they further developed and validated an error-corrected next-generation sequencing method, “CarcSeq”, to analyze panels of hCDMs. CarcSeq appears to be a promising approach to advance cancer risk assessment and carcinogenicity testing practices. *Environmental Molecular Mutagenesis*: January 2020 and September 2020.

Select Projects in 2021

- In collaboration with CBER, NCTR scientists received two years of PHCE funding to develop a human in vitro transplacental model of drug transport.
- Two collaborative projects with CBER received MCMi funding for:
  1. Continuing development of a microphysiological system for evaluating Zika virus sexual transmission using a non-human primate testicular model.

2. Development of a microphysiological system for evaluating antibody therapies targeting Zika viral infections during pregnancy.

Division of Microbiology

About the Division

The mission of the Division of Microbiology (DM) is to serve a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of FDA's responsibility in toxicology and regulatory science. To meet this mission, DM's projects involve multidisciplinary research approaches that address a variety of FDA issues with special emphasis on these division research themes.

Select DM Accomplishments in 2020

Food Safety and Virology: Antimicrobial Resistance and Virulence

- Developed and optimized genetic databases and matching algorithms to identify *Salmonella enterica* virulence factors and plasmid transfer genes from whole-genome sequence data that serves to improve the safety foods and feed. *Genes*.
- Researched the contributions of bacterial plasmids to spread antimicrobial resistance and how these plasmids can also contribute to increased virulence in bacterial pathogens. *Poultry Science*, *BMC Microbiology*, and *Microbiology Resource Announcements*.
- Developed spore preparation methods for *Bacillus spp*. which will aid in the establishment of standardized methods for sporicidal efficacy assessment that will support FDA's regulation of drug compounding.
- Produced recombinant Coronavirus spike proteins that are important resources for ongoing the evaluation of viral-host cell interactions and facilitate the assessment of antibody-dependent enhancement which can lead to deleterious disease outcomes.

Microbiome and Biological Interactions

- Studied the effects of antimicrobial drug and drug residues to determine the impact on the human gastrointestinal tract microbiota composition and intestinal permeability. Higher exposures were found to lead to alterations of the microbiota and intestinal permeability. A review article describing the dynamics of antimicrobial residues in food on the microbiota and an update on evaluating the safety in toxicology risk assessments is available in the *Journal of Veterinary Pharmacology and Therapeutics*.
- Assessed the impact of exposure of xenobiotic compounds including arsenic, bisphenol AF and triclosan on the microbiome and host responses through **National Toxicological Program** funded efforts. These efforts are informing risk assessments of the test compounds. *Toxicological Sciences*.
- Evaluated the impact of sex-related differences on microbial dysbiosis, inflammatory responses and epithelial cell permeability following exposure to xenobiotic compounds. *Frontiers in Pharmacology* and *International Journal of Molecular Sciences*.

Microbial Contaminants Detection

- Identified enhanced methods for the detection of *Burkholderia cepacia* complex (BCC) in pharmaceutical-grade water and antiseptics. Improved detection of BCC in products is a key public health concern due to disease outbreaks in susceptible individuals and immunocompromised patients. *Journal of Industrial Microbiology & Biotechnology*.

- Developed methods for the detection of microbial contaminants in tattoo and permanent make-up inks and completed two surveys on the microorganisms present. Organisms that have the potential to be opportunistic bacterial pathogens were identified in some products. *Letter in Applied Microbiology*.

- Validated new sample preparation procedures for the detection of *Salmonella* in spices. This method with its increased sensitivity has been incorporated into the FDA Bacteriological Analytical Manual (BAM) Chapter 5.

Division of Microbiology Science Advisory Board (SAB) Subcommittee Review

- Division scientists presented 13 talks and 10 posters highlighting select accomplishments of the division over the past 7 years and plans for the future.

- Click to listen to the Division of Microbiology SAB review.

Select Projects in 2021

- A Recombinant Coronavirus Spike Protein to Generate Reagents, Study Cell Interactions and Antibody-Dependent Enhancement
- Assessment of the Role that the Microbiome May Play in the Toxicity of Xenobiotics
- Studies on the Intrinsic Structural Multidrug Efflux Pump Mechanisms on Antimicrobial Resistant *Salmonella enterica* and Their Role in Antimicrobial Resistance
- Detection of Microbial Contaminants, including Anaerobic Bacteria, in Tattoo Inks and other Related Products
- Assessment of the Interactions of Nanoscale Materials used in Sunscreens on the Skin Microbiome
- Nonclinical Modeling and Risk Assessment of FDA-Regulated Drug-Nanocrystals
- Multi-Omics Approach to Identify an Antimicrobial Resistance Marker of *Staphylococcus aureus* Associated with Antimicrobial-Coated Medical Devices in a Biofilm Reactor
- A Plasmid Analyses Toolbox: Approaches and Tools to Efficiently Assess Antimicrobial Resistance and Pathogenicity-Related Functions of Plasmids in Bacterial Pathogens
- Development of in vitro Vaginal Tract Models to Assess the Biotherapeutic Potential of *Lactobacillus* Toward Toxic Shock Syndrome Toxin-1 Producing *Staphylococcus aureus*
- Comparative Methods Study for the Detection of *Burkholderia cepacia* Complex from Non-Sterile Pharmaceutical Products

Division of Neurotoxicology

About the Division

The Division of Neurotoxicology (DNT) focuses on understanding of the processes associated with neurotoxic outcomes—harmful effects associated with the brain and nervous system. Knowledge of these processes is used to perform neurotoxicological studies to aid the agency in decision making. 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.

Select DNT Accomplishments in 2020

Exploring How Arsenic Causes Neurotoxicity

A focus within the division is the developmental toxicity of inorganic arsenic exposure. Arsenic occurs naturally in groundwater and can find its way into various foods including rice, a popular ingredient in baby foods. Researchers at NCTR and CFSAN are assessing the neurotoxic potential of arsenic exposures. These findings will help to better inform FDA on acceptable levels of arsenic exposure in children. This work was performed in the rat, a powerful, but slow, predictive research model. Division researchers, in collaboration with PHCE, are using the zebrafish model to explore how arsenic causes neurotoxicity and will also evaluate the effectiveness of this model for future toxicity assessments. The zebrafish model may reduce the cost and duration of future toxicity assessments.

Blood-Brain-Barrier's Response to Alzheimer's Disease (AD)

A growing focus for the division is studying blood-brain barrier (BBB)-related neurotoxicity. The brain is a sensitive organ that exists in a micro-environment maintained by the BBB. Failure of the BBB will compromise this micro-environment and can lead to brain damage. Division scientists, in collaboration with the Office of Women's Health (OWH) and the NCTR/ORA Nanotechnology Core Facility, are studying how sex-linked differences in inflammation change the BBB's response to AD. Specifically, researchers are assessing the impact of sex on BBB function, disease progression, and cognitive function. NCTR's Divisions of Neurotoxicology and Microbiology are studying how AD can influence the stomach microbiome, and if associated differences in inflammation can impact the BBB. To complement these animal studies, division researchers are also starting an exciting series of studies using the "brain-on-a-chip" technology to better understand the mechanisms behind BBB dysfunction. These and other studies will help the division understand how disease and FDA-regulated products can impact the BBB and provide a way to study BBB-related neurotoxicity.

Assessing the Neurotoxic Potential of Ketamine in a Pediatric Population

For over a decade, the Division of Neurotoxicology has been leading the field of developmental anesthesia-related neurotoxicity research. In 2020, the division continued this rich tradition by performing formal toxicity assessments, working to understand the conditions that lead to toxicity, and describing the mechanisms of toxicity. DNT and CDER scientists are assessing the neurotoxic potential of ketamine in a pediatric population. The division is also working with CDER to understand how changes in lipid composition and microRNAs contribute to

anesthesia-related neurotoxicity. Moreover, researchers are using alternative in vitro models to investigate the toxicity of different anesthetic regimens. Through this work, the division seeks to understand the conditions needed for anesthesia-related neurotoxicity and better define the risk associated with general anesthesia in a pediatric population.

Select Projects in 2021

- Publication of an In-Depth Review of the Feasibility of SARS-CoV-2 Infection of the CNS, and an Assessment of the Validity of Various Animal Models to Study CNS Infiltration
- Studying Vascular Dysfunction in Brain of Two Transgenic Rodent Models of Alzheimer's Disease (AD): Dietary Impact and Relevance to Human AD
- Evaluation of the Correlation of Ethnicity-Related Neuroinflammation Differences in Alzheimer's Disease with ER Stress-Induced Endothelial Dysfunction in the Brain
- Implementation and Validation of a Rodent Model of Traumatic Brain Injury
- Behavioral and Neurochemical Evaluation of the Developmental Neurotoxicity of Inorganic Arsenic Exposure in the Rat
- Behavioral and Neurochemical Evaluation of the Developmental Neurotoxicity of Cannabidiol (CBD) Exposure in the Rat
- Multi-Year Assessment of Toxicity Associated with Long-Term Methylphenidate Use in the Non-Human Primate
- Quantifying the Importance of Reactive Oxygen Species (ROS) and Immunomodulation in Drug Effects — In Vivo Studies Using Zebrafish Embryos
- Utilization of Neural Stem Cell Models and Biomarkers in Assessing the Developmental Neurotoxicity of Pediatric General Anesthetics
- Non-Invasive Evaluation of Anesthesia Induced Apoptosis in the Rat Using microPET
- MRI Assessment of the Long-Term Effects of Perinatal Exposure to Gaseous Anesthesia in Adult Animals
- Evaluating the Contribution of Acute Hypoxia to Models of Early Life General Anesthesia in the Rodent
- Development of T2 MRI as a Biomarker of Neurotoxicity
- Evaluation and Application of Tissue Clearing Techniques for Neurotoxicity Assessments in the Rodent
- Collaborative Research Agreement with the Icahn School of Medicine at Mount Sinai to Study the Health Impact of Chemicals, Nutrition, and other Factors on Cognitive Ability Later in Life

Division of Systems Biology

About the Division

The Division of Systems Biology (DSB) focuses on the development and evaluation of new technologies and the identification of new biomarkers (disease indicators) to support FDA's mission. The division's mission is to address problems of food, drug, and medical-product safety using systems biology approaches and innovative technology.

Select DSB Accomplishments in 2020

Using Computational Models to Assess Structure of Addictive Chemicals

Opioid-addiction and related deaths remain a serious crisis in the US, and FDA is particularly concerned with evaluating new pain-relieving medications for addiction potential. NCTR scientists have developed a set of unique computational models to assess the structure of addictive chemicals. This project should create a better understanding of the structural requirements associated with a strong addiction potential and would allow an accurate prediction of this potential for opioids, cannabinoids, and other structurally diverse chemicals. This technology could be used to prioritize the testing of chemicals with strong addiction potentials (such as synthetic opioids and cannabinoids), thus shortening the FDA regulatory-review process. This project is expected to be completed in 2021. *Archives of Toxicology*.

DSB Scientists and CDER's Office of New Drugs Form Montelukast Working Group

Montelukast pulmonary therapeutics are used chronically by millions of Americans, primarily pediatrics, and are associated with concerns of neuropsychiatric events that were discussed at the September 2019 Pediatric Advisory Committee and Drug Safety and Risk Management Advisory Committee meeting. To address these concerns and coordinate research investigating drug-related neurological effects, a Montelukast Working Group (MWG) was formed between DSB scientists and CDER Pharmacology and Toxicology nonclinical reviewers in the Office of New Drugs. The MWG designed studies that will investigate potential mechanisms of central nervous system (CNS) effects, CNS exposure, drug accumulation potential, blood brain barrier transport, withdrawal and long-term sequelae with chronic montelukast administration. The cumulative scientific information obtained by this work will directly address the regulatory neuropsychiatric safety concerns, may impact labeling decisions for montelukast products, and will better inform healthcare providers and patients of the associated risks that should be considered for individual disease management and treatment action plans with chronic montelukast treatments.

Studies Examine the Effects of Anti-Cancer Agents on Mitochondrial Functions

The adverse reactions to many drugs, including effects to the liver and heart, are the result of their unintended interference with mitochondria, the microscopic powerplants of all cells. DSB completed the studies examining the effects of a series of anti-cancer agents (tyrosine kinase inhibitors) on mitochondrial functions. The drug pexidartinib was identified as a strong inhibitor of key mitochondrial functions at clinically relevant concentrations. This study suggests that examining drugs at an early stage of development for such effects can help predict potential

toxicities. The studies have been described in a review manuscript in *Expert Opinion on Drug Metabolism and Toxicology*.

In Vitro Model to Improve Prediction of Drug Cardiotoxicity

Cardiotoxicity remains a primary cause of drug withdrawal from the market due to the prevalence of adverse events not detected during drug development. Preclinical assessments to determine potential risks for cardiotoxicity are predominantly dependent on in vitro human gene assays and in vivo animal studies. However, to better predict cardiotoxic potential at early stages of drug development, an in vitro model using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to identify drug-related effects on myocyte function is being explored. As part of an ongoing collaboration with the Medical College of Wisconsin, the effects of various kinase inhibitors, with known cardiotoxicity in some patients, on hiPSC-CMs from multiple donors are being evaluated to determine if individual patient-derived cell lines can recapitulate the heterogeneity found in the population. Further study is being done to analyze the role of other functional parameters and gene expression to better understand the inter-individual variability. Initial results presented at national and international scientific conferences and a review article was published in *Current Opinion in Toxicology*.

PHCE Develops 3D Human Placental Barrier Model

A placenta has many tasks, including the transfer of nutrients and oxygen to the barrier. Between 50-70% of pregnant women take at least one medication. Through placental transfer, a fetus may be exposed to a drug that a mother was given, resulting in an increased risk of birth defects. However, current models for assessing toxicity of placental drug transfer do not mimic the placental transfer system in humans. To explore the similar human placental transfer model for assessing placenta toxicity testing, a study funded by PHCE is developing a human placental barrier model using a 3D microphysiological system and the translative correlation to in vivo data is being determined. The collection of in vivo measurement of drug concentration and Pharmacokinetic (PK) analysis has been completed at NCTR and a placenta-on-a-chip has been developed and deployed to evaluate in vitro drug concentration in media and PK analysis. A manuscript about placenta-on-a-chip and associated in vitro PK analysis is currently being written. A follow up study, “Evaluation of drug toxicity on placenta immunity using a microphysiological human placental barrier model,” has been funded by PHCE in collaboration with CBER.

Select Projects in 2021

Investigating Virus Inactivation to Enhance Safety and Design of Future Research Labs

Much is unknown about the SARS-Cov2 virus responsible for the devastating Covid-19 epidemic. Because of its infectious nature, research on this virus must be conducted in specialized containment laboratories (BSL-3). Samples from such research could be analyzed elsewhere if procedures could be established that definitively inactivated the virus. To address this question DSB initiated a project with the University of Tennessee Health Sciences Center (UTHSC) to investigate virus inactivation, using samples generated in UTHSC’s BSL-3 facility, subjected to different inactivation procedures, and then analyzed at NCTR laboratories. Results of this project will be used to inform the safety and design of future research.

Office of Scientific Coordination

The components of the Office of Scientific Coordination (OSC) that facilitate the FDA/ NCTR research mission are:

Research and Support Services

Veterinary Services

Veterinary Services staff play a key role in the research at NCTR, where they ensure the health and welfare of all animals used in research. The veterinarians participate in the review and monitoring of animal use through the Institutional Animal Care and Use Committee (IACUC). They participate in the research by advising study scientists regarding study design, performing surgery on animals, and monitoring the overall health of the animal program. Veterinary Services also includes the Microbiology Surveillance support staff. This facility has been Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited since 1977.

Microbiological Surveillance

Microbiological Surveillance staff ensure the animals, environment, food, bedding, and test articles are free from opportunistic pathogens. They are a resource for scientists investigating suspected contaminated cell lines. They support personnel health by microbiological testing of NCTR water and environmental samples.

Analytical Chemistry

Analytical Chemistry research and support are conducted using trained staff and state-of-the-art instrumentation for processing of a wide range of samples. Test articles and their metabolites are assayed in blood, tissues, or urine to provide measures of exposure, and for genotoxic compounds, DNA adducts are measured. The high quality of studies at NCTR is ensured by 1) verification of test-article identity and purity, 2) certification of concentration and stability of test articles in dosing solutions and vehicles, and 3) surveillance of animal study materials (bedding, water, and diet) performed routinely.

Statistics

The Statistical Support staff provides traditional statistical support for the various toxicity studies conducted at NCTR. The services provided include: statistical consultation during protocol development, statistical randomization, statistical analysis, and statistical reporting.

Experimental Support

Experimental Support staff provide computer-based support for animal studies. The staff reviews study protocols and works with research and support staff to enter study parameters in the animal data collection system, reviews data, and generates reports at the conclusion of the study.

Nanotechnology

NCTR and the Office of Regulatory Affairs (ORA) jointly operate and maintain the NCTR/ORA Nanotechnology Core Facility (Nanocore). This core facility is equipped with the appropriate

equipment and trained staff to properly characterize and detect nanoscale materials. The Nanocore has been designed to support research investigators with nanomaterial characterization, develop standard operating procedures for consistent measurement of the nanomaterials, and develop new procedures to detect nanomaterials in biological matrices in support of toxicology studies in vitro and in vivo.

Animal Care Contract

NCTR maintains an on-site contract with trained and proficient staff to provide study support including husbandry, environmental enrichment of all animals, formulation and administration of test articles, sample collection, and data collection. The contractor works with the Veterinary Services veterinarians and the IACUC to ensure the health and welfare of the animals.

Pathology Services Contract

NCTR maintains an on-site pathology contract for veterinary pathology and histopathology services. The contractor maintains a staff of three veterinary pathologists and a highly trained staff that provide NCTR with services including: clinical pathology, histopathology slide preparation, rigorous pathology examination, and complete histopathology and pathology reports for each study.

Equipment Maintenance and Repair Contract

NCTR maintains a contract for equipment maintenance and repair that supports the routine preventative maintenance and calibration of equipment, manufacture of minor equipment to support customized research needs, and repair of equipment that is not on a service agreement with the manufacturer.