NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

(Dollars in Thousands)	FY 2018 Enacted	FY 2018 Actual	FY 2019 Annualized CR	FY 2020	
				President's Budget	+/- FY 2019 Annualized CR
National Center for Toxicological Research (BA only).....	64,512	64,512	64,512	66,512	2,000
FTE.....	301	301	301	301	---

Authorizing Legislation: Federal Food, Drug, and Cosmetic Act (21 U.S.C. 393(b) (1)); Food and Drug Administration Modernization Act; Food and Drug Administration Amendments Act of 2007; FDA Food Safety Modernization Act (P.L. 111-353)

Allocation Methods: Direct Federal/Intramural

PROGRAM DESCRIPTION AND ACCOMPLISHMENTS

The National Center for Toxicological Research (NCTR) was established in 1971. As a national scientific resource, NCTR conducts peer-reviewed research to 1) Protect and Promote the Safety and Health of Families, 2) Foster Competition and Innovation, 3) Empower Consumers and Patients, and 4) Strengthen Science and Efficient Risk-Based Decision Making. NCTR enhances FDA's basis for science-based regulatory decisions by conducting collaborative research to:

- expedite the translation of laboratory findings to the clinic and regulatory application
- identify adverse effects earlier in product development and understand the risks and benefits of nanomaterials used in FDA-regulated products
- provide strategies to reduce and rapidly detect contaminants in FDA-regulated products
- use biomarkers—biological indicators of disease—to foster precision medicine
- accelerate FDA's capability to manage and analyze research data using bioinformatics
- reduce costly and dangerous surgeries by expanding minimally invasive imaging capabilities.

The following selected accomplishments demonstrate NCTR's delivery of its regulatory and public-health responsibilities.

Foster Competition and Innovation

Public health protection is the core driver of the NCTR research which aims to ensure that medical products are properly tested for safety and efficacy. NCTR conducts research to evaluate FDA-regulated products in a more predictable, consistent, and efficient way and is often sought as a collaborator and advisor due to its exemplary reputation in the research community. Within this area, examples of NCTR research include text mining to help facilitate efficient access to data that can help resolve public-health issues and participating in global scientific collaborations to foster and leverage novel approaches and research standards.

Antimicrobial Resistance (AMR) and the Human Microbiome

The CDC 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 AMR genes and bacterial

pathogens in feed, foods, clinical and environmental samples; and the potential effects of transmission of resistant bacteria on human health.

NCTR scientists have 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*[66].

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 human 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.

In FY 2018, NCTR published an article, in collaboration with CVM, related to antimicrobial drug residues and the human intestinal microbiome permeability in *Anaerobe*[67]. 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*.

Figure 12*Salmonella* is one of the most widely studied bacteria on Earth.

[66] For more information please visit: http://For more information please visit: https://www.sciencedirect.com/science/article/pii/S0168160518300345?via%3Dihub

[67] For more information please visit: https://www.sciencedirect.com/science/article/pii/S1075996417302342?via%3Dihub

In support of pathogen reduction, NCTR is starting a research study that explores and provides research data on how fecal microbial transplant is an effective treatment for bacterial infections such as *Clostridium difficile*.

Text Mining

Text-mining methods apply computation approaches to text for word recognition, frequency of use, and association—identifying similarities between documents, such as the words used. A simple example of text mining is the identification of e-mail messages containing certain words. Text-mining allows scientists to organize and search large datasets, many of which already exist, and may lead to finding new or hidden information that benefits public health.

NCTR scientists, as requested by CDER reviewers, are applying text-mining techniques including pattern-matching and natural-language processing to extract information from FDA approval letters for New Drug Applications and Biologic License Applications. A relational database and web-based application have been developed to host the information for better query, view, and analysis by FDA reviewers. NCTR scientists are also using pattern-matching and natural-language processing to map free-text drug indications to standardized nomenclatures used in approval letters and other regulatory documents. A description of this research effort was published in June 2018 and can be found in *Drug Discovery Today*.[68]

NCTR recently started a collaborative bioinformatics project with CDER to develop a database for the study of cancer predisposition in rare diseases. The study will investigate whether the genetic mutations from both cancer and rare diseases interact with the same functional protein domain and will look for correlation among the data. In other words, the database will theoretically detect if a person with a rare disease is more likely to develop cancer or vice versa.

In 2018, to enhance available bioinformatics tools, NCTR implemented technical assistance avenues. Additionally, other mechanisms, such as surveys and trainings, have been developed and implemented for FDALabel to improve the tool by collecting user feedback, usage data, and communicating directly with FDALabel customers.

Collaborations

A critical component of NCTR's and FDA's science portfolio is collaborations with other entities to leverage knowledge and to establish partnerships where expertise from each entity can contribute to regulatory-science research projects. 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 and provide FDA with access to cutting-edge science. Support of this important strategic priority is reflected in the following highlighted collaborations.

Global Summit on Regulatory Science

Because of the importance for international regulators, policy makers, and scientists to exchange views on how to develop and implement innovative methodologies into regulatory assessments, NCTR established an annual internationally renowned Global Summit on Regulatory Science. Now in its ninth year, the Global Summit's goal is to engage the global community and

[68] For more information please visit: https://www.sciencedirect.com/science/article/pii/S1359644617305858

harmonize research strategies via collaborations that aim to build knowledge of and promote regulatory science, define research needs, and seek to strengthen product safety worldwide by training regulatory scientists.

The Global Summit is led by the Global Coalition 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 2018 Global Summit on Regulatory Science was held September 26-27, 2018, in Beijing, China. The theme was Risk/Benefit of Dietary Supplements and Herbal Medicine in the Era of Data Science. The Summit had representatives from FDA and over 20 countries. The Summit reinforced the need for and initiation of scientific exchange and collaboration.

The 9th Annual 2019 Global Summit for Regulatory Science (GSRS19) will be held in Italy, in September 2019, at the Joint Research Centre – European Commission in Ispra. The meeting will focus on nanotechnology and nanoplastics. Additional details are forthcoming. For updates, abstract guidelines, agenda, and more visit www.fda.gov/globalsummit.

Bioinformatics Collaborations

NCTR and the Arkansas state university system held the fourth annual Arkansas Bioinformatics Consortium conference in April 2018 to leverage statewide bioinformatics capabilities. The conference—organized by NCTR, Arkansas Research Alliance, and the Arkansas Bioinformatics Consortium—focused on the "Data Analytics: Genomics and Beyond."

NCTR scientists led two of the four workshops and two of the breakout sessions at the 14th Annual Mid-South Computational Biology and Bioinformatics Society Conference. The theme of the conference was, "Make Them Safer Make Them Better: Bioinformatics and the Development of Therapeuticals." There were approximately 200 conference participants with 68 poster presentations, 62 oral presentations, and a wide array of guest speakers.

On March 20, 2018, two Research Collaboration Agreements (RCA) were signed between FDA/NCTR and the Food Safety Commission of Japan (FSCJ) to work on two joint research projects. Today's consumer products are increasingly globalized, impacting public health worldwide and posing new challenges for regulatory authorities. Descriptions of these two research projects are shown below:

- The first project will develop knowledge-based tools that can help international risk-assessment agencies who work on chemical toxicological prediction.
- The second project will provide the most appropriate tools for use in risk assessment of contaminants in food (e.g. acrylamide, furan, bisphenol A, and arsenic species).

NCTR conducted research, in collaboration with CDER on the development and evaluation of predictive models that can improve the assessment of drug-induced liver injury (DILI) risk during the Investigative New Drug (IND) phase. Scientists worked on DILI caused by bile acids for which CDER scientists have great interest based on their review process of new drug candidates. In collaboration with CDER, an article was published in *Alimentary Pharmacology & Therapeutics*. Another manuscript entitled "The influence of drug properties and host factors on delayed onset of symptoms in drug-induced liver injury" in collaboration with the Spanish DILI registry and Duke University was recently accepted by *Liver International*.

Nanotechnology Collaborations

The NCTR/ORA Nanotechnology Core Facility (NanoCore) supports collaborative efforts within FDA, other U.S. government agencies, and with university researchers providing analytical project support. NCTR and the NanoCore conduct regulatory science research for nanotechnology investigative projects with FDA Product Centers CDRH, CDER, CFSAN, CVM, and ORA. This work informs FDA and other U.S. government agencies on the toxicity and safety of nanotechnology-based materials. Nanocore develops standards in collaboration with the National Toxicology Program and international standards development organizations to ensure data quality and minimize iterations for industry submissions, speeding up the review process.

Through a Memorandum of Understanding between the state of Arkansas and FDA, a consortium of five Arkansas research universities provided FDA with comprehensive data on the synthesis and detection of graphene, and the study continues into FY 2020.

Empower Consumers and Patients

NCTR's research allows FDA to focus on promoting public health by empowering patients and consumers to make well-informed choices about their medical care including patient-focused medical product development. Within this area, NCTR will implement a Perinatal Health Center of Excellence and conduct research through a Memorandum of Understanding with CDER on over-the-counter drug review. Additionally NCTR will:

- identify human cancer mutations that can be used as biomarkers to potentially speed the development of effective personalized cancer treatments
- finalize research regarding the toxic potential of silver nanoparticles in feminine-hygiene products using 3D mucosal models and animal models.

With Congressional support, NCTR will fully implement the Virtual Center of Excellence for Perinatal and Maternal Pharmacology and Toxicology – also known as the FDA Perinatal Health Center of Excellence (PHCE). In FY 2018, the PHCE was accepted by the FDA Centers and ORA representatives with the goal to strengthen the scientific bases of decision making of FDA-regulated products used during pregnancy and in premature infants, newborns, and children. The PHCE council, with representatives from all FDA Centers and ORA, developed and accepted a framework for the development, review and selection of research projects. This critical area will be able to move forward with sustained Congressional support.

Over the Counter (OTC) Drug Review

In FY 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 will have also summarized ~300 relevant references on chlorine safety.

Strengthen Science and Efficient Risk-Based Decision Making

NCTR's research provides FDA regulatory science to inform standards development, analysis, and decision-making for the safety of FDA-regulated products. NCTR conducts a full range of studies in support of FDA's product portfolio. Within this area, examples of NCTR research include perinatal opioid exposure; detection of bacterial and microbial contamination; antimicrobial resistance and the human microbiome; and pathogen reduction.

Perinatal Opioid Exposure

The FDA Opioid Action Plan[69] 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 conduct lab-based *in vitro* research rather than relying solely on whole animal (*in vivo*) research. NCTR scientists are conducting research to assess perinatal opioid exposure, a concern shared in the perinatal-related FDA Drug Safety Communication.[70] NCTR, in collaboration with CDER, is finalizing the data on opioid exposure to brain cells during perinatal development.

Rapid Detection of Bacterial and Microbial Contamination

NCTR scientists 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*[71].

Figure 13*E. coli* is one of the most commonly found bacteria in foods

NCTR 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 the Center for Food Safety and Nutrition (CFSAN) scientists include:

- detecting *Listeria monocytogenes* faster using genetic tags

[69] For more information visit: https://www.fda.gov/Drugs/DrugSafety/InformationbyDrugClass/ucm484714.htm

[70] For more information visit: https://www.fda.gov/Drugs/DrugSafety/InformationbyDrugClass/ucm484714.htm

[71] For more information please visit: https://www.frontiersin.org/articles/10.3389/fmicb.2017.01493/full

- 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.

NCTR and CFSAN's collaborative tattoo research aims to 1) survey tattoo inks previously used in NCTR toxicology tests for microbial contamination and 2) develop a reliable method for the rapid detection and evaluation of pathogenic mycobacteria, including *Mycobacterium chelonae*, in 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 FY 2018 in the *Journal of Applied Microbiology*. [72] This study continues with the goals of:

- ensuring test and control groups are not affected by microbial contamination of tattoo inks used in animal studies, which may change the research outcome and interpretation
- 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

Artificial Intelligence (AI) System — DeepSafe

The Office of Regulatory Affairs (ORA) reviews import entry from foreign countries with over 40 million entry lines each year. This big data represents a critical regulatory challenge, but also offers tremendous opportunity to develop artificial intelligence capable of preventing the entry of adulterated, misbranded, or other violative goods. The current screening uses various rules to determine a risk score that determines if the goods will require a manual review. In collaboration with ORA, NCTR is conducting research using AI for screening and assessing FDA-regulated food imports. This research aims to develop an intelligent system by applying deep learning for screening and assessing import entry known as DeepSAFE. This AI system will make the product evaluation and risk-assessment process at the ports of entry more robust.

Strengthen Science and Efficient Risk-Based Decision Making

NCTR's research supports FDA to use regulatory science to strengthen risk-based decision making. NCTR brings modern scientific tools into FDA to maintain FDA's gold standard for product review, and to ensure FDA risk management is efficient and up-to-date. As the products that FDA is asked to review become more complex and specialized, there is a larger demand to develop innovative technologies and methods. Some of the research that supports this area includes informing standards development and using *in silico* tools for improving medical product development and making regulation more efficient. Within this area, examples of NCTR research include perinatal, pediatric, and maternal medicine; cancer-drug toxicity; precision medicine; biomarkers; nanotechnology; bioimaging; and bioinformatics.
In support of this priority, NCTR will:

- develop novel *in silico* (computer-based) methods to supplement animal models
- in collaboration with CDER, deploy an updated scientific database containing Marketing Application information on new molecular entities and biologics, and incorporating additional data about Breakthrough Therapy Designations

[72] For more information please visit: https://onlinelibrary.wiley.com/doi/abs/10.1111/jam.13713

- facilitate the development of a high-throughput and high-content genotoxicity assessment to evaluate the safety of FDA-regulated products.

Perinatal, Pediatric, and Maternal Medicine

NCTR is providing the 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 susceptible populations. These susceptible populations include pregnant women and infants—focusing on the perinatal period—the period-of-time including pregnancy, child birth, and infant/child development. 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 needed for drug approval in these populations. Therefore, these populations represent a vastly understudied stage of development.

Advancements at NCTR's bio-imaging facility allow FDA to translate imaging technologies from the laboratory animal to the clinical setting and to gather information not previously obtainable. This information helps the medical community understand pediatric-anesthetic use and reduce its adverse effects on children. These effects are assessed using minimally-invasive imaging technology, allowing visualization of biological processes in real-time, with as little interference as possible with life processes. NCTR research on pediatric anesthetics led to an FY 2017 FDA Drug Safety Communications where FDA approved label changes for the use of general anesthetics and sedation drugs in young children to include a warning about cumulative exposures that may affect the developing brain. A pediatric anesthetic-related ongoing study is evaluating the utility of neural stem-cell models to predict the effects of pediatric anesthetics sevoflurane and isoflurane in combination with nitrous oxide.

Scientists from NCTR; the Mayo Clinic in Rochester, Minnesota; and Baylor College of Medicine in Houston, Texas recently published results from neuropsychological tests that were conducted on school-age children who were given anesthesia during one or more surgeries that occurred before their third birthday. This study determined whether there are significant adverse effects of general anesthesia on subsequent brain function when given in the important period of rapid brain development after birth. This information may inform agency decisions about labeling and/or best practices for pediatric general anesthesia. A summary of this research was published in July 2018 and can be found at *Anesthesiology*.[73] NCTR also conducted research to evaluate the methods used to measure growth of *S. aureus* and the production of toxic shock syndrome as influenced by menstrual tampons. The manuscript titled, "Assessment of the Syringe Method for Testing Tampon-associated *Staphylococcus aureus* Growth and Toxic Shock Syndrome Toxin-1 Production" is undergoing internal review. The research concluded August 31, 2018 and should improve CDRH's review of tampon devices, provide a safer product, and increase consumer confidence.

Cancer-Drug Toxicity

Despite two decades of worldwide intensive research efforts to understand cancer biology for advancing anticancer-drug development, only ~200 anticancer drugs have been made available to cancer patients. This low throughput is in large part due to anticancer-drug development suffering high failure-rates during the later phases of clinical development.

[73] For more information visit: http://anesthesiology.pubs.asahq.org/article.aspx?articleid=2679328

NCTR, in collaboration with scientists from University of Birmingham in the United Kingdom and University of Arkansas at Little Rock summarized both successful and failed attempts in anticancer-drug development over the past 20 years. This collaborative review helped to identify why the current development model may be less than ideal. Furthermore, potential strategies for improvement of anticancer-drug development are offered in the publication, available in *Trends in Pharmacological Sciences.*[74] 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 helps 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.*[75]

NCTR also conducted research to develop a more rapid and sensitive *in vitro* (non-animal) assay for the identification of cancerous substances as an alternative to the much longer classical animal bioassay. One article was published in April 2018 in *Toxicological Sciences* and one article has been accepted for publication in *Food and Chemical Toxicology*. This research utilized a much faster method to identify potentially cancerous substances as opposed to classical 2-year animal methods.

In FY18, NCTR completed a study that will promote women's health by facilitating the development of personalized approaches to treat breast cancer. NCTR, in collaboration with the Office of Women's Health, completed a study to quantify genetic markers in difficult to treat breast cancers. An invited manuscript is in preparation for the Special Issue "Molecular Biology and Pathology of Breast Cancer" in the *International Journal of Molecular Scie*nces.

NCTR is conducting research to identify new clinical biomarkers that predict chemotherapy-induced cardiotoxicity prior to the occurrence of overt cardiac-tissue damage. NCTR published findings related to the identification of biomarkers that may predict chemotherapy-induced cardiotoxicity. The results were published in the February 2018 issue of *Experimental Biology and Medicine*. NCTR also identified additional biomarkers with the potential to predict cardiotoxicity before patient treatment or during early stages of chemotherapy. An abstract of the recent results has been accepted and will be presented in the Global Cardio-Oncology Summit 2018 as a poster.

Doxorubicin Research

Doxorubicin (DOX) is an effective chemotherapy treatment that is limited by its chronic cardiotoxicity—toxicity of the heart—which is dose-dependent, cumulative, and irreversible. Because early biomarkers of drug-induced cardiotoxicity could enable a precision medicine-based approach to chemotherapy treatment, NCTR scientists are studying DOX using various research approaches.

[74] For more information please visit: https://www.frontiersin.org/articles/10.3389/fmicb.2017.01493/full

[75] For more information please visit: https://www.sciencedirect.com/science/article/pii/S0378427418301395?via%3Dihub

A continuing DOX-related study involves scientists from NCTR, National Cancer Institute, Korea University, and CDER. In a previously completed study, it was found that males may be more susceptible than females to DOX toxicity. This current study specifically seeks to understand the molecular basis for different susceptibility to DOX toxicity between males and females. Further investigations will involve detection methods to determine where DOX and its metabolites may be localized in the heart. The most recent information about this research was presented at the 2018 Society of Toxicology[76] meeting. This study continues into FY 2019.

NCTR completed a study aimed at testing cardiotoxicity of various drugs including those used in chemotherapy. The goal of this preliminary study was to develop a mouse model of DOX-induced delayed cardiotoxicity in mice that will facilitate identification of early biomarkers for predicting risk of delayed cardiotoxicity. The mouse model is designed to mimic the delayed cardiotoxicity observed in human cancer survivors. This study may give rise to a larger study using the same model of cardiotoxicity.

Cyclophosphamide Research

Cyclophosphamide is a drug used to treat cancers and autoimmune diseases, by quickly controlling disease. However, because of its toxicity, it is replaced as soon as possible by less toxic drugs. Regular and frequent laboratory evaluations are required to monitor kidney function, avoid drug-induced bladder complications, and screen for bone-marrow toxicity.

Scientists from NCTR, CDER, and the University of Arkansas for Medical Sciences have demonstrated that the two commonly used chemotherapeutics discussed above (cyclophosphamide and doxorubicin), administered alone or in combination, did not induce behavioral alterations in a female-mouse model reflective of human breast-cancer patients. The study, which was completed in FY 2018, investigated the memory and attention problems that some female breast-cancer patients experience after chemotherapy—sometimes known as "chemo" brain. Thus, the selected chemotherapeutics were administered intravenously at clinically-relevant doses and the mice were assessed using a comprehensive test battery to detect effects on learning and memory, general activity, and motor coordination. The study results show no significant behavioral alterations and provide new insights into two commonly used chemotherapeutics. The NCTR lead author of the article was awarded the Developmental Neurotoxicology Society's "2018 Richard Butcher New Investigator Award" for this publication. The article is available online at *Toxicological Sciences.*[77]

Precision Medicine and Genetic Prediction

Biomarker development is a method for predicting FDA-regulated product toxicity and providing precision-medicine solutions such as individually-tailored therapeutic drug regimens. NCTR scientists continue research to identify new biomarkers—a biological indicator of a biological state or condition—that can be used to:

- identify populations susceptible to drug side-effects
- predict harmful effects of drugs during safety evaluations
- reduce or reverse cardiac injury

[76] For more information please visit: http://www.toxicology.org/pubs/docs/Tox/2018Tox.pdf

[77] For more information please visit: https://academic.oup.com/toxsci/article/162/2/462/4706007

- improve therapeutic patient treatments.

Examples of NCTR biomarker-development research to improve therapeutic patient treatments are shown below.

Genes are found in the DNA of every human cell and control how the cell functions—including how quickly it grows, how often it divides, and how long it lives. Despite all that is known about genes and their relationship to disease, more research is needed to better understand how genetic changes affect cells and disease, such as cancer. This knowledge may lead to improvements in the ability to develop personalized treatment plans.

Figure 14 NCTR scientist looks at cells through a microscope.

In an FY 2018 consortium effort with the Health and Environmental Sciences Institute, NCTR scientists collaborated with experts from other government agencies, academia, and industry to search for non-invasive biomarkers of neurotoxicity that can be evaluated in circulating biofluids, such as serum, plasma, urine, and cerebrospinal fluid. In the first set of studies, several unique biomarker candidates were identified, and an initial summary of these findings was published in *Experimental Biology and Medicine*[78]. Preliminary findings from this work were presented at the 2018 Society of Toxicology Annual Meeting. This collaborative study continues.

NCTR scientists used a well-known chemical that is toxic to the liver—thioacetamide—which could be a liver carcinogen (cancer-causing agent) in humans, to discover microRNA biomarkers of liver toxicity. They also used next-generation sequencing to discover early and sensitive microRNA biomarkers for liver injury and tumor progression. These biomarkers could improve cancer diagnosis, prognosis, and management. A paper summarizing the findings of this study can be found in *Scientific Reports*[79] and research on this topic continues.

In support of precision medicine, NCTR scientists presented a webcast lecture titled, “Ethnicity- and Gender-Related Differences in Alzheimer’s Disease (AD),” as part of the FDA Grand Rounds series. AD has a higher incidence in women at later ages and poses a greater threat to African-American and Hispanic communities. This presentation discussed NCTR’s novel research into proteins implicated in AD and their levels in post-mortem African-American and Caucasian brain tissues from both genders to explore ethnicity- and gender-related differences. Research studies like these are crucial to a precision-medicine approach treating neurodegenerative diseases like AD. A recording of the presentation and a brief synopsis can be found on FDA.gov.

NCTR is conducting a study specifically tailored to precision-medicine solutions for FDA entitled, "Sequencing Quality Control Phase 2 (SEQC2): A Consortium Effort to Assess Next-Generation Sequencing (NGS) for Enhanced Regulatory Science Research and Precision

[78] For more information please visit: http://journals.sagepub.com/doi/full/10.1177/1535370217739859

[79] For more information please visit: http://www.nature.com/articles/s41598-017-02798-7.pdf

Medicine." This effort seeks to develop quality metrics and standard analysis protocols for NGS and other similar technologies frequently encountered in regulatory applications and research. Thus, the outcome from SEQC2 has the potential to significantly impact FDA projects and practices and to prepare FDA for the effective use and review of NGS data. Three recent publications related to this research can be found in *Pediatric Investigation*[80], *Nature Biotechnology*[81], and *Experimental Biology and Medicine*[82].

Nanotechnology

The NCTR/ORA Nanotechnology Core Facility (NanoCore) supports collaborative research efforts within FDA, and between FDA and other government agencies and universities. This work provides information on issues related to the safety of nanotechnology-based materials that may be used in FDA-regulated products.

Research being conducted at the NanoCore to better understand the attributes of these emerging materials, their safety, and efficacy are listed below.

- With CDER, NCTR is studying how nanomaterial-containing drug distribute to different parts of the body, to determine their safety and efficacy, using rodent animal models.
- With OWH, NCTR is evaluating the potential migration and toxicity to the vaginal tissue of silver nanoparticles when used in feminine-hygiene products.
- With the National Toxicology Program, NCTR is developing documentation standards to shorten FDA review times for industry submissions of scientific nanomaterial data.

A continuing study at NCTR involves determining the effect of silver nanoparticles on the intestinal virome—the collection of viruses in and on the human body. The virome is thought to affect the overall human microbiome which is an integral part of understanding toxicity of regulated products. The rise in use of nanoparticles in many types of regulated products has made this issue vital to FDA. The most recent publication related to this study can be found in the *International Journal of Nanomedicine.*[83]

Another ongoing NCTR study deals with the impact that nanomaterials may or may not have on the formation of biofilm on the surface of teeth. Nanomaterials are being considered to combat the dental formation of biofilms which are precursors to tooth decay. Initial data reveals that low-concentrations of each of the tested nanomaterials have minimal effect on the growth of biofilms. This research was presented in June 2018 at the 118th General Meeting of the American Society of Microbiology. Further research is needed to study other nanomaterial candidates and their effect on biofilms.

Magnetic Resonance Imaging (MRI)

Full-brain MRI imaging offers the potential to dramatically improve detection of neurotoxicity produced by new drugs and to spur new drug development and evaluations. NCTR continues the development of minimally-invasive diagnostic methods for identifying nervous system-tissue

[80] For more information please visit: https://onlinelibrary.wiley.com/doi/epdf/10.1002/ped4.12044

[81] For more information please visit: https://www.nature.com/articles/nbt.4029.pdf

[82] For more information please visit: http://journals.sagepub.com/doi/pdf/10.1177/1535370217750087

[83] For more information please visit: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5961469/pdf/ijn-13-2857.pdf

anomalies. This technology, derived from FDA-regulated MRI instruments, is called magnetic resonance spectroscopy (MRS).

NCTR, in collaboration with Huntington Medical Research Institute, developed a method to improve the use of MRS scans to predict or diagnose medical abnormalities without the need for biopsies. The method is being used to identify Alzheimer's disease, dementia, and mild cognitive impairment. The data obtained from these studies are being readied to support the qualification of MRI signals as brain-toxicity biomarkers. Recent publications highlighting this approach can be found in: *Journal of Magnetic Resonance in Medicine*[84], *Experimental Biology and Medicine*[85], and *Neurotoxicology*[86].

NCTR, in collaboration with FDA Product Centers, is studying the bioaccumulation of gadolinium in the brain. Gadolinium is an agent commonly used during MRI procedures. This research contributed to a FDA Drug Safety Communication[87] on May 22nd, 2017 about gadolinium-based contrast agents (GBCAs) and their retention in the brain and will continue through FY 2019.

New and continuing imaging research at NCTR includes:

- studying the relationship of MRI findings and biological fluid biomarkers of neurotoxicity
- comparing MRI results to current neurotoxicity assessment methods to assess MRI sensitivity and specificity.

Other imaging advancements include:

- Multi-center study completed in collaboration with Amgen, AstraZeneca, and GlaxoSmithKline on non-invasive MRI biomarkers of DILI. Publication in *PLoS One*[88]
- Initiated a collaboration with University of Arkansas-Fayetteville to use MRI scans of porcine mitral valve to optimize human-valve replacement. Publication in *PLoS One*[89]
- Validated a three-dimensional MRI technique to more accurately evaluate brain neurotoxicity. It will allow for minimally-invasive detection of brain lesions.

Bioinformatics

Bioinformatics uses computer software tools to develop and improve methods for storing, managing, and analyzing large quantities of biological data. NCTR develops, provides training for, and makes bioinformatics tools available to FDA and the global research community. FDA must have the software and database tools to manage the large amount of scientific data generated by new technologies required to improve product development, safety assessments, and risk analysis. Computer-based methods (*in silico*) are also important since they can, in some cases, be used as an alternative to animal methods (*in vivo*). Below are examples of NCTR's bioinformatics program.

[84] For more information please visit: https://onlinelibrary.wiley.com/doi/pdf/10.1002/jmri.25378

[85] For more information please visit: http://journals.sagepub.com/doi/abs/10.1177/1535370217739859?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed

[86] For more information please visit: https://www.sciencedirect.com/science/article/pii/S0161813X18300330?via%3Dihub

[87] For more information please visit: https://www.fda.gov/Drugs/DrugSafety/ucm455386.htm

[88] For more information please visit: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0197213

[89] For more information please visit: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0184042

Publicly Available Dataset/Database Name	Description
DILIrank	Largest publicly available annotated dataset listing 1,036 FDA-approved drugs ranked by potential to cause drug-induced liver injury (DILI). The drugs were defined and verified as shown below: • 192 "Most-DILI" concern • 278 "Less-DILI" concern • 312 "No-DILI" concern • 254 "Ambiguous-DILI" concern DILIrank is used by FDA reviewers, industry for drug development, and researchers for adverse drug reaction studies and to build scientific models. More informationcan be found at *Drug Discov ery Today*[90]
Endocrine Disruptor Knowledge Base (EDKB)[91]	Database of roughly 3,000 chemicals that interfere with the endocrine system; used to develop computer-based predictive models that are quicker and less expensive than traditional experiments. Incorporated into larger government-initiated toxicological projects, such as EPA's Tox21.
Estrogenic Activity Database (EADB)[92]	Part of EDKB that assembles data from a variety of data sources and contains 18,114 data points collected for 8,212 chemicals tested in 11 different species. Incorporated into larger government-initiated toxicological projects, such as EPA's Tox21.
FDALabel Database – Drug Labelings[93]	NCTR created and maintains two FDALabel versions that make previously unavailable information easy to access—one for the public and one for FDA staff who review drug labeling. FDALabel, allowing customizable searches of over 99,000 labeling documents is regularly used by: • researchers for adverse drug-reaction studies • FDA medical officers for drug review • pharmaceutical companies for drug development and repositioning • physicians and consumers for drug-safety information. Labeling documents with information about product indications, target populations, and adverse drug reactions are added weekly. In

[90] For more information about DILIRank visit: http://www.sciencedirect.com/science/article/pii/S1359644616300411

[91] For more information about EDKB visit: http://www.fda.gov/ScienceResearch/BioinformaticsTools/EndocrineDisruptorKnowledgebase/default.htm

[92] For more information about EADB visit: http://www.fda.gov/ScienceResearch/BioinformaticsTools/EstrogenicActivityDatabaseEADB/default.htm

[93] For more information about FDALabel visit: http://www.fda.gov/ScienceResearch/BioinformaticsTools/ucm289739.htm

	collaboration with CDER, NCTR is developing a version that will allow multiple products to be searched at the same time for use by CDER and CBER reviewers

FUNDING HISTORY[94]

Fiscal Year	Program Level	Budget Authority	User Fees
FY 2016 Actual	$63,329,000	$63,329,000	---
FY 2017 Actual	$63,331,000	$63,331,000	---
FY 2018 Actual	$64,512,000	$64,512,000	---
FY 2019 Annualized CR	$64,512,000	$64,512,000	---
FY 2020 President's Budget	$66,512,000	$66,512,000	---

BUDGET REQUEST

The FY 2020 Budget Request is $66,512,000, which is all Budget Authority. Budget Authority increases by $2,000,000 compared to the FY 2019 Annualized Continuing Resolution level. The FY 2020 Budget will allow NCTR to continue research to support emerging technologies and toxicology assessments required by FDA and increase the scope of NCTR's collaborative research. Specifically, NCTR will continue to:

- expedite the translation of scientific advancements to regulatory application
- develop new tools and approaches to assess the safety and efficacy of FDA-regulated products
- integrate toxicology safety assessments maximizing existing and emerging technologies
- provide regulatory science capacity to evaluate FDA-regulated products in a more predictable, consistent, and efficient way
- provide valuable research data on FDA-regulated products using new technologies
- help FDA to better understand and interpret diverse data submissions generated using new methodologies and techniques.
- inspire innovation and knowledge sharing through collaboration.

These research areas include, but are not limited to, the advancement of bioinformatics technologies, precision medicine, biomarkers, bio-imaging, perinatal health, neurotoxicology, human microbiome, and nanotechnology. This research will be in collaboration with scientists from around the world in government, academia, and industry to exchange views on how to develop, apply, and implement innovative methodologies into regulatory-assessments. Investments in these areas in recent years have enhanced the capabilities and expertise that allows FDA to capitalize on global scientific advancements and expand FDA’s regulatory-science capacity and, ultimately, benefit the American public. These funds will allow such efforts to continue and will give the programs and associated projects the opportunity to develop.

[94] Numbers reflect comparability adjustments for FY 2018, FY 2019, and FY 2020 consistent with budget figures.

BUDGET AUTHORITY

Medical Product Safety ($2.0 million)

Promoting Domestic Manufacturing: +$2.0 million

NCTR will help reduce the cost and uncertainty of adopting new manufacturing technologies by developing a science-based framework that includes the regulatory tools and guidance for how products will be evaluated, and by funding research, developing and testing of these technologies. NCTR will be able to pursue the following activities to focus on supporting FDA's highest priorities for FY 2020.

Virtual Center of Excellence for Perinatal and Maternal Pharmacology and Toxicology

As part of FDA's FY 2020 initiative to advance safe and effective medical products, NCTR will implement the newly established FDA Virtual Center of Excellence for Maternal and Perinatal Pharmacology and Toxicology, also known as Perinatal Health Center of Excellence (PHCE).

Participants from across the FDA Centers and ORA, along with the researchers from across the agency have already demonstrated a great deal of interest in the PHCE with NCTR receiving 20+ peer-reviewed research proposals in the planning phase. The additional funds will be used to stimulate more perinatal research efforts across FDA. FDA expertise in the field of pediatric medicine has already led to advancements in the field through faster, less expensive, and more predictive approaches and models. The virtual center will enhance the effort by harnessing the collective talents and resources of the agency and serve as a hub for research and collaborations internally and externally. The importance and potential for the PHCE is tangible and realistic given the current knowledge gaps and rapidly evolving technology. It will improve the safety and/or efficacy of FDA-regulated products in understudied populations, including pregnant women and infants — focusing on the perinatal period (the period-of-time including pregnancy, child birth, and child development).

PERFORMANCE

NCTR's performance measures focus on research to advance the safety of FDA-regulated products, to develop a strong FDA science base for emerging technologies, and to provide precision medicine solutions to protect and improve the health of the American public as represented by the following table.

Measure	Most Recent Result / Target for Recent Result	FY 2019 Target	FY 2020 Target
263103: Conduct translational and regulatory research to advance the safety of products that FDA regulates (Output)	FY 2018: In collaboration with CDER, an article was published in Alimentary Pharmacology & Therapeutics on drug-induced liver toxicity caused by bile acids. (Target Met) FY 2018: Optimized differentiation conditions of neural precursor cells to investigate the effects of opioid exposure on prenatal development. Investigation of the individual effects of opioids on neural precursor cell viability and toxicity are on-going.(Target Met)	In collaboration with CDER, finalize data on the bioaccumulation of gadolinium, a heavy metal commonly used as a contrast agent during Magnetic Resonance Imaging (MRI) procedures Finalize data on the potential neurotoxic effects of opioids during perinatal development	Develop methodology that can be used to detect *Burkholderia cepacia* in non-sterile pharmaceutical products and pharmaceutical water Gather data regarding the toxicity of 40 FDA approved small molecule kinase inhibitors—highly effective cancer fighting molecules—on the liver and heart
263201: Develop science base for supporting FDA regulatory review of new and emerging technologies (Output)	FY 2018: Findings published in Toxicological Sciences on research utilizing a much faster method to identify potentially cancerous substance as opposed to classical 2-year animal methods (Target Met)	Finalize research regarding the toxic potential of silver nanoparticles in feminine-hygiene products using 3D mucosal models	Develop a safety assessment for gene editing therapies that are pending clinical trials

Measure	Most Recent Result / Target for Recent Result	FY 2019 Target	FY 2020 Target
262401: Develop biomarkers to assist in characterizing an individual's genetic profile in order to minimize adverse events and maximize therapeutic care (Output)	FY 2018: Completed a study to quantify genetic markers in difficult to treat breast cancers. Manuscript in preparation for invited Special Issue "Molecular Biology and Pathology of Breast Cancer" in the International Journal of Molecular Sciences. (Target Met)	Identify human cancer mutations that can be used as biomarkers to potentially speed the development of effective personalized cancer treatments	Construct a database of information on how genetics effect drug efficacy and toxicity (pharmacogenomic data) in understudied minority populations that will aid in the clinical application of biomarkers In collaboration with the University of Arkansas for Medical Sciences, develop biomarkers of the heart to predict and mitigate radiation-induced heart disease
264101: Develop risk assessment methods and build biological dose-response models in support of food protection (Output)	FY 2018: NCTR published findings related to antimicrobial drug residues and their effects on human intestinal microbiome and epithelial cell permeability/wound healing in the February 2018 issue of *Anaerobe* and the November 2017 issue of *Food and Chemical Toxicology*. CVM and NCTR also presented related data at the NIH-FDA Joint Agency Microbiome meeting in December 2017. (Target Met)	Explore and provide results of research on how fecal microbial transplant is an effective treatment for bacterial infections such as *Clostridium difficile*	In collaboration with CVM, develop a database and analysis tool to better understand and control Salmonella enterica in foods and feed

Measure	Most Recent Result / Target for Recent Result	FY 2019 Target	FY 2020 Target
263104: Use new omics technologies to develop approaches that assess risk and assure the safety of products that FDA regulates (Output)	Investigators found that antibiotic-coated catheter inhibited growth of antibiotic-sensitive Staphylococcus aureus and Enterococcus faecium but not antibiotic-resistant bacteria isolated from patients. Silver-coated catheter prevented growth against both antibiotic-sensitive and -resistant bacteria.(Target Met)	Discover and validate early biomarkers of cardiotoxicity associated with an effective chemotherapy drug utilizing a variety of methods including whole RNA genome sequencing	Develop parameters to assist reviewers when evaluating applications submitted to FDA for genome assembly-based devices, products, and services
263102: Develop computer-based models and infrastructure to predict the health risk of biologically active products (Output)	FY 2018:Novel data mining and visualization methods resulted in a total of 63,082 drug adverse event pairs were identified from FAERS as the significant association between 936 drugs and 10,316 adverse events. New safety signals were identified when comparing with the currently available information in various sources. Results were presented in the Society of Toxicology 2018 Annual Meeting. (Target Met)	Facilitate the development of the quality metrics and standard analysis protocols for Next Generation Sequencing (NGS) and other similar technologies Develop novel in silico (computer-based) methods as alternatives to animal models	Examine the utility of In Vitro (lab-based) to In Vi*vo* (animal-based) Extrapolation (IVIVE) as a new tool for FDA safety assessments

Advance the Safety of FDA-Regulated Products

NCTR research is vital to ensure the safety and effectiveness of the products that FDA regulates. Two specific examples include research regarding opioids and gadolinium—a heavy metal commonly used as a contrasting agent during MRI procedures. In FY2018, in support of the FDA Opioid Action Plan, the NCTR initiated a study to investigate opiod exposure during

prenatal development. Final data on this study should be available in FY 2019. Additionally, in FY 2019, NCTR will continue to advance the safety of FDA-regulated products by providing data on the bioaccumulation of gadolinium in the brain and, in FY2020, will gather toxicity data on FDA-approved cancer fighing molecules, called kinase inhibitors.

Develop Science Base for New and Emerging Technologies

NCTR continues to develop the science base to help FDA in its regulatory review of new and emerging technologies. In FY 2018, NCTR published research utilizing a faster method to identify potentially cancerous substances than the current 2-year animal methods. In FY 2019, NCTR research regarding the toxic potential of silver nanoparticles in feminine-hygiene products using 3D mucosal models will be finalized. In FY 2020, scientists plan to develop a safety-assessment for gene editing therapies that are pending clinical trials.

Precision Medicine

NCTR continues to support FDA in its pursuit for precision medicine solutions through cutting-edge research that uses genetic information from an individual or demographic group to tailor treatment regimens to increase safety and effectiveness. NCTR investigates post-market chemotherapy drugs and new alternative drugs and methods available to cancer patients. In FY 2018, NCTR completed a study on genetic markers for difficult to treat breast cancer. Manuscripts and publications are currently in development. Work in precision medicine will continue in FY 2019 with research to identify human cancer mutations to help speed the development of personalized cancer treatments. In FY 2020, NCTR will work in collaboration with the University of Arkansas for Medical Sciences, to develop biomarkers of the heart to predict and mitigate radiation-induced heart disease.

PROGRAM ACTIVITY DATA

National Center for Toxicological Research Program Activity Data (PAD)			
Program Workload and Outputs	**FY 2018 Estimate**	**FY 2019 Estimate**	**FY 2020 Estimate**
Research Outputs			
Research Publications	170	165	160
Research Presentations	135	145	150
Patents (Industry)	5	5	5
Leveraged Research			
Federal Agencies (Interagency Agreements)	3	3	3
Nongovernmental Organizations	28	30	32
Active Research Projects	159	165	176